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Vox Sanguinis ISBT International Society of Blood Transfusion

Oral Presentations

Plenary Session 1: Climate change, pandemics, disasters: The new normal?

PL01-L01 | One health

J Amuasi

Abstract not available.

PL01-L02 | Disaster planning and preparedness in transfusion medicine

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Transfusion support is an essential element of modern healthcare and therefore should be considered in emergency and disaster planning. In addition, many national civil contingency arrangements require healthcare providers to prove that they can deal with emergencies while ensuring other critical services continue. Transfusion communities refer to these arrangements as 'blood supply contingency', 'emergency or 'disaster preparedness'. There is no consistent categorisation, but all may result in disruption of normal activity and implementation of emergency measures. Most definitions recognise that with planning and preparation, the impact may be avoided or at least mitigated. Preparedness should be considered a dynamic, collaborative process that actively identifies and manages potential and emerging threats. The threat landscape is continuingly evolving, and recent events demonstrate the 'One Health' concept that humans, animals and ecosystems are interconnected. Examples include extreme weather, earthquakes and pandemics many of which have challenged the continuity of blood supply. However, it is mass casualty events, together with changing trauma and transfusion practice, which have stimulated a renewed academic and political interest in transfusion emergency preparedness. Planning and preparation are essential to protect patients, support staff and mitigate the impact of the emergency and are increasingly mandated.

WHO and others have developed guidance to support national and international efforts in their response to blood service disruptions due to disasters and other emergencies. The aim is to provide a sufficient supply of safe blood and blood components. The role of the national blood system during emergencies must be clearly defined and needs to be an integral part of wider emergency preparedness. Preparation should be underpinned by locally sensitive risk assessments using relevant local or country level data for risks that will or may impact on transfusion services. Whereas it is not possible to predict the nature of every situation that could impact on the blood supply, local transfusion services should assess their own situation, projected demand, capabilities and resources in the development of their own response plans. Planning should consider the concurrence and combinations of events and the response should be proportionate and coordinated with others. Resilience options include the principles of Patient Blood Management and Business Continuity. The main aims are to maintain critical services during the emergency and prepare for recovery. Local success is translating this guidance and the emerging evidence base into locally sensitive practice. Societal success will require a multi-sectoral and collaborative approach together with a "whole of society" approach to health hazards.

PL01-L03 | Emergence of infectious diseases due to climate change

C Erikstrup

Abstract not available.

Plenary Session 2: Lab grown blood: Future or fantasy?

PL02-L01 | The RESTORE clinical trial

A update on the RESTORE clinical trial: Manufacture and clinical assessment of cultured red cells

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With an ageing population and developments in medicine and surgery, many heath organisations fear the future demand for blood may

exceed availability, increasing the need to find new sources of red blood cells (RBCs), especially of rare blood types. The generation of fresh RBCs grown from human blood stem cells (or immortalised cells) is one potential way to provide novel transfusion products. The use of such cells has the promise better care for those patients who require regular transfusions throughout life (e.g., thalassemia, sickle cell disease and certain cancers), because the lab grown blood cells are freshly made and so may last longer in the patients circulation. If these cells do last longer as hoped, this may reduce the number of transfusions needed and reduce iron loading of tissues. The challenge, however, is still to produce enough freshly grown red blood cells needed for a standard adult therapeutic dose. In 2011, the Douay group conducted a ground breaking proof-of-principle mini-transfusion of autologous manufactured RBC given to a single volunteer

The **RE**covery and survival of **ST**em cell **O**riginated **RE**d cells (RESTORE) clinical trial is Phase 1 trial powered to conduct the first ever clinical assessment of a mini dose transfusion of allogeneic lab grown RBCs generated from adult stem cells in healthy human volunteers. The cultured cells performance in the recipient will be compared to the performance of the standard RBCs produced by the same donor transfused into the same recipient and the trial will also test safety.

The RESTORE team recently announced that two clinical trial participants were transfused with an allogeneic mini dose of lab grown blood to world-wide media interest. The RESTORE trial is still ongoing, scheduled to complete 2024, and this presentation will discuss the challenges of setting up a phase 1 comparative study and will provide an update on trial progress.

PL02-L02 | Ex vivo platelet production system: Future perspective from first-in-human study by IPSC-PLTS

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Platelet products are provided from blood banks which collect blood from healthy donors. However, approximately 5% of platelet transfusion patients are complicated with alloimmune platelet transfusion refractoriness (allo-PTR) due to alloantibodies against class I human leukocyte antigens (HLA-I) or human platelet antigens (HPA). But for patients with rare HLA-I or HPA, donors are difficult to find. Aiming to ultimately solve this issue, we had developed *ex vivo* production system of iPS cell-derived platelet products (iPSC-PLTs), which were based on our technologies, megakaryocyte cell lines (imMKCLs) from patient iPSCs (Cell Stem Cell, 2014; Blood Advances, 2022), turbulent flow bioreactor (Cell, 2018) and new drugs (Blood Advances, 2017; Stem Cells Trans Med, 2017). The iPLAT1 study was conducted from 2019 to 2020 as the first-in-human clinical trial of iPSC-PLTs (Blood, 2022). The subject was a patient with aplastic anaemia complicated by anti-HPA-1a antibody-induced allo-PTR and had no matched HPA-1b/1b donor in Japan. Three dose cohort studies, as a dose-escalation fashion, allowed us to consider possible problems with the post-transfusion measurement method and the circulation capacity of the iPSC-PLTs. I would like to discuss scientific points towards further improvement of *ex vivo* manufacturing of iPSC-PLTs in my talk.

PL02-L03 | Large scale production of cultured red blood cells for transfusion purposes

M von Lindern

Abstract not available.

Plenary Session 3: A social scientists perspective: Current and emerging challenges in transfusion medicine

PL03-L01

Abstract not available.

PL03-L02

E Merz

Abstract not available.

PL03-L03 | The power of science, art and co-design for enhancing donor recruitment and retention

E Ferguson

Abstract not available.

Local Day Session 1: News from Nordics

LD01-L01 | Country presentations

Abstract not available.

LD01-L02 | Scandinavian collaboration on blood supply in crisis and war

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The co-operation between the Nordic countries is the world's oldest regional partnership (https://www.norden.org/en/information/official-nordic-co-operation). We share many similarities as countries. Geography, especially in Fennoscandia with long borders, large sparsely populated areas in the north and islands in the Baltic Sea as well as in the north and west of Norway, creates a challenge for the blood supply chain during normal times as well as in crises. These challenges can best be overcome with close continuous cooperation both in civilian and military preparedness plans.

Challenges

The national regulations in our countries are based on the EU Directive, but despite the same origin the translations and interpretations vary in some aspects. Donor questionnaire and testing requirements may differ. For instance, in Finland NAT hepatitis B, C and HIV are required, while in Norway and Sweden only serology testing is mandatory. National regulations may complicate import or use of blood from another country. Differences in the organization of the blood services in our countries centralised blood service in Finland and individual hospital-based blood service in Norway and Sweden, may obstruct agreements and collaboration on a national and international level. Documentation of transfusions and blood product traceability may be difficult due to different IT-systems. All these challenges exist during normal activity and must be solved to facilitate Nordic collaboration in civilian preparedness and in crises where presence of armed forces is required.

Current activities

We have initiated a civilian Nordic network in Transfusion Medicine and there is a close military collaboration between our countries. Recently, meetings have been arranged to increase awareness of the need for blood preparedness plans, on a local, national and regional level. Increased availability and sustainability of blood product supply are needed. Dry plasma, used in military forces for many years and of increasing use in civilian prehospital care, is a limited resource worldwide. In a Nordic project, initiated and led from Norway a Nordic production line of dried plasma is planned. Cold-stored and cryopreserved platelets, that can be stored for 14 days and several years respectively, may make platelets and balanced transfusion available in remote areas. Whole blood is introduced to civilian emergency medicine in Norway, Sweden and Finland and experiences of these projects are shared. Norway has introduced civilian walking blood banks in remote areas. In addition, civilian-military meetings are held to discuss blood support in the defense forces in our respective countries.

Summary

Close relationship between civilian blood services, transfusion medicine experts, the defense forces and competent authorities, are needed to ensure adequate blood supply and preparedness for crises and in war situations in the Nordic countries. Currently, collaboration is often made from personal contacts. A coordinated effort, formal agreements and authority to make decisions will strengthen the collaboration and ensure implementation of documented plans.

LD01-L03 | Blood groups and common infections

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Background: Blood group antigens are of interest to blood services because of the risk of antibody driven transfusion reactions. However, these membrane bound proteins, of which some are glycosylated, have specific functions and the difference in antigen occurrence between populations is believed to have been caused by continuous selection pressure mostly from infectious agents. The different glycoprotein blood groups are the result of glycosyltransferase enzymes that add sugars to membrane proteins. Variations in these glycoproteins are believed to be caused by structural overlap (mimicry) with surface glycoproteins on different infectious agents and consequently selection pressure towards human antigens that differ from those on infectious agents. Given this hypothesis we would expect to find associations between blood groups and different infections.

Aims: To determine the association between blood groups and common infections in Denmark.

Methods: This study was based on the Danish Blood Donor Study (DBDS), which is a nationwide blood donor cohort that comprises more than 100,000 extensively genotyped individuals linked to both blood bank data and Danish health registries including the Danish National Patient Registry, the Danish Prescription Registry and the Danish Microbiology Registry (MiBa). Blood groups were predicted using both available serological phenotypes and genetics using deep learning techniques such as denoising auto encoders and convolutional neural networks resulting in highly accurate combined predictions for A, B, D, D-weak, C/c, CW, E/e, Coa /Cob, Doa /Dob Fya /Fyb, Jka /Jkb, K/k, Kna, Kpa, Lea /Leb, Lua /Lub, M/N, S/s, P1, Vel, Yta /Ytbas well as secretor status. Associations between infections and blood groups were assessed using logistic and Andersen-Gill Cox-regression models adjusted for different covariates including sex and age.

Results: For SARS-CoV-2 infection, we could confirm the previously identified association to ABO where blood types B, A and AB were found more susceptible towards infection compared to blood type O with ORs 1.1 CI[1.06-1.14] p.

Local Day Session 2: Donors and donation in Nordics

LD02-L01 | Lessons learned and changes made based on FinDonor 10,000 study

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Introduction: The Finnish Red Cross Blood Service (FRCBS) has been active in scientific research since its foundation for 75 years ago. We are focused on two research areas: blood supply chain and new cell therapies and compatibility of organ and tissue transplantations.

Blood donation is one of the entities of Blood supply chain research. The donor research group was founded for ten years ago, and its first significant achievement was the establishment of the FinDonor 10,000 research project and the collection of the blood donor cohort in 2015–2017.

Aim: The FinDonor cohort study was established to help the FRCBS in understanding the effects of regular blood donation to iron stores and health of blood donors.

Materials and Methods: The FinDonor cohort consist of 2584 blood donors (1013 men, 1572 women) and of 8003 samples collected in the capital city region of Finland between April 2015 and December 2017. Whole blood count, CRP, ferritin and sTFR were measured from the samples, and DNA was isolated for Genome-wide association studies (GWAS). Besides blood samples also health and lifestyle related data was collected by using electronic study questionnaires. The majority of FinDonor participants have given their permission to include their cohort data into the FRCBS Biobank and therefore FinDonor participants are an important research source also in donor research projects being enabled via Biobanking.

Results: FinDonor studies showed that donation activity was the most important factor affecting blood donor iron levels, but regular blood donors still considered their health generally to be excellent. And importantly, self-reported health of donors with lower iron stores was not lower than self-reported health of donors with higher iron stores.

We detected that the youngest female age group (18–25 years) had remarkable lower iron stores than all other age groups, including the other premenopausal women. Based on these findings we started to seek international benchmarking data and to revise and strengthen iron deficiency mitigation policies with targeted measures to this specific age group. Targeted iron deficiency related information was added to the website, iron related question was added to first-time donor test ("Can I donate?"), anaemia question was added to the electronic donor health questionnaire, donor invitation practices were revised, recommendation of donation frequency was limited to one donation per year, and amount of iron provided as iron replacement after a successful donation was doubled for the young women.

After 2 years of completed policy changes the deferral rates due to too low Hb among young female repeat donors decreased from 8.4% to 3.9% and the donation activity (mean donation number per year) from 1.27 to 1.04.

Conclusion: Based on FinDonor study results several donor policy changes were introduced to better protect the iron balance of young female donors. The first results indicate results of successful direction of policy changes. Further follow up and studies are needed to verify these preliminary positive findings.

LD02-L02 | Hepatitis E virus in Finnish blood donors and symptomatic infections in the Finnish population

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Background: Hepatitis E virus (HEV) as a potential hazard for transfusion has received increasing interest in Europe during the last decade. The infection is usually mild or asymptomatic in healthy persons, but severe forms may occur in immunocompromised patients or those with a severe liver disease. Epidemiological studies on hepatitis E have been performed in several European countries in the 2010s. In Finland only restricted information has been available on the virus in the blood donors despite the findings in pig farming.

Aims: This study aims to survey the blood donors for antibodies against hepatitis E and for hepatitis E RNA. The antibody findings give information on the population prevalence of the virus and the RNA findings support a risk-based decision making concerning transfusion safety procedures. We also wanted to complement the screening information with the national infectious disease registry (FIDR) statistics on symptomatic HEV to have a broader view on this disease as a public health concern.

Methods: We analysed 23,137 blood samples from Finnish blood donors who had given a biobank consent to the Finnish Red Cross Blood Service (FRCBS) biobank. The samples were tested with Procleix HEV Assay (Grifols Diagnostics Solutions) for the RNA and with the VIDAS anti-HEV IgG assay (BioMérieux) for the HEV IgG antibodies. Further, antiHEV IgG positive samples, initially reactive HEV RNA samples and HEV RNA positive samples (altogether 88 samples) were analysed with VIDAS anti-HEV IgM assay (BioMérieux) to evaluate prevalence rate of recent infection. The database of the FIDR was surveyed for reported cases of HEV during the years 2016–2022. Results: Four samples were interpreted as HEV RNA positive resulting in HEV-RNA prevalence of 1:5784. This result is among the lowest prevalence statistics in Western European countries but higher than e.g. Australia or USA. Of the 1012 blood donor samples analysed for anti-HEV IgG 75 were positive (7.4%, 95% CI 5.9%–9.2%). This result is also at the lower end of European countries. Between 2016 and 2022, 249 hepatitis E cases were reported to FIDR. The mean annual incidence was 0.7/100,000 inhabitants. The incidence decreased from 1.0 to 0.5/100,000 in 2019–2022. The reporting of detected HEV

cases is mandatory for diagnostic laboratories in Finland. Localised small epidemics with less than 10 cases were detected during the period.

Summary/Conclusions: Prevalence of HEV RNA positivity and anti-HEV IgG among Finnish blood donors are relatively low in European comparison. There appears not to be a significant increasing trend in the symptomatic infections among Finnish general population even though studies at slaughterhouses have demonstrated a low level of risk from domestic meat products. The cumulative food derived risk from different sources can be estimated to be much higher than the risk from transfusion on the level of the Finnish population. Follow-up of the epidemiological situation and continuous awareness of HEV as a part of the transfusion risk landscape is necessary.

LD02-L03 | Long-term haemovigilance: The SCANDAT experience

G Edgren

Abstract not available.

LD02-L04 | Fetal NIPT *RHD* screening: Status and outlooks in the five Nordic countries

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Non-Invasive Prenatal Testing (NIPT) of foetal *RHD* is used to predict the foetal RhD in RhD negative pregnant women. This analysis is based on testing cell-free foetal DNA present in the maternal plasma. For non-immunised RhD negative pregnant women, this analysis is used as a screening (antenatal *RHD* screening) to guide the administration of antenatal and often also postnatal anti-D immunoglobulin prophylaxis.

Antenatal *RHD* screening allows for the administration of anti-D prophylaxis only to those women carrying an RhD positive foetus. Thus, unnecessary use of anti-D prophylaxis is avoided, and unnecessary exposure to an immunoreactive blood product is avoided.

The Nordic countries have been pioneering the implementation of antenatal *RHD* screening. In Sweden, the first local implementation was launched in 2009 in Stockholm, and the first national programme was launched in Denmark in 2010. Currently, antenatal *RHD* screening is fully implemented in all Nordic countries (Denmark, Sweden, Norway, Finland and Iceland). High method reliability has been presented for the antenatal *RHD* screening in these countries.

LD02-L05 | PBM strategies–Where are we and what is the next step

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Background: Blood transfusion is a common medical treatment, but with large variations of usage between nations, regions, hospitals and treating physicians. Patient Blood Management (PBM)–to give blood on appropriate indications and avoid not necessary transfusions were early implemented by National Blood Organisations in Australia and UK. Multidisciplinary teams made guidelines in different patient groups. In the Nordic countries we have been- and still are–high consumers of RBC transfusions.

Sweden: A PBM working group under Swedish Blood Alliance was initiated 2018. We have lectured at conferences and local meetings, informed about PBM in transfusion committees and published articles in national and international journals. We have published general guidelines of indications for RBC transfusions, transfusion thresholds, evaluation after each unit and perioperative transfusion strategies. The focus is continuous work with education and to improve statistics and follow-up of strategies. A National Group (NAG) with representatives from the authorities was formed in the beginning of 2023. 2021 35 RBC units /1000 inhabitants were transfused, with large variations between the regions.

Finland: A national PBM network with monthly meetings was established in Finland, 2021. In 2022 the network accomplished an online survey of transfusion practices and knowledge of PBM and arranged a seminar with emphasis on anaemia and multiprofessional collaboration. This year the aim is to continue the education of PBM principles, perform a survey about transfusion protocols in emergency situations and emphasise the importance of implementing PBM. The use of RBC was 32 units/1000 inhabitants in 2022.

Norway: The Norwegian PBM working group was established in 2015 by the Norwegian Association of Immunology and Transfusion Medicine. We were involved in the Norwegian "Choosing wisely campaign", where we contributed with recommendations in transfusion medicine. One recommendation was the use of single-unit RBCs in haemodynamically stable patients. The compliance of this recommendation was encouraging. The Norwegian PBM group has recently applied as an own association under the Norwegian Medical Association, giving the opportunity to invite other specialties to join the group. The PBM group plans a national survey to evaluate transfusion practices and a paper is submitted for publication to the Norwegian Medical Association, stressing the need to implement optimization of

iron and haemoglobin in patients prior to major elective surgery. The first PBM seminar in Norway will be arranged in June 2023. 2021 28 RBC units/1000 inhabitants were transfused.

Conclusion Blood is a limited resource and restricted compared to liberal transfusion strategies result in less transfusions, less complications, decreased length-of stay in hospital and decreased costs in most patient groups, shown in many publications. Transfusion indications and thresholds considering age, specific diagnoses and quality of life variables need further evidence, but in general restricted transfusion strategies should be implemented.

Local Day Session 3: Blood components in Nordics

LD03-L01.1 | Cryopreservation of platelets: Karolinska experiences

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Background: Cryopreserved platelets in 5% dimethyl sulfoxide (DMSO) has recently been implemented in routine use at Karolinska University Hospital, in the treatment of bleeding and as preparedness inventory. A comprehensive in vitro characterization was performed prior to a clinical feasibility study in remote locations.

Aims: To summarise the in vitro and in vivo data performed on cryopreserved platelets at Karolinska.

Methods: A review report including articles from Karolinska University Hospital and Karolinska Institute referenced in PubMed and published in English between 2018 and 2023 studying the effect of cryopreservation on platelets *in vitro* and *in vivo* was performed.

Results: Four in vitro studies and one clinical feasibility study in remote hospitals were performed. Ninety-two buffy coat platelet units were used in the in vitro studies and twenty-three patients were included in the clinical feasibility study, on bleeding indication. Highlighted in vitro data indicate that the use of a uncontrolled freezing rate protocol is feasible, creating a platelet product comparable to using a controlled rate freezing equipment during cryopreservation of platelets and that INTERCEPT-treated platelets appeared slightly more susceptible to freezing than conventional fresh platelets. All included papers are listed in Table 1.

Summary/Conclusions: Cryopreserved platelets show a reduced recovery and viability after freezing and thawing including several ultrastructural and phenotypic deteriorations compared with liquid-stored platelets but, importantly, all data show that cryopreserved platelets exert haemostatic potential in vitro. Cryopreserved platelets in remote hospitals are logistically feasible in the treatment of bleeding. Clinical effectiveness and safety previously shown in other studies are supported in this feasibility study.

LD03-L01.1 Table 1

Meinke	Transfusion	2018
Tynngård	Blood transfuse	2020
Tynngård	Transfusion	2021
Sandgren	Blood transfuse	2022
Wikman	Front public health	2023

LD03-L01.2 | Cryo preserved versus cold storage platelets: What way to choose?

H Braathen

Abstract not available.

LD03-L02 | Platelet lysate, where are we now?

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Human platelets are rich in growth factors needed for cell culture and have been used successfully as an animal serum replacement for cell expansion and differentiation. The decision to use platelet concentrates for the particular purpose of supplying hPL as a cell culture supplement should entail the principles and values that blood establishments hold towards the use of donated blood components for transfusion. Therefore, questions on ethics, on practical standardization of hPL production and logistics, as well as on assuring hPL quality and safety need careful consideration. High-quality platelet concentrates are produced in blood banks under strict regulations daily. However, numerous platelet concentrates are discarded periodically due to expiry. Expired platelet concentrates may represent a good source for obtaining platelets for lysate production without struggle with blood banks for platelet donors. An essential aspect of using hPL for clinical therapy, whether directly or indirectly, for example, via cell therapy and ATMP therapy, is the standardization of the production of hPL. In this presentation, I will discuss the status of hPL products and how I foresee their use in the future, both when it comes to their use in clinical protocols or research and development.

LD03-L03 | Autologous serum eye drops, experiences drawn from implementation and production of a novel blood component at a university hospital in Sweden

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Autologous serum eye drops, manufactured from the patient's own blood plasma, are used to treat ocular surface disorders such as keratoconjunctivitis sicca and epithelial corneal defects. The rationale for treatments are the many similarities between the composition of natural tears and serum, for proteins, lipids and growth factors. The Stemcell Laboratory at Clinical Immunology and Transfusion medicine, Sahlgrenska University Hospital Gothenburg, a clean-room GMP facility, has manufactured autologous eye drops after referrals from eye clinics since 2016. Eye drops of three types are produced; 100% serum, 20% serum and platelet-rich plasma eye drops (PRP). Venipuncture is performed at blood donation centres in the Gothenburg region, safeguarding traceability and adequate skin disinfection. Samples are transported to the GMP facility, centrifuged and aliquoted into pipettes in a closed system (COL system 10/20, Biomed Device, Modena Italy), frozen and stored at −20°C until release for use after negative microbial culture and virology results. The number of pipettes is adequate for approximately 2 months of treatment. Between 2016 and 2021, 45 patients have received treatment, 53% with 20% serum, 40% with PRP and 7% with 100% serum eye drops. The number of consecutive treatments per patient have varied between 1 and 50, in median 3. A 31% of patients have received ≥7 treatments, that is, were treated for more than 12 months. The number of referrals increased 45% between 2019 and 2020 and 36% between 2020 and 2021.

LD03-L04 | Hypoxic erythrocytes in clinical use

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Background: Anaemia is a common symptom of hematologic malignancies due to reduction in volume of functional erythrocytes (RBCs) in bone marrow. RBC transfusion is primary supportive treatment, which can be associated with adverse events (AEs), and often leads to transfusion dependence. Hypoxic processing and storage of RBCs may benefit those requiring frequent transfusion by increasing interval between transfusions and reducing release of free iron into circulation and iron overload. A CE mark certified device to process and store RBCs hypoxically –CPD/PAGGSM leukocytes-reduced (LR), O_2/CO_2 reduced–which may potentially reduce transfusion burden in transfusion-dependent patients and attenuate oxidative stress associated with acute major bleeding, has been developed (Hemanext, Inc., Lexington, MA, USA). A validation study of the *in vitro* performance of the RBCs was conducted at Department of Immunology and Transfusion Medicine, Oslo University Hospital, Ullevål, Norway.

Aims: A clinical investigation assessing safety of single administration of hypoxic RBCs in transfusion-dependent patients with haematological malignancies in Norway is underway.

Methods: Patients aged ≥18 years with Hb ≤9 g/dL and requiring ≥2 RBC units in a single transfusion event received hypoxic RBCs as part of an observational pilot safety study, which will include 10 patients with haematological malignancies and 10 patients with burns. This scheduled interim safety analysis reports on the first 5 patients in the haematological malignancy cohort to receive hypoxic RBCs. All patients received one transfusion episode of two units of hypoxic RBCs produced using the CPD/PAGGSM LR, O_2/CO_2 reduced system instead of their usual transfusion of conventionally stored RBCs. The primary objective was number of adverse events (AEs) up to 24 h following transfusion initiation and up to 7 days (±1 day) post-transfusion. Secondary objectives included assessment of AEs up to subsequent transfusion episode or 28 days (±1 day) post-transfusion, whichever occurred first. Additionally, changes in Hb levels post-transfusion were assessed.

Results: At this interim analysis, three patients were diagnosed with MDS, 1 with myelofibrosis and 1 with AML (80% male, mean [±SD] age 69.8 ± 19.3 years; mean BMI 24.8 ± 3.4 kg/m^2). Patients had been receiving conventional erythrocyte transfusions every 2–3 prior to study initiation, and all patients received the hypoxic RBCs without complication. One AE (rhinovirus) was reported 2 days post-transfusion. This was deemed mild in severity and unrelated to treatment. No further AEs were reported up to 28 days. Hb levels increased by 17% following the administration of hypoxic RBCs from a pre-transfusion mean of 7.7 ± 0.5 to 9.0 ± 0.9 g/dL post-transfusion.

Summary/Conclusion: This interim analysis showed that transfusion with hypoxic RBCs processed with the CPD/PAGGSM LR, O_2/CO_2 reduced system was well tolerated in patients with hematologic malignancies. Hb levels were within the target range at follow-up, suggesting that hypoxically stored RBCs function appropriately. The overall clinical program will assess the benefit of hypoxic RBCs versus conventional RBCs in patients requiring acute and chronic transfusion.

LD03-L05 | Plasma collections: National goals–local implementation

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Background: In recent decades, Denmark has had a national contract, which stipulates the delivery of plasma, in exchange for derived immunoglobulins (IG) and albumin. Up until 2007, the country was

producing enough plasma for the manufacturing of IG and albumin to meet demand. However, an increase in the use of IG, a reduction in the use of red blood cells and a subsequent reduction in recovered plasma available, have resulted in the loss of plasma self-sufficiency and a dependence on foreign plasma.

Aims: In order to fulfil the obligations of the contract with the fractionator, regarding the amount of plasma, a plasmapheresis program was initiated in 2013. A goal was set to obtain 20 tons of plasma by plasmapheresis, to supplement 60 tons of recovered plasma. During the COVID-19 pandemic, Denmark experienced severe shortages of IG. Because of this, the responsible politicians in June 2021 decided that 100% plasma self-sufficiency for the production of IG should be achieved. However, no timelines were stipulated.

Methods: A national tender for plasmapheresis machines and consumables was issued in 2013. By late 2015, the five regional blood establishments had initiated their plasmapheresis programs. In 2017, a working group of the Association of the Danish Regions prepared a report on plasmapheresis, which took lessons from setups around the world, including considerations of optimal sizes for plasmapheresis centres. The blood establishments developed business cases for centres of two different sizes. A second tender for plasmapheresis equipment was issued in 2020. National statistics for plasma production and the use of IG were collated from the annual national reports regarding the blood domain.

Results: Business cases for plasma centres, with 12 and 24 beds respectively, were developed, including rooms for various purposes, outdoor areas, equipment, supplies, opening hours and staffing. The annual amount of recovered plasma for fractionation decreased from 83 tons 2005 to 41 ton in 2022 whereas source plasma increased from zero in 2014 to 77.3 tons in 2022. The amount of delivered plasma per 1000 inhabitants varied considerably among the five regions (11.4, 19.5, 21.0, 22.7 and 29.5 per 1000 in 2022), depending on the development of their plasmapheresis programs. If all regions performed as the best, 180 tons of plasma should be available. Furthermore, the best performing region plans to increase the delivery of plasma by 50% within the next 3–4 years. For all regions, facilities for plasmapheresis were the bottleneck, rather than a lack of donors. From 2010 to 2020 the annual use of IG increased from 410 to 1187 kg. With a yield of 5 g IG/kg plasma the amount of plasma necessary for self-sufficiency would be 237 or 119 tons more than that which is currently delivered. With an average of 0.7 kg plasma per procedure, a further 170,000 plasma collections will be needed annually, in addition to the 110,000 collections performed in 2022.

Conclusion: National plasma self-sufficiency for IG with unpaid, uncompensated donors appears to be feasible, as soon as the necessary plasma centre facilities have been established. Eight years after starting from scratch, plasma collection has improved to a level where 42% of the required plasma is being obtained. It is expected that, within the next five years, almost 100% of the annual target amount of plasma will be collected.

Local Day Session 4: Future in blood banking

LD04-L01 | Extracellular vesicles, current status and role in blood banking

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Blood is one of the richest sources of extracellular vesicles (EV). As blood is vital and needed for different transfusion products the role of EV content has evoked interest both in biomarker development for diagnostic and treatment follow-up purposes, but increasingly also as a source of novel therapeutic components. Blood services follow rigor manufacturing processes and functions under tight legislation in specific regulatory environment. Using decades of established knowhow and expertise in personalised cell therapies, EVs form potential continuation in this success story of saving lives. Our example in Finnish Red Cross Blood Service shows that also in very conservative highly regulated environment it is important to look beyond and help the cell biological sciences to develop further. Blood services can help the field of EV research to move forward, but EVs can also provide new cost-efficient therapies for diseases waiting for breakthrough. Could EVs be the next generation products from blood?

LD04-L02 | Reintroducing whole blood for transfusions, pros and cons

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Background: International civilian and military guidelines recommend balanced transfusion to patients with haemorrhagic shock, and the timing of transfusion is of importance for improved survival. Whole blood has been reintroduced for patients with massive bleeding because it affords plasma, red blood cells and platelets in a balanced ratio in a logistically advantageous way.

Current and future use: Whole blood is currently being used for bleeding patients in both military and civilian health care services both in low-income and high-income countries. It is used in level 1 trauma centres, smaller rural hospitals, prehospital ambulance services and municipal health services have implemented whole blood. Emergency collection from pre-screened emergency donor panels (walking blood banks) have been implemented both in military and civilian services.

Pros and cons: Prospective observation studies suggest improved survival and reduced blood usage by use of whole blood. However, more information is needed and prospective randomised trials are undergoing both for prehospital and in-hospital use. Low titter group O whole blood (LTOWB) and also ABO type-like whole blood transfusions are

currently used. Concerns regarding donor availability and donor selection prevent implementation of whole blood in many institutions. However, both blood bank staff and clinical personnel report easier and faster issue and managing of massive transfusions when using whole blood as compared to blood components in massive transfusion packages. Implementation requires validation and quality control. Several in-vitro studies have been published showing preserved quality of whole blood during storage; however, platelet function is reduced during storage.

Preparedness: In an emergency, a wide range of blood products is needed. Whole blood can be used in blood preparedness on all levels of health care and is beneficial for situations when personnel resources are limited and disruptions in delivery of consumables or facilities occur. It may serve as a contingency product for smaller hospitals with no platelet inventory as it can be stored longer than ordinary platelet concentrates. It also provides an alternative for transfusion of patients in haemorrhagic shock in case of delayed medical evacuation. Advanced medical facilities and trauma centres may be overwhelmed by a high number of patients in mass casualty events or catastrophes and need to have plans that ensure access to large amounts of blood for bleeding patients. In these situations whole blood can be of importance as a supplement to blood components. For situations like nuclear events and far forward treatment of bleeding patients, access to blood components like platelets and (dried) plasma are also important.

Summary: Whole Blood has been reintroduced as a logistically feasible alternative for early balanced transfusion in both prehospital and hospital services. Discussions and clinical studies are ongoing on the use of whole blood in treatment of patients with massive haemorrhage. Inclusion of whole blood in blood preparedness planning is recommended.

LD04-L03 | Prediction models for blood bankers

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Background: Blood products are valuable, perishable goods, whose waste must be minimised for ethical and economic sustainability. Variation in the blood product demand creates considerable uncertainty in managing the blood supply chain, complicating adequate preparation. On the supply side of the chain, in blood collection, it is important to minimise is Hb deferrals. In the EU blood donors' Hb levels must be monitored, and those with inadequate Hb levels are deferred. The Finnish Red Cross Blood Service (FRCBS) is responsible for organizing blood donations and distributing blood products in Finland and carries out scientific research to develop its activities.

Aims: We aim to develop computational prediction models for blood demand and Hb deferral prediction to optimise blood banking operations.

Methods: To predict blood demand using historical demand data, we have developed a time-series analysis approach (https://github.com/FRCBS/production_forecasts). For demand predictions based on patient and operational hospital data, we will employ various machine learning methods. We have also developed random forest and linear mixed models approaches to estimate Hb deferrals, using blood donation history data. These models have been evaluated in 5 countries (https://github.com/FRCBS/Hb_predictor_container).

Results: We find that time series analysis of blood demand is useful for planning and budgeting of blood banking activities (https://pubmed.ncbi.nlm.nih.gov/35383944/). However, it is obvious that further progress requires richer data. To this end, we are currently pursuing analysis of electronic health care data of all blood product usage at Helsinki University Hospital since 2017.

Using blood donation history data, we have shown that cost-saving Hb deferral prediction models can be developed (https://pubmed.ncbi.nlm.nih.gov/34825380/). Furthermore, blood establishments with higher Hb deferral rates are more likely to benefit from such models. (https://pubmed.ncbi.nlm.nih.gov/36924102/). To improve the performance of these models, we are currently exploring the use of genetic, ferritin, and menstruation data.

Summary: Elective surgical operations, which may require blood products, are often scheduled well in advance. Additionally, patient characteristics can help predict their need for blood products. Hence, it is very likely that hospital-based blood demand prediction models could inform blood collection activities and reduce waste. The recent pandemic drove many blood establishments to prefer prebooked donations. Integrating Hb deferral predictions into donation booking systems has the potential to reduce Hb deferral and save both time and costs for blood establishments and donors.

Academy Session A1: Developing research skills

AD01-L01 | How to critically review scientific data

E van der Schoot

Abstract not available.

AD01-L02 | How to write a systematic review

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Systematic reviews are considered the most powerful tool to synthesise and quality appraise the evidence available within a specific topic. They constitute the base for the preparation of guidelines, and are commonly used in the clinical settings. Methodology is well defined and can be easily accessed via online free tools and availability of

Cochrane centres. Cochrane is the leading organisation in developing and updating the methodology of systematic reviews. Since 2018, Cochrane offers the possibility to get involved in mentoring programmes ("Cochrane International Mobility"), open to both junior and senior researchers.

In this presentation, examples will be used to show the main steps in conducting systematic reviews, including the definition of the research question, the pre-registration of the protocol, the choice of the eligibility criteria, the literature search strategies, risk of bias assessment, data synthesis, certainty of the evidence. Moreover, practical aspects will be discussed, such as the requirements for the team of authors of the review and how to contact Cochrane to register a review or receive support.

AD01-L03 | Big data–What is it? How can I get it? What can I do with it?

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Big Data is a term often appended to large data sets too complex to analyse with traditional methods. Large-scale blood donor and transfusion medicine research projects have been ongoing for multiple years, in the form of, for example, the Recipient Epidemiology and Donor Evaluation Study (REDS) Program, the Scandinavian Donation and Transfusion (SCANDAT) Database, the INTERVAL Study, and The Danish Blood Donor Study (DBDS).

In this presentation I will use DBDS as an example of a Big Data project and show and discuss challenges and projects within DBDS. DBDS has included more than 160,000 blood donors and collected more than 2.4 million plasma samples from the participants. All participants have been chip-genotyped for 650,000 SNPs and a large subsample is undergoing whole genome sequencing. More than 400,000 questionnaires have been collected and the data is linked to the extensive national health registers with complete follow-up on diagnosis codes for all hospital contacts, filled prescriptions, socioeconomic data and granulated data from the laboratory databases and microbiology databases. Recently, similar data from blood recipients have been linked as well.

DBDS provides extensive possibilities for research and collaborations but the presentation will also include a focus on some of the challenges with large-scale studies: A strong governance structure must be build assuring compliance with the scientific ethical law and the data protection act. Careful attention to communication with donors and the Danish Blood Donor Association is required to assure agreement on the research projects carried out. With genetic testing, the first incidental findings have now appeared mandating procedures to contact and refer donors for further work-up. Finally, Big Data requires the use of new data analysis methods often performed in a high-performance computing environment.

In the blood service we have an infrastructure, which can be used to perform large-scale studies with immense possibilities for research that ultimately contribute to improve donor management and patient care.

Academy Session A2: Neonatal and paediatric transfusions

AD02-L01 | Use of whole blood in paediatrics

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Background: Whole blood was one of the initial haemostatic resuscitation products commonly used for traumatic haemorrhagic shock and immediate life-threatening injuries. Presently whole blood is not routinely available since there is a wide availability of blood components. In recent times there is a resurgence of whole-blood use in both military and civilian life-threatening bleeds. Recent data have shown that there is also a growing interest in using low-titter cold-stored type O whole blood (LTOWB) for the paediatric group of patients.

Aims: The aim of this presentation is to evaluate the recent renewed interest and indications of whole-blood use in paediatrics.

Methods: The presentation discusses the published data on the use of whole blood (LTOWB) in paediatrics and neonates. There is a detailed discussion on the concept of LTOWB, indications of use and possible focus areas for future research.

Results: A recent survey of 36 USA children's hospitals showed that there is a willingness to participate in a study to determine the use of LTOWB in paediatrics. Another retrospective study showed the use of LTOWB in 56 injured children showed better outcomes when compared to the component therapy. A recent observational study also showed improved outcomes for 80 children receiving LTOWB post-trauma (AOR 0.23) as compared to component therapy (AOR 0.41). One recent case report also showed a positive outcome of transfusing cold-stored LTOWB re-warmed (via fluid warmer) to a preterm neonate.

Summary/Conclusion: There is a growing interest in using LTOWB as compared to component therapy, in paediatric and neonatal patients with life-threatening bleeds but there is limited data on efficacy, utility and overall benefit. There is the scope of identifying the group of patients which will benefit the most from the transfusion of LTOWB.

AD02-L02 | Optimising blood components for neonates and children

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Preterm neonates have a relatively high rate of transfusion and are vulnerable recipients. They are developmentally immature, with differing physiology and haemostasis compared to adults which may put them at higher risk of transfusion-associated adverse events. As a result, Blood Establishments frequently provide 'neonatal' components with additional safety features compared to standard components. In addition, guidelines on shelf-life and optimum usage of blood components for neonates and older children may differ from those for adult recipients (New et al., 2016; NBA, 2016).

A number of surveys have demonstrated international variation in processing and shelf-life of neonatal and paediatric blood components (Reeves et al., 2021; Arora et al., 2023). This variation partly reflects the lack of specific evidence on optimal practice in several areas. For example, although there are many case reports of hyperkalaemia following large volume transfusions to neonates and infants (Yamada et al., 2021), systematic review of the literature has found little quality comparative evidence to support specific mitigation measures (Wolf et al., 2022).

There is evidence of potential harm of transfusion of prophylactic platelets to neonates from the PlaNet2/MATISSE trial (Curley et al., 2019), but the reason for this is not well understood. One possibility is that it could result from the mismatch between adult donor-derived platelets and the neonatal haemostatic system, raising the question of whether there could be a benefit of using neonatal derived components. This possibility is already being explored for neonatal red cells, with the publication of preliminary studies using cord blood-derived components with the aim of reducing adverse outcomes such as retinopathy of prematurity.

AD02-L03 | From drip to drop: Clinical blood conservation innovation in paediatrics

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Young children and babies are at heightened risk of healthcare-associated anaemia, in comparison to other healthcare users. Over the last two decades there has been increased awareness by clinicians and researchers of this iatrogenic harm, including its sequelae. In particular, critically ill children and babies, especially at extreme birth weights and prematurity are at particular risk for adverse consequences from anaemia. This has spurred innovation and collaboration that has successfully bridged the bench-bedside divide.

There are safe and effective multi-modal strategies aimed to minimise phlebotomy losses within and beyond critical care settings. These include those focussed on human factors engineering, point of care and micro-sampling technologies, and laboratory partnership.

Considerable improvements in blood conservation can be achieved if these high quality and innovative practices are sustainably implemented throughout paediatric and neonatal healthcare.

Academy Session A3: Challenges in managing a blood service

AD03-L01 | Strategies for managing staff shortages

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Hiring qualified staff for the transfusion service is a challenge. Maintaining a safe, timely and high quality service may require changes in work practices to allow your laboratory to do more with less staff. Strategies that may help in planning and working through periods of staff shortage have been grouped into five broad categories.

1. The first involves optimizing the efficiency of your current workplace. Organizing the work space to ensure efficient workflow: organizing operating procedures to ensure they are easy to find and to follow; and simplifying protocols when possible. Improving communication between staff so that everyone knows what to do and when, is also an important organizational tool.
2. Second is reviewing what you do, and ensuring that all tasks are really needed. There may be tests that are obsolete or unhelpful, or strategies that are unnecessarily complex. This review may benefit from an outside observer to audit and advise on what could be changed or abandoned. Visits to other transfusion services and conversations with colleagues can help to identify different ways of working.
3. A third step involves reviewing what tasks may be done by someone else on the team. Laboratory assistants or others trained on the job may be able to manage sample intake, undertake maintenance tasks and triage phone calls, diminishing the burden for testing staff and increasing the pool of people to hire. Clinical staff or pharmacy may be able to pool or reconstitute blood rather than having the laboratory staff do so, acknowledging that clinical teams may also be significantly short staffed.
4. Fourth involves sharing the burden. Whenever possible, consider sharing procedures and writing tasks with other similar facilities. Use of standardised testing menus, SOPs and strategies can allow sharing of staff to work on these items and decrease the workload for both. If regional standardization is

feasible, the administrative burden can be significantly decreased for all of the laboratories involved. One or more laboratories may specialise in some tasks and could serve as a referral site in times of shortage. This is especially helpful for complex or rarely done tests, where efficiency is better if a particular test can be routed to one site where expertise and competency are maintained. Plan ahead on where you could send samples for testing if a severe staff shortage arose. It is more efficient to plan for such a contingency rather than to undertake planning only when you need it.

5. Last, to the extent possible automate tasks and interface results reporting. Tools for automation can be expensive and time consuming to implement, but once in place can save significantly in terms of the need for staff to perform basic tasks. With automated testing platforms samples can be batched and managed by a single individual leaving others with time to evaluate and complete complex analyses or reports. Often, when preparing for automation or for an interface to a laboratory information management system, other efficiencies in work flow and test menu can also be identified.

Many transfusion services struggle with staff shortages. A number of practical strategies may be employed to decrease the workload burden for transfusion service staff while maintaining a high quality service for patients in need.

AD03-L02 | Strategies to maintain safe blood during emergency & disaster

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Blood services must be prepared to move quickly in response to changes, during which blood sufficiency is most likely to be affected. The word "disaster" generally refers to any situation that temporarily restricts or eliminates the ability of the service to maintain its blood supply or a situation that creates a sudden demand for blood higher than usual or a massive influx of donors posing difficulties to the blood collection system. Managing the blood system in disasters is one of the main challenges for any blood service exposed to natural hazards such as earthquakes, floods, biological threats such what happened during COVID pandemic as well as manmade disruptions and terrorism.

A national rather than sub-national or local approach should be adopted for coordination of health services to ensure public confidence in blood safety and supply. Blood services should be included in the national outbreak response, through experts linked to the national emergency response team. Coordination during a disaster among local blood services; national blood organizations; and federal and local government is required to determine the medical need for blood, facilitate transportation of blood from one facility to another, communicate a common message to the national blood community and the public about the status of the blood supply in the disaster-affected area. Supply chain strategies is an important part of disaster planning to ensure the availability of critical material, reagents and consumables for a period of time. Strategies should include staff availability and training as another essential part of planning.

The blood collection facilities need to communicate with donors registered in their database and public in general during disaster to reflect the need for donations. Best communication tool to be selected by the collection centre.

Planning for disaster should involve a coordinated, multidisciplinary approach to define and document different tasks and responsibilities.

AD03-L03 | Managing blood services in times of uncertainty: Lessons learned from the COVID-19 pandemic

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Blood transfusion is an essential part of the health care service. To secure a safe and adequate blood supply, blood services rely on an uninterrupted flow of complex activities: collection, screening, processing, storage and distribution of blood and blood components for clinical use. The blood supply chain can be adversely affected by various factors such as shrinkage of donor pool or increase in blood demand as a result of population ageing. It can also be disrupted by unexpected external hazards that can be natural or man-made. The still ongoing COVID-19 outbreak, declared as a pandemic on March 11, 2020 by the World Health Organization (WHO), had major implications on blood services operations worldwide. With the advent of globalization, the world has become more susceptible to infections and more outbreaks of infectious diseases that may develop into pandemics are inevitable. Lessons learned from the COVID-19 pandemic will hopefully make blood services and national health authorities be better prepared for the next pandemic.

To mitigate the impact of a pandemic on blood services operation a national approach should be adopted for coherence and coordination. This Academy session will discuss precautionary safety measures to mitigate the risk of donors and staff to exposure to the infectious agent and the potential transmission of the infectious agent through transfusion and also discuss measures to balance blood demand and supply.

Academy Session A4: TTID impact and surveillance

AD04-L01 | Strategies to managing emerging transfusion transmissible infectious diseases

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Since the 1970s, introduction of serological assays targeting virus-specific antibodies and antigens has been effective in identifying

blood donations infected with the classic transfusion-transmitted infectious agents (TTIs; hepatitis B virus [HBV], HIV, human T-cell lymphotropic virus types I and II, hepatitis C virus [HCV]). Subsequently, progressive implementation of nucleic acid amplification technology (NAT) screening for HIV, HCV, and HBV has reduced the residual risk of infectious window-period donations, such that per unit risks are <1 in 1,000,000 in the United States and other high-income countries, although risk of classic TTIs remains a problem in developing countries with inadequate testing resources or infrastructure. Although there is continual need for and programs that monitor current risks due to established TTI, ongoing challenges in blood safety primarily relate to surveillance for emerging and re-emerging infectious diseases (EIDs) coupled with development of rapid response mechanisms when such agents are identified. NAT screening has emerged as a preferred option for detection of newer potential TTIs including West Nile virus, Hepatitis E Virus, Zika virus and *Babesia microti*. Recent progress in development and implementation of pathogen-reduction technologies also provide the opportunity for proactive rather than reactive response to blood-safety threats. This presentation will summarise these advances in TTI safety and EID surveillance and review several recent responses to EID threats including WNV, XMRV, Dengue, Chikungunya and Zika viruses and epidemic respiratory viruses including SARS-CoV-2.

AD04-L02 | Introduction of new assays for emerging infectious agents

J Levi[1,2]

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Transmission of infectious agents has been noticed since blood transfusion was incorporated into medical science. Selection of donors based on epidemiology and questionnaires on risk sources is insufficient to prevent transfusion transmission of microorganisms (TT). Laboratory screening of the donated blood is central to transfusion safety. Methods for blood screening require high-throughput capacity, short turnaround time and high sensitivity. These methods are constantly evolving, fostered by a quest for an unachievable "zero" risk. An evolutionary line can be traced beginning with morphological methods like microscopy, progressing to serology and finally incorporating molecular tools (Nucleic Acid Testing). So far, the definition of the agents to be screened is based on the current knowledge of their transmissibility and pathogenicity through the blood transfusion route. The possibility of recognizing all organisms in the donor's blood is nowadays provided by unbiased next-generation sequencing (NGS), namely metagenomics. As NGS becomes cheaper and prone to automation, it may replace all other laboratory methods while providing genetic information useful to transfusion services like blood groups, HLA and other antigens involved in the matching of blood units, identifying rare and emerging unsuspected infections. It is likely that serology, that is, seeking for specific antibodies, will remain in use providing another layer of safety. Currently, there is one assay for each agent but new multiplex technologies are in development and may allow testing at once for several specific antibodies.

AD04-L03 | Blood donor surveillance, risk assessment and policy

S O'Brien[1]

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Blood donor infectious disease marker surveillance is foundational to monitoring blood safety. Donor surveillance, research study data and public health data are used in mathematical models to quantify risks to blood safety to inform policy. The ISBT Transfusion Transmitted Infectious Diseases Working Party aims to evaluate and advance safety of blood transfusion by analysing transfusion transmitted infectious disease data, coordinating international studies and publishing scientific reports. There are four subgroups which focus on bacteria, virology, parasites and surveillance, risk assessment & policy (SRAP). This presentation aims to describe the Surveillance, Risk Assessment and Policy subgroup's activities, explain underlying concepts of surveillance and risk assessment in relation to informing policy and discuss the potential for blood operators to contribute to public health surveillance.

Monitoring infection in blood donors requires data collection and organization into a database which includes all blood donations, dates of donation, infectious disease markers and demographic variables such as age and sex. Prevalence is usually expressed as positive donations per hundred thousand donations. Prevalence counts both new and old infections. For long duration infections such as HIV prevalence tends to be higher in first time donors because they may have been infected at any time in the past. Incidence is the rate of new infections, usually defined as donations with a positive test for which a previous donation was negative and is expressed as rate per person-years. Incidence is generally much lower than prevalence. Incidence and prevalence allow us to make comparisons over time and between regions.

Quantitative risk is estimated using mathematical models based on observed data. For transfusion safety the risk measured is usually the residual risk of an infection after screening questions and testing have culled most of the infections. Tested blood could be infectious due to errors in testing or erroneously releasing a test-positive unit but the greatest risk is usually from very recent infections. Very recent infections can be in the "window period" of the assay when they may not be detected. The SRAP mathematical modelling team developed a model to estimate residual risk from shortening deferral time periods. The model is adaptable for any blood transmissible infection and any time deferral. The model was used to assess the residual risk of HIV from shortening the time deferral for gay and bisexual men who have sex with men in Canada, and was part of the evidence in the policy decision to reduce and ultimately to remove the deferral altogether and replace it with sexual behaviour risk questions.

The potential for blood suppliers to contribute to public health surveillance has seen renewed interest since the COVID-19 pandemic began. During the pandemic blood suppliers around the world leveraged their capacity to carry out SARS-CoV-2 sero-surveillance to inform public health policy. The SRAP sub-group members developed a tool-kit now posted on the ISBT website which provides information on what blood suppliers can offer and how to support emerging outbreak surveillance.

Academy Session A5: Donor & donation challenges

AD05-L01 | What do haemoglobin measurements tell us about blood donors?

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Blood donor selection criteria aim to protect donor health, to ensure safe transfusions for recipients and to achieve blood products of appropriate quality. For donation suitable haemoglobin (Hb) value is one of the few selection criteria that takes account all these three perspectives. For donor perspectives too low Hb is the most common reason for deferral and therefore the requirement of measuring Hb at every donation is often simplified to "the method of avoiding individuals with anaemia to donate blood".

Blood donors' Hb values are measured by using point of care methods and/or analysing blood count in laboratories. There is no single golden standard for Hb measurement method or policy in donor selection process. The result of Hb measurement gives as a minimum an answer (within the level of precision of the chosen method) whether the donor has a high enough Hb value for donation. By understanding the limitations of measurement method in use and developing the Hb determination processes, the result of Hb measurement can be more conclusive and contain valuable information of risks related to health or to donation.

For the citizen/donor, a normal Hb value represents one general important measure of good health and therefore Hb measurement performed in blood donation adds value as such to the donor.

For the operative activities in blood establishments, the measured donor Hb values play an important role in determining the appropriate further paths for each donor and the counselling needs by healthcare professionals.

To develop policies and strategies in mitigating donor health risks and unnecessary donor deferrals blood establishments need to collect data of Hb values among their donors and have information of the Hb values in the general population. Reference data from other blood establishment is important in benchmarking Hb values and deferrals and to detect possible spots for improvements.

AD05-L02 | Moving to risk based sexual behaviour criteria for all donors

M Goldman[1]
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Introduction: Screening donors for high risk sexual behaviours is important to ensure recipient safety, since some pathogens, such as HIV, may be transmitted by both transfusion and sexual routes. Policies vary internationally, in part related to the epidemiology of HIV in different countries. In the last few years, several countries have moved away from time based deferrals for men having sex with men, to approaches based on sexual risk behaviours in all donors. These changes are due in part to consultation with communities who felt stigmatised by previous policies and efforts to promote a more diverse and inclusive donor base.

Methods: The various approaches blood services use to screen donors for sexual risk will be reviewed, as well as their evolution over time. The presentation will summarise the evidence used to assess the safety of moving from a time based deferral to a risk based approach, considerations on implementation of policy changes, and methods of post-implementation monitoring.

Results: In Canada, as in many other countries, gay, bisexual, and other men who have sex with men (gbMSM) are a higher risk group for HIV, and men who have sex with men were deferred first indefinitely, and then for progressively shorter periods of time. In Sept, 2022, the 3 months deferral for MSM was replaced by deferral of all donors who had anal sex with a new sexual partner or more than one sexual partner in the last 3 months. Evidence used to support this change included: surveillance of known and emerging pathogens, assessment of donor compliance, modelling of the risk increment of the proposed change, review of epidemiologic studies of HIV transmission, a cohort study of HIV transmission in a gbMSM population, and assessment of the possible impact on adequacy of supply. Preliminary data from implementation of very similar criteria in the UK were extremely reassuring. Implementation of criteria changes involved engagement with 2SLGBTQIA+ communities and staff members, discussion with blood recipient groups, and staff and volunteer training. Current donors were also informed of the upcoming change. Post-implementation monitoring will include following infectious disease marker rates in donors, studies of donor compliance, and monitoring of deferral rates.

Several other countries have also recently adopted or will soon adopt changes in screening for sexual risk, including the UK, France, the Netherlands, Israel and the US.

Conclusions: To date, implementation of sexual risk based criteria has not changed extremely low HIV rates in Canadian donors, and has resulted in manageable donor deferral rates of approximately 0.08%. A longer observation period and planned compliance studies would strengthen these preliminary observations.

AD05-L03 | Update on iron supplementation for whole blood donors

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Whole blood donations are associated with haemoglobin-bound iron loss and subsequent iron store depletion. Insufficient iron stores may reduce the recovery of haemoglobin after donation. Repeat donors therefore risk developing iron deficiency and iron deficiency anaemia. Ferritin measurements are increasingly applied by blood establishments in order to identify donors that should be deferred from donating or supplemented with iron.

This presentation will provide an update on considerations with regards to donor iron monitoring policies, in particular those including ferritin measurements and iron supplementation. Are certain subgroups of donors more at risk of developing iron deficiency (anaemia) than others? Which measures can and do blood establishments take, and what are the pros and cons? Which considerations should be taken into account, according to existing literature and practice?

A specific focus will be on the iron supplementation trial 'FORTE', currently being carried out at Sanquin in the Netherlands, and on associated qualitative and quantitative studies investigating donor and personnel perceptions regarding iron supplementation as a blood establishment policy.

Academy Session A6: Drug-related antibodies in immunohematology

AD06-L01 | Anti-CD38 and anti-CD47; considerations in immunohaematology testing

S Grimsley

Abstract not available.

AD06-L02 | Immunohaematological considerations regarding drug-induced antibodies

S Johnson

Abstract not available.

Academy Session A7: Alternative approaches to blood use and blood banking

AD07-L01 | Blood product revival: The "old" is "new" again

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With increasing evidence that prehospital blood products and early balanced transfusions may save lives in bleeding patients, blood products with improved availability and sustainability are required. Whole blood, dried plasma, cold-stored platelets and cryopreserved platelets are "old" blood products with increasing requests on "new" indications. The products are important as part of preparedness plans.

The experience of whole blood transfusions comes from trauma in war and are introduced in the treatment of civilian bleeding, initially in rescue helicopters and ambulances but of increasing use in-hospital. The advantages are logistics and quality variables; fresh red blood cells, platelets and coagulation proteins in high concentration in one unit. The disadvantage is challenge for the blood bank with an additional production line and difficulties to predict the need and minimise wastage. Low-titter O whole blood (LTOWB) is a limited resource, especially if only RhD negative donors are used. Freeze-dried or spray-dried plasma (DP) has been used since the second world war, until recently mainly in military forces. It is an increasing request of DP in civilian prehospital care since this product does not need cold-chain and can be stored for at least two years. Today, DP is of very limited supply, but new products are coming, both as pharmaceutical products and blood products- produced in the blood bank. The production lines differ; single or pooled plasma, blood type specific or universal, with or without pathogen inactivation and different methods of pathogen inactivation. Platelets are critical to ensure haemostasis in bleeding patients, but the inventory of platelets are often limited, especially in small hospitals. Platelets are normally stored in room temperature for up to seven days and are associated to high wastage and high costs. Due to that, remote hospitals often have no platelets in stock, despite occasionally treating bleeding patients. With several hours before platelets are available, the patients are not optimally treated. Cold platelets are stored in fridge for up 14 days and are shown to be haemostatically active in *in vitro* studies and *in vivo* in the treatment of bleeding. Cryopreserved platelets are frozen in a small volume (10 mL) and can be stored in −80°C for several years. At request they are quickly thawed and reconstituted in plasma. They have been studied extensively *in vitro* and clinically evaluated with good results. Randomised clinical trials are on-going in Australia and

New Zealand. Both cold-stored and cryopreserved platelets have been described since long, and used on special indications, but are now of increasing interest in the treatment of bleeding and as back-up inventory in preparedness plans.

In the presentation the indications and evidence as well as the challenges and quality requirements of these "old-new" blood products will be discussed.

AD07-L02 | Update on the collection and use of granulocyte concentrates

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Granulocyte concentrates (GC) are used 10,000 times less frequently than red cell concentrates. This partly reflects the rarity of the indication: neutropenic, drug-refractory infections and severe granulocyte function disorders. In addition, bone marrow transplant patients are occasionally bridged with GC. Indications can develop within a few days and require timely care. This is not always possible and, in fact, is the most common reason for not transfusing GC, as their half-life is short, usually 24 h, and preparation by apheresis requires specialised skills for donor recruitment, medical pre-treatment and sedimentation agents. Alternatively, GC can be prepared from extracted pooled buffy coats. Again, the required expertise and regulatory approvals are limited to specialised centres.

To date, there is no single best practise for producing GC, as all methods have their own drawbacks. In pooled buffy coat GC, patients are exposed to many donors, which may lead to immunisation and refractoriness. In single-donor apheresis GC, on the other hand, donors are subjected to single-dose pre-treatment with for example, dexamethasone and G-CSF. In addition, a sedimentation agent must be used, as granulocytes cannot simply be separated by gravity from equally dense red blood, but require counter current flow in the apheresis chamber onto a fast-moving red cell bed under the buffy coat. The easiest way to achieve this is by adding high molecular weight hydroxyethyl starch (HES). However, this substance has been shown to have unfavourable effects in sick patients and accumulates in the donor's interstitium, including the bone marrow, resulting in a half-life of up to months. As a result, the availability of high-molecular-weight HES has become uncertain. Low molecular weight HES and modified liquid gelatin (MFG), on the other hand, have lower numerical collection efficiencies. At least MFG does not appear to be inferior in terms of its effects on granulocyte biological function. In general, it is impaired in all types of production, which may explain why the indication for GC transfusion is controversial. The considerable differences between observed patient outcomes in different centres can be partly explained by timely delivery, ability to achieve sufficient doses and gentle manufacturing, but there may be other factors on top of these.

AD07-L03 | Walking blood banks in the civilian population

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Background: Early blood transfusion is recommended in Civilian and Military guidelines for patients with haemorrhagic shock. The Norwegian Blood Preparedness Program seeks to establish a system that ensures the supply of blood for treatment of patients with life-threatening bleeding at all levels of health care regardless of aetiology. To provide the best care to patients in regions with reduced access to medical evacuation, blood transfusion must be available locally. A decentralised blood preparedness program is needed to ensure self-sufficiency in emergencies. This can be accomplished by establishing a system for emergency whole blood collection and transfusion in the framework of a community based civilian walking blood bank.

Definition: In a walking blood bank, whole blood is collected on site from a pre-screened emergency donor panel for immediate life-saving transfusion to patients in haemorrhagic shock.

Methods: Establishing a civilian walking blood bank requires a planned and rehearsed system, which includes procedures on donor selection, whole blood collection and emergency transfusion. A system for education, training, supervision and revision must be established. In Norway, the civilian walking blood banks are subject to the supervision from local hospital-based blood services, the "Mother Blood Bank", and are regulated by national regulations. Donors are selected based on the same criteria as regular blood donors, which includes donor interview, physical examination and laboratory investigations. It is important to educate the emergency blood donors in donation routines and donor selection criteria, so that the safety of donors and patients are maintained. Group O low titter donors of both RhD types and genders are included. Transfusion transmittable disease (TTD) testing and donor interviews are performed at the inclusion, at regular intervals (every sixth month) and at donation. Trained personnel perform donor collection. The WB collection bag has integral access ports for connection of infusion sets to enable immediate transfusion. No further processing of the whole blood units is performed as the blood will be transfused immediately. Blood bags must be labelled to ensure traceability including a unique donation identification number that can be traced back to the donor. Adverse donor or patient events are to be recorded and monitored according to national/regional guidelines.

Summary: In this presentation, the Norwegian system for emergency whole blood collections and transfusion in the framework of a civilian walking blood bank is described. We conclude that implementation of a civilian Whole Blood based emergency collection and transfusion program for early balanced transfusion is possible in regions with reduced access to medical evacuation.

Academy Session A8: Quality control assessment–Principles and practice

AD08-L01 | Quality assurance and assessment in serology testing

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Serology testing within blood transfusion laboratories includes ABO and D grouping, red cell phenotyping, antibody detection and identification, compatibility testing and antibody titration. The quality of blood transfusion testing is a vital part of ensuring that patients receive appropriately matched blood for transfusion. Measures to ensure this happens include a functioning quality management system with suitable internal quality controls and external quality assessment (EQA) / proficiency testing.

Over many years EQA providers have sent samples to laboratories to assess their testing processes; reports are issued together with discussions of errors made, and commentary provided on how laboratories can improve. EQA has identified many procedural errors and serological testing errors within laboratories and highlighted deficiencies in knowledge and training.

EQA providers have a wealth of information on common errors made, and this presentation will provide an overview on the performance of transfusion medicine laboratories in various serological EQA schemes together with identifying some of the gaps in knowledge and understanding, and the potential risks these pose in a clinical service.

AD08-L02 | QA in blood processing

R Cardigan

Abstract not available.

AD08-L03 | The role of ICCBBA in blood banking

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Background: The ISBT 128 Standard, first published in 1994, describes the methodology to ensure globally unique identification for blood and other medical products of human origin (MPHO). ICCBBA is the international non-profit organization that maintains the ISBT 128 Standard with the assistance of volunteer technical advisory groups.

Traceability: The key elements of traceability are globally unique donation identification, division identification, product code, and processing facility identification (if applicable). The ICCBBA issued Facility Identification Number (FIN), combined with the year and a sequence number provides globally unique identification for each donation. This donation identification number (DIN), combined with a standardised product description code and division identifier, provides globally unique identification for each component prepared from a blood donation.

Harmonised Terminology: ICCBBA maintains a database of standardised product description codes, constructed from descriptive classes, modifiers, core conditions, and variable attributes. Multiple volunteer Technical Advisory Groups consisting of representatives, technical experts, liaisons, and observers, guide the development of the terminology to meet evolving labelling and coding needs for blood. Users may search the database for appropriate codes and request new codes be defined as needed. For example, many new product description codes were developed to support accurate labelling of COVID-19 convalescent plasma.

In addition to product description codes, ICCBBA also defines data structures and Harmonised terminology for many other blood product characteristics, including, but not limited to blood groups, transfusion transmitted infection markers, red cell antigens, collection date and time, and expiration date and time.

Electronic Data Transfer of Information: Since its inception, the ISBT 128 standard has utilised data identifiers to distinguish different data structures. This provides the mechanism for blood establishment computer systems to ensure that the bar code being scanned is the correct type and that the length and format of the data content match that defined for the data structure.

The data structures were designed to provide significant information within a confined space on a blood product label. For example, the product code data structure includes the product description code, information about the type of collection, and the division identifier. With the advent of direct electronic transfer of information about medical products of human origin, these data structures have been separated into discrete data elements that can be utilised with electronic messaging systems.

Conclusion: The ongoing maintenance and development of the ISBT 128 Standard continues to enhance patient safety by providing globally unique blood product identification that supports both traceability and biovigilance.

Parallel Session 1: New findings in established blood group systems

PA01-L01 | Knops blood group system: A molecular view

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The antigens of the Knops blood group system (KN; ISBT 022) are located on the complement receptor 1 (CR1), a large glycoprotein consisting of 2039 amino acids. Accordingly, the *CR1* gene encompasses a large genomic region of more than 145 kbp and 39 exons. CR1 is a single pass membrane protein with more than 95% outside of the cell. It has structural features typical for complement proteins such as Complement Control Protein (CCP) domains each consisting of 60 to 71 amino acids. CR1 contains 30 CCPs in a repetitive order of 4 Long-Homologous-Repeats (LHR-A, -B, -C, -D). All KN antigens identified so far are located in LHR-C and LHR-D. Antibodies to KN antigens are usually not clinically significant but they are common in patients. The introduction of Massively Parallel Sequencing (MPS) to immunohematologic case work enabled a time and cost effective analysis of large genomic regions including the *CR1* gene. In patients with a suspected antibody against a high prevalence KN antigen the serologic and molecular investigation clarified the antibodies' specificity and the underlying gene variants. Most interestingly, the allele frequency of several *CR1* variants show remarkable differences between populations, that is, Africans and Europeans. This is also true for some variants that encode KN antigens. For example, the *CR1 c.4801A>G* variant encoding the Vil (KN7) antigen is frequent in Africans (allele frequency 0.6222) but very rare in Europeans (0.0029). Thus, Vil is a low prevalence antigen in Europeans but not in Africans. The differences in antigen prevalence harbours a significant risk of alloimmunization, for example, when an African patient is transfused with blood from an European donor. In our immunohematologic case work most of the cases with suspected KN antibody were transfused patients with African origin. Very recently, the alloimmunization of a transfused African patient led to the identification of a new KN antigen, named KNMB. The underlying *CR1* variants c.3290T>C (p.Leu1097Pro; rs200111726) and c.3298A>G (p.Arg1100Gly; rs202070239) are very rare in Europeans (0.00025) but not in Africans (0.04). The antibody forming index patient was homozygous for the variants and, therefore, negative for the high prevalence antigen KNMB. MPS simplified the molecular analysis especially of blood group systems with large genes such as *CR1* for the KN system. The systematic case work, presumably, will lead to the identification of new antigens.

PA01-L02 | A new knops antigen located on the long homologous repeat C

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Background: The Knops blood group system (KN; ISBT 022) consists of the antithetical antigens Kn$^{a/b}$, McC$^{a/b}$, Sl^{a}/Vil, KCAM/KDAS, and DACY/YCAD and two high prevalence antigens (Yk^{a} and Sl3). They are located in the long homologous repeats (LHR) C and D of complement receptor 1 (CR1). Antibodies to Knops antigens are usually not clinically significant but they are common in patients.

Aims: In a previously transfused female patient of Ethiopian origin with a severe COVID pneumonia we found an antibody to a high frequency antigen with a new specificity which could be inhibited by the Kn(a)/DACY recombinant protein.

Methods: For the antibody identification commercially available panels (BioRad, CH) and different test red cells negative for high frequency antigens were used. The identification was performed in the indirect antiglobulin test with untreated and papain treated red cells in the gel technique. Patient's Knops genotype was determined using in-house PCR-SSP. Following recombinant proteins were used for the inhibition assays of the patient´s plasma: Chido, Rodgers, JMH, Knops(a) and DACY. Massive parallel sequencing (MPS) of a blood group gene panel including all exons of the genes encoding the blood group systems ISBT001 to 043 was performed on the patient and a single non-related African patient non-reactive with patient´s plasma. PCR-SSP methods were established to confirm the mutations in CR1.

Results: The antibody of the patient was reactive with all test cells of commercial antibody identification panels and 29 test red cells negative for different high frequency antigens. It was non-reactive with red cells of a single unrelated patient of African origin only and with all papain-treated red cells. The patient's *KN* genotype revealed homozygosity for the *KN*01.07*, *KN*01.10* and *KN*01.12* alleles determining the phenotype Kn^{a+b-}, McC^{a+b-}, Yk^{a+}, Sl3+, Sl^{a-}/Vil+, KCAM-/KDAS+, DACY-/YCAD+. An antibody to one of the known Knops antigens, including anti-KCAM and anti-DACY, was ruled out. The antibody was inhibited by the DACY recombinant protein only, indicating that the corresponding antigen was probably located on the LHR-C part of the Knops protein. Recombinant Knops(a), Chido, Rodgers and JMH did not inhibit the antibody. In both unrelated patients

MPS identified two homozygous missense mutations c.3290T>C (p.Leu1097Pro; rs200111726) and c.3298A>G (p.Arg1100Gly; rs202070239) in exon 21 of CR1. Both variants are very rare in Europeans (MAF 0.00025) but frequent in Africans (MAF 0.04). The PCR-SSP confirmed the genotype of both mutations in the two patients.

Summary/Conclusions: Using an antibody present in the plasma of a previously transfused patient of Ethiopian origin we identified a new Knops antigen located on the long homologous repeat C (LHR-C). The provisional antigen name (KNMB) was derived from the initials of the antibody producer. KNMB is defined by two adjacent amino acids, that is, Leu at position 1097 and Arg at position 1100. Homozygosity for the two missense mutations in both patients caused absence of the two amino acids and the KNMB-negative phenotype.

PA01-L03 | Disruption of a KLF motif in intron 1 of *RHCE*c* alleles alters recruitment of transcription factors and causes C/c-related changes of *RH* gene and protein expression

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Background: Polymorphism in the regulatory regions of blood group genes is increasingly recognised to be an important factor in blood group antigen expression. In the Rh blood group system, the coding region variations that underlie the molecular bases of the 56 Rh antigens are well understood, from a single nucleotide variant (SNV) to complex *RHD/RHCE* gene rearrangements. However, variation in antigen expression, such as the "Ceppellini effect", remains unexplained. We hypothesised that an uncharacterised regulatory polymorphism in a DNA-binding protein motif may contribute.

Aims: To identify putative regulatory motifs disrupted by a *RHCE*C/c* polymorphism and investigate whether it affects gene and antigen expression.

Methods: *RHD* and *RHCE* were bioinformatically analysed to identify putative regulatory regions using the JASPAR database (GRCh38). Sanger sequencing of one blood sample from each R1R1, R2R2, r'r and rr phenotype was performed using *RHCE*-gene specific primers to confirm the motif. Electrophoretic mobility shift assays (EMSA) and mass spectrometry (MS) were performed to assess binding of nuclear proteins from the human erythroleukemia cell line, HEL, to biotinylated probes containing either intact or disrupted motifs. Dual luciferase reporter assays were performed using HEL cells. Constructs with the intact or disrupted motif in both forward and reverse orientations were compared to the baseline "*RH* promoter" construct to assess motif directionality. *RHCE*-mRNA levels were measured from serologically R1R1, R1R2, R2R2 and rr donor red blood cells (RBCs; four samples each) and normalised to *GAPDH*. RhCE expression on 11 of 16 RBC samples were analysed by flow cytometry with an Rh17-like mouse monoclonal (MIMA-51).

Results: A putative KLF region was identified in *RHCE*C* intron 1 (chr1:25412568-25412576). This was disrupted at chr1:25412573 by a C>T SNV (rs12048617), which was confirmed to be *RHCE*c*-associated by sequencing representative samples. EMSA showed HEL nuclear extract proteins binding to the *RHCE*C* probes with the intact KLF motif, but not to the *RHCE*c* disrupted motif. MS identified 59 differentially-bound proteins on the biotinylated probes. Of note, Sp1- and KLF1-derived peptides, along with Sp3 and KLF3 in lower amounts, were identified only on probes with the intact motif. Relative luciferase activity showed intact KLF constructs were similar to the baseline construct in either direction. In contrast, a 2-fold increase was observed with the disrupted KLF motif in the forward but not the reverse orientation, indicating that direction-dependent loss of KLF binding resulted in increased gene expression. Normalised *RHCE*-mRNA levels increased according to the serological c phenotype: R1R1<R1R2<R2R2<rr. While flow cytometry results did not correlate fully, R2R2 RBCs had the highest Rh17-like antigen expression compared to R1R1, R1R2 and rr RBCs.

Summary/Conclusions: Our findings suggest that the increased RhCE expression found in c-positive samples is due to a KLF motif disrupted by rs12048617:T on *RHCE*c* alleles. We speculate that KLF1/Sp1 proteins compete with KLF3/Sp3 at this motif to cause variable RhCE expression. It is currently unclear how each KLF protein functions and how it recruits its Sp protein. Further work is needed to test for this SNV in a larger cohort and whether it also affects RhD expression, thereby representing the mechanism underlying the well-known but enigmatic "Ceppellini effect".

PA01-L04 | A missense single nucleotide variant, c.305G>A (p. Gly102Asp), in the *GYPA*01* gene disrupts the dimerization motif of glycophorin A, resulting in a weakened M phenotype.

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Background: M and N antigens of the MNS blood group system are carried on glycophorin A (GPA), which is the most abundant sialoglycoprotein existing as a monomer, homodimer, or heterodimer with glycophorin B (GPB) on the membrane of red blood cells (RBCs).

Aims: We aimed to characterise the variant GPA.M encoded by a newly identified *GYPA*01* gene having a c.305G>A (p.Gly102Asp, rs574776481) missense single nucleotide variant (SNV) that may affect the GlyxxxGly dimerization motif at amino acid positions 98 through 102 of immature GPA.

Methods: Blood donor samples were screened by an automated blood typing system (PK7300) using in-house monoclonal anti-M antibodies. When weak or unusual reaction patterns were observed, further tube tests and flow cytometry analyses were performed using in-house monoclonal anti-M and commercial polyclonal anti-M antibodies. The *GYPA* gene was analysed using polymerase-chain reaction followed by

Sanger sequencing. After sodium dodecyl sulphate polyacrylamide gel electrophoresis of the RBC membranes, immunoblotting using anti-M and anti-N antibodies was performed to examine the presence of monomeric and dimeric forms of GPA and GPA-GPB.

Results: Among the blood samples that exhibited weak or unusual reaction patterns with anti-M antibodies, one blood sample showed weak agglutination with a monoclonal anti-M antibody by PK7300 and tube tests, although it was typed as M+N+. Flow cytometry analysis using another three monoclonal anti-M antibodies indicated that the proband's RBCs expressed weakened M antigen compared with control M+N+ RBCs. Sanger sequencing revealed that the *GYPA*01* gene of the proband had a c.305G>A (p.Gly102Asp) missense SNV in exon 5. This SNV is predicted to change the GlyxxxGly dimerization motif to GlyxxxAsp at amino acid positions 98 through 102 of immature GPA. Immunoblot analysis of the proband's RBC membranes did not show any dimeric forms of GPA.M but showed dimeric forms of GPA.N and GPA.N-GPB.

Summary/Conclusions: We identified a novel missense SNV c.305G>A, which disrupts the dimerization motif of the GPA.M. This disruption of the dimerization motif may affect the total amount of GPA on RBCs, resulting in a weakened M phenotype.

PA01-L05 | The expression of *BCAM* c.674G>A in K562 and HEK293T cell lines helps to define a novel Lutheran antigen LUOM

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Background: The Lutheran blood group system is comprised of 26 antigens, of which there are currently four pairs of antithetical and 18 high prevalence antigens. All Lutheran antigens are carried on type I membrane glycoproteins encoded by a single gene *BCAM* located on chromosome 19. Alternative splicing of *BCAM* results in the expression of two glycoprotein isoforms on the red cell surface, 85 kD Lu glycoprotein and 75 kD basal cell adhesion molecule (B-CAM). Both isoforms have five immunoglobulin-like extracellular domains, bind the alpha five subunit of the extracellular matrix protein laminin with high affinity and may mediate intracellular signalling. Both isoforms are expressed on erythrocytes and both carry Lutheran antigens. Recently, we have described a novel high prevalence antigen of the Lutheran system in an Omani female with a history of recurrent abortions and a full-term neonatal death. The patient lacked this high prevalence antigen, proposed to be called LUOM, arising from a mutation c.674G>A in her *BCAM* gene, resulting in a single amino acid change p.Arg225Gln in the Lu glycoproteins (AlSubhi et al., Transfusion Medicine 32(S2):13, 2022).

Aims: We report here the amino acid requirement for the expression of the novel high prevalence antigen LUOM, which was defined by generating a mutant c.674A construct using site directed mutagenesis with wild-type *BCAM* cDNA as a template and expressed either in K562 cell line or in HEK293T cell line as a Fc fusion protein.

Methods: Clones containing the c.674G>A mutation observed in the LUOM-negative patient were produced by site-directed mutagenesis of Lu^b *BCAM* cDNA within the pBabe and pIg plasmids. The pBabe construct was used to transfect K562 cells to produce stable cell lines which were used in a flow cytometry assay with Lutheran-specific antisera (anti-Lu^a and anti-Lu^b), the antibody produced by the patient (anti-LUOM) and Lutheran-specific murine antibodies (BRIC108, BRIC221 and BRIC224). K562 cell line expressing Lu^b was used as control. In addition, the pIg construct was used to transfect HEK293T cells to produce a LUOM Fc fusion protein for testing with the Lutheran specific antisera by ELISA using Lu^b Fc fusion protein as a control.

Results: Native K562 cells do not express Lutheran glycoproteins and thus did not react with any anti-Lutheran antibodies. Cells expressing wild-type Lu^b construct did not react with anti-Lu^a but reacted with anti-Lu^b and anti-LUOM. Transfected K562 cells expressing the Gln225 (LUOM-) did not react with anti-Lu^a or with anti-LUOM and reacted with anti-Lu^b. These results were in agreement with previously reported red cell serology, indicating that the LUOM antigen specifically requires the residue Arg225 for its expression, as anti-LUOM binding is significantly reduced when Gln replaces Arg at residue 225. Furthermore, ELISA was used to confirm the flow cytometry results and the two detection methods were compared and contrasted, with flow cytometry being decided a more practical approach in a red cell reference laboratory due to the more conservative use of human anti-sera.

Summary/Conclusions: This study confirms that the LUOM requires the amino acid arginine at position 225 of the Lu glycoprotein and demonstrates the usefulness of site-directed mutagenesis followed by expression studies in the investigation of novel high prevalence antigens.

Parallel Session 2: Blood stop and go

PA02-L01 | Pre-hospital use of blood components

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Background: During the last decades, there has been a paradigm shift in the initial treatment of patients with critical haemorrhage. Whereas earlier clear fluids were considered the preferred choice, now early balanced resuscitation with blood components is shown to improve patient survival. "Early" is key: The importance of early intervention has led to major changes in both civilian and military transfusion practices: In the military settings the walking blood bank principle has

been introduced and in civilian medicine pre-hospital transfusion has been introduced in many countries worldwide.

It is important to underline that the use of pre-hospital blood transfusion is controversial. Just as this abstract is prepared, on March 1, the Sixth Edition on European guidelines on management of major bleedings and coagulopathy is published in the Critical Care journal: "No clear recommendation or suggestion in favour or against the use of pre-hospital blood products can be provided at this time". The aim of this presentation is accordingly to point to issues that must be clarified before the final recommendations are made.

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Aims:

1. Define the need for pre-hospital blood components
1. Define the blood products that shall be used.
1. Define the blood group criteria
1. Define the need for training of personnel
1. Ensure the storage conditions, resupply systems and cold chain requirements
1. Documentation and haemovigilance

It is essential that the activities are documented in similar systems as for in-hospital transfusions.

Methods: See aims.

Results: See aims.

Summary/Conclusions: Each of these points will be illustrated by examples from personal experience and relevant literature. The presentation may be biased by case stories that ended well, and a provocative question may be: If the patient does need transfusion in-hospital, why not also pre-hospital?

PA02-L02 | A large multicentre evaluation of massive haemorrhage protocol performance and compliance across adult and paediatric hospitals

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Background: Massive Haemorrhage Protocols (MHPs) allow for rapid and standardised delivery of blood components to patients with critical bleeding. To address variability of MHP performance and standardise the approach to massive haemorrhage, the Ontario Regional Blood Coordinating Network, released an MHP toolkit developed by experts, comprising 42 evidence-based principles and nine quality metrics. We conducted a multicentre cohort study to evaluate compliance with the quality metrics in practice.

Aims: To evaluate MHP performance across adult and paediatric patients in Ontario, Canada including the impact of timely haemostatic and transfusion therapies, and blood product wastage.

Methods: We reviewed consecutive MHP activations across 15 hospital sites from January 01, 2019 to July 31, 2022 ($n = 1844$), and collected demographic data, outcomes, and MHP quality metrics using an electronic data collection tool (REDCap®). The primary objective was to measure overall MHP performance using a validated MHP case score, calculated as a proportional score out of a maximum of nine quality metrics. Not all activations could be evaluated for all nine metrics (e.g., timely patient transfer out was not evaluated at all tertiary care centres). Categorical data were analysed using Pearson's Chi Squared test and Fisher's exact tests. Logistic regression analyses were conducted to identify risk factors for in-hospital death at 24 h and up to 28 days. Appropriate MHP activations were defined as >6 red blood cell (RBC) units (>40 mL/kg of RBCs in paediatric patients) within 24 h from the time of activation or haemorrhagic death in the first 24 h.

Results: We analysed MHPs for patients <18 years ($n = 86$, 5%), 18–65 years ($n = 1228$, 66%) and >65 years ($n = 530$, 29%); 1250 (68%) were male. Most MHP activations occurred in the emergency department (51%) and were triggered by trauma related injuries (45%). In-hospital 28-day mortality was 37% ($n = 679$) overall, and highest among patients >65 years (47%) compared with younger adults (32%) and children (38%). Of all MHP activations, 40% were considered inappropriate and 25% were associated with blood product wastage,

especially plasma (717 units). In multivariate analysis, improved survival was associated with a haemoglobin (Hgb) level above 60 g/L for first 24 h but below 110 g/L at 24 h, patient temperature ≥35°C at MHP termination, and administration of tranexamic acid (TXA) within 1 hr (Table 1).

PA02-L02 - Table 1: Multivariate logistic regression analysis for the association between quality metric performance and death

MHP quality metrics	OR (95% CI)	*p*-value
TXA within 1 h of MHP activation	0.67 (0.55 to 0.83)	<0.001
RBCs administered within 15 min of MHP activation	1.09 (0.83 to 1.44)	0.52
Patient transfer out within 1 h of MHP activation	1.46 (0.44 to 4.76)	0.53
Temperature ≥35°C at termination of MHP	0.16 (0.12 to 0.19)	<0.001
Hgb maintained over 60 g/L in the first 24 h	0.07 (0.05 to 0.09)	<0.001
Hgb below 110 g/L at 24 h	0.41 (0.33 to 0.50)	<0.001
Transition to group specific blood products within 90 min	0.93 (0.76 to 1.13)	0.46

Summary/Conclusions: In this large, multicenter study, mortality from massive haemorrhage was highest among patients >65 years, necessitating a better understanding of how to optimise care for these patients. MHP over activation and blood product wastage were common. Administration of TXA within 1 h, haemoglobin levels above 60 g/L (in the first 24 h) but less than 110 g/L (at 24 h), and patient temperature ≥35°C were associated with improved survival.

PA02-L03 | Massive transfusion–can we agree on a definition?

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Background: Recognizing the importance of blood transfusion for improving outcomes of major bleeding, many studies use massive transfusion (MT) as an outcome measure. However, no standardised definition for MT currently exists.

Aims: As part of a collaboration to investigate an international consensus definition for MT, we performed a systematic review of the literature to identify MT definitions used in randomised control trials (RCTs).

Methods: We searched relevant databases from inception to August 2022. To be eligible for inclusion, studies had to: **(1)** be an RCT; (2) include adult patient population with an acquired bleeding disorder in any clinical setting who had received, or were anticipated to receive, MT; and (3) specify an MT definition. In cases where trial protocol and results were published separately, only the latter was included.

Results: 8640 references were identified, of which 19 published and 11 ongoing RCTs fulfilled our criteria, published between 1986 and 2022. Trauma (13/19) and obstetrics/gynaecology (5/11) were the most common settings for published and ongoing RCTs, respectively. We identified 15 distinct MT definitions. The most common was ≥10 units of red blood cells (RBC) in 24 h ($n = 10$: trauma 9/10), followed by 10 units of RBC without any defined time (trauma [2], mixed [2], orthopaedics [1], cardiothoracic surgery [1]) and others (Table 1). We identified 3 key themes: lack of other blood components/products included in MT definitions, more recent definitions moving towards fewer RBC units given over a shorter period, and more non-trauma RCTs being performed.

Summary/Conclusions: Lack of standardisation of MT definitions precludes the comparison of outcomes between trials and limits the synthesis of evidence to inform future research and guideline development. Our findings will help international transfusion/clinical communities to define key elements of MT for future use, including whether a 'one-size-fits-all' definition should be used across all clinical settings and whether the definition should be RBC-focussed or include other blood components/products.

PA02-L04 | Comparison of ROTEM and conventional coagulation tests in identifying trauma-induced coagulopathy during massive haemorrhage protocol

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Background: Trauma-induced coagulopathy (TIC) resulting from massive haemorrhage can be fatal but preventable if recognised early. Evidence has shown that viscoelastic haemostatic assays, such as rotational thromboelastometry (ROTEM), are sensitive and guide goal-directed transfusion; but comparatively expensive leading to limited availability. With its emerging use in trauma, we believe ROTEM can identify TIC more rapidly than conventional coagulation tests (CCTs).

Aims: We evaluated TIC-defining parameters using the ROTEM and CCT results at Vancouver General Hospital (VGH), the primary trauma

centre in British Columbia, Canada, to determine their correlation with blood component utilisation and clinical outcomes during trauma-based massive haemorrhage protocol (MHP) activations.

Methods: This was a retrospective observational study of trauma patients who received transfusion as per 1:1:1 ratio-based MHP at VGH from June 1, 2020, to May 31, 2022. Patient characteristics, CCT and ROTEM results, transfusion data and 24-h and 28-day mortalities were collected from patient and blood bank disposition records. We defined TIC based on institutional algorithms using ROTEM and CCT transfusion triggers in the MHP, including (A) ROTEM-based results of Extem A10 < 40 mm, Extem CT > 100 s, Extem maximum lysis (ML) > 10%, Fibtem A10 < 10 mm; and (B) CCT-based results of INR > 1.8, PTT > 1.5 times of upper normal limit, platelets < 50×10^9/L, and Clauss fibrinogen level < 1.5 g/L. Lab findings of TIC were assessed for their correlation with blood component utilisation and mortality using univariate analysis. Continuous variables were compared by using an independent *t*-test.

Results: Sixty-eight patients had CCT and ROTEM testing performed during a trauma MHP. Thirty-one patients (46%) did not have TIC defined by initial CCT and/or ROTEM results. Twenty-four patients (35%) presented with abnormal ROTEM alone, and 13 patients (19%) had both abnormal CCTs and ROTEM. Of 55 patients with no additional blood components suggested by CCTs, the median number of red blood cells (RBC), frozen plasma (FP), platelet units, and grams of fibrinogen concentrate (FC) transfused within the first 4 hours of MHP was significantly higher in those who had abnormal initial ROTEM (median RBC: 7.8 vs. 4.3, FP: 5.5 vs. 2.6, platelets: 1.3 vs. 0.4, FC: 4.2 vs. 1.4; $p < 0.05$).

Comparing fibrinogen results between ROTEM and CCT, 20 of 68 patients (29%) had hyperfibrinogenaemia based on fibrinogen < 1.5 g/L by CCTs versus 30 of 68 patients (49%) had hyperfibrinogenaemia based on ROTEM within the first 24 h of MHP activation. Those with hyperfibrinogenaemia per CCT had significantly higher 24-h and 28-day mortalities compared to patients with fibrinogen > 1.5 g/L [24 h: 6/20 (30%) vs. 4/48 (8%), $p = 0.028$; 28 days: 11/20 (55%) vs. 8/48 (17%), $p = 0.001$]. However, those with hyperfibrinogenaemia based on ROTEM Fibtem A10 had no significant difference in 24-h and 28-day mortalities [24 h: 6/33 (18%) vs. 4/35 (11%), $p = 0.507$; 28 days: 10/33 (30%) vs. 9/35 (26%), $p = 0.673$], but had significantly higher transfusion requirements within the first 4 and 24 h of MHP compared to the normal Fibtem A10 group.

Finally, all blood component requirements within the first 4 h of MHP were significantly higher in patients with a CCT fibrinogen level of 1.5–1.9 compared to those >1.9 g/L (*p*-values < 0.05).

Summary/Conclusions: ROTEM, particularly FIBTEM A10, was more sensitive in identifying hyperfibrinogenaemia and TIC than CCTs. There was a significant association between increased blood component usage in patients with TIC defined by ROTEM but not by CCTs, and Clauss fibrinogen < 1.9 g/L. Thus, TIC identified by ROTEM and higher cut-offs for Clauss fibrinogen may be more sensitive indicators for transfusion, which could benefit patient outcomes, but this requires further study.

PA02-L05 | Did critically bleeding RhD negative patients receive RhD matched RBCs? Data from the Australian and New Zealand massive transfusion registry

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Background: RhD negative (neg) is a low frequency phenotype and RhD neg RBC supply may be exhausted in critical bleeding requiring massive transfusion (MT). Limited data exist on the frequency and outcomes of RhD mismatched transfusions in the setting of critical bleeding in RhD neg patients, particularly females of childbearing potential (≤50 years). The Australian and New Zealand Massive Transfusion Registry (ANZ-MTR) captures hospital electronic data on patients who received MT (≥5 RBCs in any 4 h period) for any type of critical bleeding during their admission.

Aims: Using ANZ-MTR data, investigate the incidence and patterns in the use of RhD mismatched RBC transfusions in critically bleeding RhD neg patients.

Methods: All MT cases at participating ANZ-MTR hospitals during 2011 to 2018 were included. Variables collected included patient sex, age, ABO/RhD group, bleeding context, number and blood group of transfused RBCs and time of issue. Alloantibody data were from the New Zealand Blood Service. Data were analysed by statistical software (Stata). Significance was defined as $p < 0.05$.

Results: Of 9013 MT cases, 1207 (13.4%) were RhD neg patients (data from 28 hospitals). Of these, 939 (77.8%) received RhD neg RBCs only (matched); 268 (22.2%) received ≥1 RhD positive (pos) RBCs (mismatched) (23 hospitals).

The mismatched group, compared to the matched group, had higher incidence of: male recipients (76.5% vs. 61.6%, $p < 0.001$), out-of-hours MT onset ($p = 0.05$), MT for trauma bleeding (29.1% vs. 18%, $p < 0.001$), early MT within ≤2 h of hospital admission (32.8% vs. 22.4%, $p < 0.001$) and in-hospital mortality (26.1% vs. 20.4%, $p = 0.047$).

The total RBCs transfused during the MT admission episode was higher in the mismatched group than the matched group (14 [9, 22] vs. 10 [7, 14], $p < 0.001$). The mismatched group received median 6 [3, 11] RhD pos RBCs in total during their admission episode. Twenty RhD neg patients (18 male, 2 female) received exclusively RhD pos RBCs.

Mean (SD) age of all RhD neg patients was 61 (18) years with no difference between mismatched and matched groups. There were 323 patients (187 male, 136 female) (26.8%) aged ≤50 years, of whom 59 (56 males, 3 females) (18.3%) were in the mismatched group.

Of the 3 RhD mismatched younger females, mean (SD) age was 43 (5) years. One received 40 RBCs (18 RhD pos) for major trauma and died in hospital. Another received 15 RBCs (8 RhD pos) for terminal metastatic cancer bleeding; she survived the MT, but died within

months. The third received 19 RBCs (3 RhD pos) for liver transplant surgery and survived.

Evaluable RBC alloantibody test results were available for 16/30 (53.3%) New Zealand RhD neg patients who received RhD pos RBCs. Of the 16 patients, 5 (31%) made a new anti-D antibody after the MT episode.

Summary/Conclusions: Of the total RhD neg patients requiring MT, 22.2% received a median of 6 RhD pos RBCs.

RhD neg females ≤50 years were almost always managed with RhD matched RBCs, suggesting close adherence to current transfusion policy (O RhD neg RBCs for patients with blood group unknown) for this specific patient demographic.

RhD neg patients who received RhD pos RBCs were more likely to have early commencement of MT within 2 h of hospital admission, receive more RBCs and to die in hospital, indicative of the emergency nature and severity of the critical bleeding. The high induction rate (31%) of new anti-D alloantibody in the small subset of evaluable cases is concerning and warrants further consideration.

Parallel Session 3: Change management–Evidence based decisions

PA03-L01 | Modelling the effect of an individual-risk based deferral policy for sexual behaviours on blood donations in the US

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Background: The blood donor deferral policy in the US to reduce the risk of HIV transmission by blood and blood components has evolved from an indefinite deferral of men who have sex with men (MSM) that was introduced in 1983, to a time-limited deferral after the last sexual contact, of 12 months in 2015 and 3 months in 2020. The UK and Canada have recently replaced the time-limited deferral for MSM with an individual-risk based approach for HIV sexual behavioural risk for all potential donors, regardless of sex/gender. FDA proposed draft recommendations in January 2023 that eliminate the time-based MSM deferral for MSM and related deferral for women who have sex with MSM, and instead ask all presenting blood donors the same risk-based screening questions and defer individuals who have had a new sexual partner or multiple sexual partners, and anal sex, in the past 3 months.

PA03-L01 – Table 1

Estimates of percent current donor deferral

Scenario	Mean (%)	95% CI
New or multiple sexual partners past 3 months + anal sex past year	1.16%	1.02%–1.31%
New or multiple sexual partners past 3 months + anal sex past month	0.35%	0.30%–0.40%

Aims: We calculated the effect of the individual-risk based deferrals on the current donor base, the 3-month donor deferral for MSM and the industry practice of deferral for pre-exposure prophylaxis (PrEP), and modelled the changes expected after implementation of a 3-month deferral for all presenting donors based on sexual behaviour with new and/or multiple partners and anal sex.

Methods: We developed a computational model to incorporate information about donors, sexual partners and sexual behaviours, including age- and sex-distributions and dependencies, assuming an independent relationship between the risk factors. In our model, both new or multiple partners and anal sexual behaviour must have been exhibited in the past 3 months to be considered a deferred donor. For new and multiple partners, we used the data for the past year to extrapolate for the last 3-month period. For anal sex, we applied the data for the past year and the past month to estimate possible maximum and minimum values for the 3-month period. Our model used 2021 blood donor age and sex distribution data from the US Transfusion Transmissible Infectious Disease Monitoring System (TTIMS) and survey data on US sexual behaviour from the National Health and Nutrition Examination Survey (NHANES 2015–2016), the National Survey of Family Growth (2015–2019), and the National Survey of Sexual Health and Behavior (2009).

Results: Our model estimates that an additional 0.35% to 1.16% of presenting donors would be deferred under the new recommendations (Table). We verified our results with pre-COVID-19 pandemic demographic data from TTIMS (2019).

Summary/Conclusions: The model estimates that the effect of an individual-risk based deferral for sexual behaviour instead of the time-based deferrals for MSM is relatively small, and would only affect 0.35% to 1.16% of the current donor base. Our results are consistent with a 2018 Canadian donor survey (Caffrey, Transfusion Medicine, 2022) that estimated the donor loss of the individual-risk based deferrals as 0.7%.

The additional donor deferrals predicted in the US may be offset by gains from potential donors responding to a more inclusive donor assessment policy, although further studies are needed to assess the effect of the new policy on blood donation in the US.

PA03-L02 | Understanding preferences for advancing inclusion of trans and gender diverse people in blood donation in Australia

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Background: Australian Red Cross Lifeblood is working to advance inclusion of trans and gender diverse (TGD) people in blood donation. Currently blood collection agencies, including Lifeblood, experience challenges in adapting their systems and processes to be inclusive of TGD people, including limitations of donor registration software to binary gender options; how questions about sex and gender identity are asked and recorded; which donor screening questions are asked and how eligibility to donate is managed.

Local data about the experiences of TGD donors is lacking and is needed to optimise Lifeblood policies and procedures. For example, at Lifeblood, donors can register with their preferred gender, and this can be self-reported, however sex at birth is not collected. Further, TGD donors who report sex with a male, trans or gender diverse partner are deferred for 3-months.

To ensure findings addressed experiences and concerns of community members, we established a stakeholder group to inform research focus and design.

Aims: To work with stakeholders to understand issues on TGD blood donation and inform research.

To understand the perspectives and experiences of TGD people who have interest in donating, have attempted donating or who have donated blood.

To propose recommendations based on stakeholder feedback and interview findings.

Methods: Following ethical approval from Lifeblood, nine stakeholder group members were recruited through community groups, donor feedback and donor centre staff.

Interview participants were recruited through social media. 34 expressed interest and semi-structured interviews were completed with 14 people. Of these 11 were current/past donors and 3 non-donors. Interviews followed a journey-mapping approach.

Interviews were recorded, transcribed and analysed using thematic analysis.

Results: Stakeholders identified barriers for TGD people at each stage of donor recruitment and the pre-donation process. These data informed the interview guide.

Registration

Interviewees expressed a preference for separate fields to capture sex assigned at birth and current gender identity, and for gender identity to include transgender man, transgender woman and non-binary, as a minimum. They felt this information would improve processes for assessing product use and criteria for haemoglobin and total blood volume, and capture non-binary people in the donor database. Participants recommended explaining why sex assigned at birth was being asked, as this information may be sensitive.

Donor questionnaire

Participants suggested introducing the capability for donors to record preferred pronouns and name. Further, they indicated that the donor questionnaire should only ask what is necessary for blood donation, omitting titles or binary gendered language. These changes were perceived to reduce the risk of incorrect pronouns, names or inappropriately gendered language being used. Participants recommended changing how recent pregnancy is asked about to be inclusive.

Assessing eligibility

All participants thought that eligibility to donate blood should be based on sexual behaviour rather than gender identity, as behaviour was perceived to more accurately capture risk. Adopting an individual risk assessment approach was broadly supported.

Other

Non-donor and past-donor participants identified deterrents including: fear of incorrect name being used, uncertainty about procedures, and eligibility criteria. Participants suggested including more information on the website about procedures and to let TGD people know they are welcome to donate.

Summary/Conclusions: Our study identified several options to improve inclusion of TGD people in blood donation in Australia. We continue to work with stakeholders to develop recommendations for implementing our findings into operational practice.

PA03-L03 | Safety profile of plasma for fractionation donated in the United Kingdom, with respect to variant Creutzfeldt-Jakob disease

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Background: Plasma-derived medicinal products (PDMPs) are life-saving and life-improving therapies, but demand is rising and the

starting material is in short supply: Europe depends on importation of PDMPs manufactured with plasma from countries including the United States of America (US). Plasma from United Kingdom (UK) resident donors has not been fractionated since 1999 when this precautionary measure was introduced in response to the outbreak of variant Creutzfeldt-Jakob Disease (vCJD).

Aims: We gathered the latest evidence on the safety of UK plasma for fractionation and present it for review.

Methods: The epidemiology of vCJD over the last 23 years was reviewed, as were PDMP manufacturing processes. We reviewed recent risk assessments and regulatory policy changes in the UK and other jurisdictions, and performed a risk analysis using mathematical modelling. Blood service, industry and patient viewpoints were included, and ethical issues were considered.

Results: The vCJD outbreak was much smaller than had been feared and there have been no transfusion transmissions since 1999, when leucodepletion was introduced (allowing for an assumed eight-year incubation period more than 40M components were transfused up to 2015).

There are numerous and effective vCJD risk-reduction steps in the plasma donation and manufacturing process of PMDPs, and effective sanitisation processes in place in fractionation facilities. In February 2021, after a review by the UK Medicines and Healthcare products Regulatory Agency, the UK Government authorised the use of UK plasma to manufacture immunoglobulin for UK patients, and extended this to albumin in February 2023. Following separate reviews concluding no significant difference in the risk posed, the US, Australia, Ireland, Hong Kong and Israel also lifted their deferrals of blood donors with a history of living in the UK.

Mathematical modelling showed that the risk from each unit of donated plasma that is fractionated, using a process with a 4-log prion reduction factor (the industry minimum requirement), is over 7000 times less likely to lead to a vCJD transmission than if that unit was used for transfusion. This suggests that there would be less than one death from vCJD for every 36.4 billion units of UK plasma that are fractionated—this approximates to one possible death from vCJD transmission every 33,000 years. There is considerable uncertainty in the precision of this modelling and these numbers should be viewed with some caution but it is clear that the probability of vCJD transmission through the use of UK plasma for fractionation is extremely low.

Industry and patient groups support the review of guidelines that may enable the use of UK plasma and would bring significant immediate benefits to patients and to the resilience of the European supply chain. It is ethical to do so give the opportunity to provide significant benefits to patients currently in need of treatment.

Summary/Conclusions: UK Plasma is safe for fractionation and blood regulators and operators are strongly encouraged to review their guidelines in the context of current safety evidence and the rising demand for PDMPs in Europe.

PA03-L04 | Risk of variant Creutzfeldt-Jakob disease for the Canadian blood supply

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Background: Despite the >20-year absence of new transfusion-associated cases of variant Creutzfeldt-Jakob disease (vCJD), transfusion-transmission remains theoretically possible due to asymptomatic carriers. Blood services have introduced donor deferrals to mitigate this risk, but this measure limits the donor base.

Aims: To estimate the risk of a donation being contaminated with vCJD ("risk of vCJD") in Canada should current vCJD deferral criteria be lifted.

Methods: The model used Bayesian networks and a Markov chain Monte Carlo method to simulate a cohort of 10 million blood donors observed between 2023 and 2120. The model accounted for several variables that may influence the risk of vCJD, including the number of "at-risk" donors (i.e., those with an extended travel/immigration history in Western Europe), *PRNP* genotype at codon 129, demographics, and the type of labile blood product donated. Donors were no longer considered at risk past the incubation period, as they should be deferred by pre-donation screening. Moreover, donors were assumed to be infectious only in the late phase of the incubation period. Separate models were developed for the two blood services in Canada, that is, Héma-Québec (HQ, which operates in Québec) and Canadian Blood Services (CBS, which operates in other provinces). Two estimates of vCJD prevalence were considered: a high estimate of 1 case in 2028 individuals derived from the Appendix-II study, and a low estimate of 1 case in 588,235 individuals derived from Garske to Ghani's stochastic model. Three scenario analyses were conducted to test the sensitivity of model outputs to assumptions on vCJD prevalence (i.e., high vs. low estimate); the presence or absence of a second wave; and the proportion of deferred at-risk donors, which was reduced by 20% relative to the base case in the optimistic scenario (**Table 1**).

PA03-L04 - Table 1. Model scenario

Scenario	vCJD prevalence in the UK	Second wave	% of at-risk donors
Base case	Low (1 in 588,235)	Yes	HQ = 1.90% CBS = 3.00%
Optimistic	Low (1 in 588,235)	Yes	HQ = 1.52%[a] CBS = 2.40%[a]
Pessimistic i	High (1 in 2028)	No	HQ = 1.90% CBS = 3.00%
Pessimistic ii	High (1 in 2028)	Yes	HQ = 1.90% CBS = 3.00%

Results: In the base case, the simulated HQ cohort comprised 188,825 (1.9%) at-risk donors, and the CBS cohort comprised 324,313 (3.2%) such donors. Over 98 years of observation, no donors were predicted to be infectious at either blood service, and no donations would be contaminated. At HQ, the risk of vCJD was estimated at 0.0, or ≤1 in 294 million based on the upper bound of the 95% confidence interval (CI). At CBS, this risk was also estimated at 0.0, or ≤1 in 312 million based on the upper bound of the 95% CI. Similar results were obtained in the optimistic scenario, but not in pessimistic scenarios i (only for CBS) and ii. In pessimistic scenario i, at HQ, the risk of having a contaminated donation was estimated at 0.0, or ≤1 in 294 million based on the upper bound of the 95% CI. At CBS, this risk was ≤1 in 50 million based on the upper bound of the 95% CI. In pessimistic scenario ii, under the same assumption, at HQ, the risk of having a contaminated donation was estimated at ≤1 in 77 million based on the upper bound of the 95% CI. At CBS, this risk was ≤1 in 16 million based on the upper bound of the 95% CI.

Summary/Conclusions: Given that there are roughly 230,000 whole blood donations each year at Héma-Québec and 800,000 at CBS, the risk of having a vCJD-contaminated donation appears minimal in Canada (i.e., based on the 95% CI upper bound of the pessimistic scenario ii, 1 in 335 years and 1 in 20 years, respectively). These results thus support lifting current vCJD deferral criteria.

PA03-L05 | Is dual testing for Hepatitis C necessary? Risk modelling and cost effectiveness of removing Hepatitis C antibody testing for Australian blood donors

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Background: Parallel testing of blood donations for hepatitis C virus (HCV) antibody and HCV RNA by Nucleic Acid Testing (NAT) has been standard practice in Australia since 2000. Meanwhile, NAT technologies have improved, and HCV has become a curable disease. This has resulted in a significant reduction in the risk and clinical consequences of HCV transfusion-transmission.

Aims: This study aimed to estimate the residual risk under various testing options, conduct a cost-effectiveness analysis and complete a risk assessment to determine the optimal testing strategy.

Methods: A deterministic risk model was used to calculate the residual risk of HCV transmission for four testing strategies. A low, mid and high estimate of the residual risk was calculated for each. The testing strategies modelled were as follows: universal dual testing targeted dual testing for higher risk groups (first-time donors or transfusible component donations) and universal NAT-only. A decision-analytic model was developed to assess the cost-effectiveness of alternative HCV testing strategies.

Results: The mid estimate of the residual risk was 1 in 151 million for universal dual testing, 1 in 111 million for targeted dual testing of first-time donors, 1 in 151 million for targeted dual testing for transfusible component donations, and 1 in 66 million for universal NAT-only. Universal testing with NAT only was the most cost-effective strategy due to the lowest testing cost and the number of transfusion-transmitted cases approximating zero with all testing strategies.

Summary/Conclusions: Antibody testing in addition to NAT does not materially change the risk profile. Even conservative estimates for the cessation of anti-HCV predicts an HCV transmission risk substantially below 1 in 1 million. Therefore, given it is not contributing to blood safety in Australia but consuming resources, anti-HCV can safely be discontinued.

PA03-L06 | Advancing inclusivity and equity for trans, nonbinary, two-spirit and other gender-diverse donors

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Background: Trans, nonbinary, Two-Spirit and other gender-diverse donors (henceforth 'gender-diverse donors') face unique challenges in blood donation and have increasingly advocated for more inclusive and affirming donation policies and practices. Many blood operators recognise the importance of addressing challenges for gender-diverse donors given their commitment to inclusivity and equity. Moreover, data from several countries suggest that the number of people who are gender diverse is increasing. This has important implications for blood operators as they aim to maintain sufficiency of the blood supply into the future. Expanding gender options for donors in blood systems is one important area for blood operators to consider in their efforts to advance inclusivity.

Aims: To examine (1) the general donor population's and (2) gender-diverse donors' views on a two-step question for all donors (i.e., asking their gender *and* sex assigned at birth, SAAB) and expanding gender options for donors.

Methods: We report results from two studies: Study 1- mixed-methods study with the general donor population and Study 2-qualitative study with gender-diverse donors. Participants were recruited from the Canadian Blood Services' donor database. Study 1 examined understanding and acceptability of a two-step question in current donors through qualitative cognitive interviews ($n = 40$, Jan–Mar 2021) followed by a quantitative survey. Qualitative results are presented here. Study 2 examined acceptability of a two-step question and views on expanding gender options with gender-diverse donors though semi-structured interviews ($n = 85$, July–Oct 2022). Thematic analysis was conducted.

Results: Study 1: all participants reported understanding what was being asked with a two-step question. Participants reported that a

two-step question is acceptable and comfortable to answer because they view the questions as "not affecting" them, understand why the questions are asked, and recognise changing social norms. Participants recommended examining gender-diverse donors' views since they are the people to be most affected by the questions.

Study 2: gender-diverse participants had mixed views on whether a two-step question is an improvement on the current single question asking a donor's gender. Most participants who viewed this as an improvement did so because they assumed that it is necessary for the blood operator to know a donor's gender and SAAB. Participants who were not in favour did not assume that it is necessary for the blood operator to know a donor's SAAB and were concerned that a two-step question would "out" gender-diverse donors and compromise their safety. All participants were in favour of expanding gender options beyond the binary to address the erasure of nonbinary donors.

Summary/Conclusions: If a two-step question is implemented, results suggest that donors would understand the questions and find them acceptable. However, gender-diverse donors, the population of donors whose views on this topic should be prioritised, do not necessarily see this as an improvement on the current single question. Results suggest that rather than a two-step question, a better option may be to expand gender options to be more inclusive of gender-diverse donors. When considering expanding gender options, blood operators should collect only necessary information, seek communities' input and provide clear explanation to donors on how the information will be used.

Parallel Session 4: Factors affecting quality of red blood cell products

PA04-L01 | Recycling apparent waste into biologicals: The case of umbilical cord blood for preterm neonates' transfusion

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Preterm neonates frequently require repeated red blood cell (RBC) transfusions as a treatment for prematurity anaemia. In particular, neonates born before 28 weeks of gestation (extremely low gestational age neonates, ELGAN), are prone to receive more blood products, and at an earlier age. The physiological switch from foetal (HbF) to adult haemoglobin (HbA) synthesis occurs several weeks after premature birth. Indeed, RBC transfusions produce in these patients an abrupt substitution of HbF by HbA. The increased oxygen delivery, due to the lower oxygen affinity of HbA, may therefore result at the cellular level in a dangerous condition of hyperoxia, with consequent overproduction of reactive oxygen species. The physiopathology of prematurity-related diseases relies on the extreme susceptibility to oxidative stress, due to the imbalance between the immature antioxidant defense and the high burden of pro-oxidant stimuli produced as a consequence of oxygen therapy, mechanical ventilation, infections, anaemia itself and blood product transfusion. Indeed, it is not surprising that a growing body of evidence connects the decreased levels of HbF to the severity of prematurity-associated diseases such as retinopathy of prematurity or bronchopulmonary dysplasia. Transfusing preterm neonates with cord blood (CB) might be a strategy to raise haemoglobin concentration without depleting the physiological HbF reservoir. Autologous CB in full-term neonates has been used to cover or reduce the transfusion needs during surgery. In the setting of prematurity anaemia of ELGAN, requiring a median number of 4 RBC transfusions, the autologous approach is impracticable. Unrelated CB banks worldwide support transplant activities for patients with malignant and non-malignant diseases. The progressive refinement of banking criteria, mainly regarding the total nucleated cell content (TNC), has been paralleled by the narrowing of probabilities that collected units are suitable for long-term storage and selected for transplantation. In Italy, as well as in other European countries, the ratio between units fulfilling the TNC criterion and total collected units is 1 to 10, or even lower. There is a growing interest in recycling part of the discarded units into CB RBC concentrated. This approach has been demonstrated safe and feasible, but clinical advantages in preterm neonates still remain speculative and need to be documented. Currently, the randomised controlled multicenter clinical trial BORN (NCT05100212), aiming to assess if CB RBC transfusions reduce the severity of retinopathy in ELGANs, is still recruiting. Hopefully, the results of the planned interim analysis will provide useful information. Despite starting from recycled units, processing whole cord blood into RBC concentrates is time-consuming and has additional costs in terms of personnel, consumables and microbial testing. These drawbacks could further affect the economic sustainability of public CB banks and need to be balanced by the evidence of the beneficial effect of the HbF-enriched transfusions in preterm neonates.

PA04-L02 | The quality of leukoreduced red blood cells isolated from cord blood can be maintained during 21 days of storage

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Background: Premature neonates frequently develop anaemia. Treatment is transfusion with standard leukocyte-depleted red cell concentrates (RCC) from adult whole blood. However, adult red blood cell (RBC) haemoglobin consists of >95% HbA, while RBC of preterm neonates contain >90% HbF. Because HbA has a lower oxygen affinity than HbF, higher oxygen release can occur after a transfusion with

adult RBCs. Higher oxygen tension in the retina can be associated with retinopathy. RBCs obtained from umbilical cord blood (CB) contain HbF. Transfusion with HbF containing RBC may therefore have advantages over transfusion with adult RBC.

Aims: The aim of this study was to evaluate the quality of CB-derived, leukodepleted-RCC during storage at 2–6°C RCC.

Methods: CB were collected in CPD and those with a volume of >65 mL were processed to CB-RCCs. CB ($n = 9$) was filtered over a whole blood leukoreduction filter to remove leukocytes and platelets. Filtered CB was centrifuged and the plasma removed. RBCs were diluted with SAGM storage solution to a haematocrit (Ht) of 50%–65%. The CB-RCCs, with a volume of approximately 20 mL, were stored in 100 mL DINCH-PVC bags at 2–6°C for 21 days. The CB-RCCs were sampled weekly for analysis of quality parameters.

Results: CB units ($n = 9$) with a volume of 77 ± 14 mL were processed to CB-RCC with a volume of 21 ± 1 mL and an Ht of 58% ± 2%. The haemoglobin level on day 1 was 3.7 ± 0.2 g. The RBC count remained constant during storage, MCV and haematocrit increased during storage. On day 1, haemolysis amounted to 0.14% ± 0.04%. During storage, haemolysis increased to 0.57% ± 0.16% at day 21. All CB-RCCs met the target value of <0.8% haemolysis. During storage, glucose was consumed and lactate produced. The ATP concentration on day 1 was 4.1 ± 0.8 and decreased during storage to 2.6 ± 0.7 µmol/g Hb on day 21. The ADP and AMP increased during storage. At day 21 of storage, total adenylate, an important measure for RBC energy status and *in vivo* survival, was still 93% ± 12% of the day 1 value.

Summary/Conclusions: Umbilical cord blood can be successfully processed into leukoreduced CB-RCCs. CB-RCCs in SAGM can be stored for up to 21 days in DEHP-free containers with preservation of integrity (haemolysis <0.8%) and energy status. During the processing of the CB, a relative large amount of RBCs were lost. Future research will therefore, in addition to extending the shelf life of CB-RCC, also focus on increasing the RBC recovery during processing of the CB. In addition, second generation storage solutions will be used to increase the ATP concentration.

PA04-L03 | Knock-out of miRNA-30a-5p promotes differentiation and enucleation capacity of erythroblast cell line "imBMEP-TUD-BGO" (imBMEP-A).

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Background: In vitro manufacturing of red blood cells (RBCs) from an unlimited source represents a promising complement to conventional transfusion medicine and cell therapy. Several strategies were described that allow the generation of immortalised erythroid cell lines from different sources, such as embryonic stem cells, induced pluripotent stem cells or hematopoietic stem cells (HSCs). All these approaches have in common, that elaborate differentiation cultures are needed while the yield of enucleated RBCs is inefficient.

Aims: In an attempt to reduce cultivation effort and to increase the enucleation efficiency, we have further engineered an erythroblast cell line previously established in our lab (imBMEP-A). Therefore we investigated the impact of miRNA-30a-5p K.O. on the proliferation and differentiation capacity of the erythroblast cell line, since this miRNA is described as a key inhibitor of erythroid enucleation in human embryonal cell lines.

Methods: The erythroblast cell line imBMEP-A has been established from bone marrow derived $CD71^+$ erythroid progenitor cells from a male donor with blood group O ccD.EE K^-k^+. Via lentiviral transduction of c-myc-p2A-BCL-XL integrated into a doxycycline-inducible expression system, cells were immortalised and a monoclonal cell line derived, which is stably in culture for >3 years under supplementation of 1 µg/mL doxycycline. Since the cells showed impaired terminal erythropoiesis, we aimed to enhance enucleation rate by electroporation-based CRISPR/Cas9 K.O. of the miR-30a-5p gene locus whose complete deletion was confirmed by sequencing. Original and miR-30a-5p-K.O. imBMEP-A cells were compared according to their proliferation and differentiation capacity. Differentiation was induced by EPO-supplemented cultivation (3 U/mL) in absence of doxycycline and erythroid parameters were examined over a course of 10 days.

Results: K.O. of miR-30a-5p did not affect morphology and erythroid surface marker expression in undifferentiated imBMEP-A cells. While proliferation rate and doubling time remained unchanged, miR-30a-5p K.O. resulted in a significant increase in terminal erythropoiesis capacity with ~75% orthochromatic erythroblasts, as compared to 55%–65% in miR30a-5p-expressing imBMEP-A cells after 10 days of differentiation culture. Additionally, enucleation was positively influenced by the K.O., as is evident from a 2.7-fold increase of enucleation rate up to 8.9% ± 1.7%. Furthermore, miRNA30a-5p-K.O. induced a temporary switch of haemoglobin type from foetal to adult during differentiation culture.

Summary/Conclusions: miR-30a-5p K.O. in imBMEP-A cells resulted in promotion of terminal erythropoiesis in terms of increased yield of orthochromatic erythroblasts and enucleated cells, confirming our previous published findings in K562 erythroleukemia cell line. In addition, a promoting effect on the switch from foetal to adult haemoglobin production induced by the miR-K.O. was observed. While the role of miR-30a-5p as inhibitor of terminal erythroid differentiation could be corroborated, K.O. of the miR alone does not enhance the enucleation rate in imBMEP-A to a sufficient extent. In order to address the low yield of enucleation in immortalised cell lines, we are therefore currently employing a systematic shot gun approach, to identify factors that account for the enucleation inefficacy as compared to primary hematopoietic cells, where 80% enucleation rates are common during in vitro culture.

PA04-L04 | Pre-transfusion storage alters proteostasis and circulation capacity of a metabolically- and morphologically-altered red cell subpopulation, but maintains other red cell properties

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Background: Maintaining the availability of red blood cells (RBCs) for transfusion depends on refrigerated storage of RBC concentrates for up to 42 days. During this time, part of the RBC metabolism shifts from glycolysis to the pentose phosphate pathway, leading to progressive decreases in intracellular ATP. Thus, these RBCs are less able to cope with the oxidative stress generated by storage, thereby affecting RBC integrity. These storage lesions are responsible for clearance of up to 25% of the RBCs in the hours following transfusion. We developed a staining protocol to allow sorting of two >95% pure subpopulations of morphologically-distinct RBCs: storage-induced micro-erythrocytes (SMEs) and morphologically-normal RBCs. The SME fraction increases during storage and negatively correlates with post-transfusion recovery, suggesting that SMEs have characteristics inducing their rapid clearance from the circulation.

Aims: Our aim was to obtain a multi-parametric characterization of two long-stored RBC subpopulations (i.e., morphologically-altered SMEs and normal RBCs) to identify storage properties leading to post-transfusion RBC clearance.

Methods: RBC concentrates stored for 42 days at 4°C were stained and sorted by flow cytometry to obtain $CFSE^{high}$ RBCs (i.e., SMEs) and $CFSE^{low}$ RBCs (morphologically-normal). These subpopulations were analysed by an *ex vivo* perfused human spleen model, for deformability (using a splenic biomimetic filter), phosphatidylserine (PS) exposure, osmotic fragility, endothelial cell adherence, intracellular ATP, and by metabolomic and proteomic analysis. In some cases, RBC concentrates stored for <7 days were used as controls.

Results: The proportion of circulating $CFSE^{high}$ RBCs decreased by 57% during *ex vivo* human spleen perfusion, whereas $CFSE^{low}$ RBCs were retained similarly to short-stored RBCs (20% vs. 15%, respectively). Compared to $CFSE^{low}$ RBCs, $CFSE^{high}$ RBCs had decreased filterability; increased PS exposure, osmotic fragility, and adherence to endothelial cells; and very low ATP content.

By metabolomics, 27 metabolites varied between long-stored $CFSE^{high}$ and $CFSE^{low}$ RBCs, including decreases in reduced glutathione (GSH) and increases in its oxidised form (GSSG). Acyl-carnitines (implicated in phospholipid repair) and glyceraldehyde-3-phosphate (essential for ATP synthesis) were decreased. The metabolome did not significantly differ between long-stored $CFSE^{low}$ RBCs and short-stored RBCs.

By proteomics, 96 proteins were overrepresented in $CFSE^{high}$ RBC plasma membranes, with no changes in whole RBC amounts, indicating major protein re-localization to the membrane in these morphologically-altered RBCs. In particular, 44% of these were in the proteostasis family, comprising most 19S proteasome subunits and chaperone proteins. However, the proteome did not significantly differ between long-stored $CFSE^{low}$ RBCs and short-stored RBCs.

Summary/Conclusions: $CFSE^{high}$ RBCs are morphologically-altered, accumulate during storage and were preferentially cleared from the circulation in an *ex vivo* perfused human spleen model. Storage lesions mainly affected this subpopulation, whereas long-stored, morphologically-normal RBCs were similar to short-stored RBCs. The proteome and metabolome of $CFSE^{high}$ RBC were extensively modified, with alterations of oxidative and energetic pathways and dramatic membrane re-localization of proteostasis proteins, suggesting their importance in maintaining RBC integrity.

PA04-L05 | Evaluation of in vitro quality of red cell concentrates derived from lipemic whole blood donations

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Background: Haemolysis in red cell concentrates (RCC) during storage is one of the quality parameters and should be <0.8% of red cell mass in minimum 90% of units tested at the end of storage period according to European guidelines (EDQM). Several studies have indicated an association between lipaemia and red cell haemolysis during storage. A study conducted in Netherland "Lara A.E. ISBT Science Series-2018" reported that RCC derived from whole blood (WB) donations had significantly higher haemolysis as compared to RCC derived from normal plasma at the end of storage. They decided not to use lipaemic

WB donations to produce cellular components as a result of this study.

Norway does not have any guidelines specifying production and shelf life of RCC and platelets produced from lipaemic WB donations. Norway sends all their plasma for fractionation abroad and presence of lipaemia in plasma can interfere with the plasma fractionator's testing. Plasma with high grade of lipaemia (>6.8 mmol/L) is therefore discarded and cannot be delivered for fractionation.

Aims: To compare haemolysis between RCC derived from lipaemic donations and RCC derived from non- lipaemic donations during storage. Further, we aimed to assess if RCC produced from lipaemic donations show increase in haemolysis during storage.

Methods: Data protection officer approved this study and consent was obtained from the blood donors. Donors age, gender and time of collection was noted. Terumo BCT Reveos LR Ref.4FG456S0 donation system was used. Initially leukoreduced RCC (450 mL) derived from lipaemic WB donations ($n = 10$) were included and as a control RCC from non-lipaemic donations ($n = 10$) collected in the same time period were used. RCC were stored and tested on day 1, 14 and 34/35 post collection. Haematological analysis (HCT, MCHC) were analysed on Sysmex XM-9100. Hb samples from RCC were centrifuged for 6 min at 3800 RPM on Eppendorf centrifuge 5810R, supernatant centrifuged once again and Hb was analysed on Haemocue Low Hb system and the results were calculated as percentage of red cell mass. In addition, triglyceride levels were analysed in blood samples from both donor groups. In the second part of our study 21 RCC from lipaemic donations were included and tested for haemolysis on day 21 in addition to day 1, 14 or 35 post collection. Lipemic and control samples were compared using a one-sided unequal variances *t*-test and haemolysis at different days were compared using a one-sided paired *t*-test. $p < 0.05$ was considered significant.

Results: At the end of storage (day 35), average haemolysis in the lipaemic group (0.66 ± 0.45) was significantly higher compared to control group (0.16 ± 0.05) ($p = <0.004$). When haemolysis was calculated at different time periods during storage, both the number of RCC outside haemolysis criteria of <0.8% and the average rate of haemolysis increased significantly between day 21 and day 35 ($p = <0.0004$). Triglyceride levels in the lipaemic group were on average 6.77 mmol/L and the average for the control group was 1.7 mmol/L. There was a male predominance among donors with lipemic donations (87.1% / 12.9%) with a mean age of 41 years and 94% of the lipaemic donations were collected after 12 pm.

Summary/Conclusions: Haemolysis at the end storage period when compared between the two donor groups was increased in the lipaemic donor group. This is in agreement with the findings of the study in Netherland. Haemolysis during storage was significantly increased between day 21 and day 35. These findings did not represent the regular quality control tests conducted in our blood bank. Based on our findings we decided that RCC derived from lipaemic donations are suitable for transfusion with a reduced shelf life of 21 days instead of 35 days.

Parallel Session 5: Technology to improve the donor experience (and blood supply)

PA05-L01 | Remote monitoring of vital and activity parameters in chronic transfusion dependent patients

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Introduction There are no completed trials investigating the value of transfusion in the outpatient setting for chronic transfusion-dependent patients, and little data in general about this subject. Adverse events of red cell (RBC) transfusions, like transfusion reactions and iron overload are reasons for considering a restrictive transfusion strategy. Optimising RBC transfusion however requires an understanding of not only the risks but also the benefits. With no objective parameters to measure the benefits of RBC transfusions, this may however not be the optimal strategy for patients who require transfusion on a regular basis. Transfusions are ordered primarily based on haemoglobin concentrations and a subjective interpretation of symptoms of anaemia. Endpoints that may be more clinically relevant include quality of life measurements, vital signs, functional activity and cognition. To date, there has been little systematic assessment of tools that assess these measures and no published trials that have examined the impact of transfusions on functional outcomes in transfusion-dependent patients.

Aim The aim of our studies is to compare the effects of different dosages of RBC transfusion on patients, and to understand how wearable devices and web-based cognitive and quality of life testing can be used to measure physiological changes and functional activity in a cohort of transfusion-dependent MDS/MPN patients.

REMOTE pilot We first performed a pilot with 5 patients: Withings Steel Smartwatch captured data was 98.9% high quality and usable. The sent out web-based CANTAB tasks and questionnaires were all completed on the appointed days. Heart rate and sustained attention are significantly and reversibly affected by RBC transfusions, corrected for predefined confounders: Mean daytime heart rate was 76.5 ± 2.8 bpm 3 days before and 72.3 ± 2.8 3 days after transfusion ($P = 0.002$). Sustained attention increased from 8586 ± 140 to 9141 ± 183 after transfusion ($P = 0.004$). Activity and quality of life measures did not yield transfusion-induced changes.

REMOTE 2 We are currently monitoring 24 MDS/MPN patients who chronically receive transfusions with the Withings Steel HR. Patients complete CANTAB cognitive tasks, QUALMS and PROMIS quality of life questionnaires through web-based testing. We aim to evaluate transfusion-induced changes in (1) heart rate, (2) activity, (3) sustained attention and (4) patient-reported quality of life. Patients receive three study transfusions: (1) One standard of care transfusion, the

standard amount for the individual patient-specific transfusion regimen, (2) a standard transfusion with one extra RBC concentrate, and (3) a standard transfusion with two extra RBC concentrates.

Conclusion Withings Steel HR and the CANTAB platform are usable for extracting and analysing data on physiology and cognition. RBC transfusions significantly and reversibly decrease heart rate and increase sustained attention in our cohort of 5 RBC transfusion-dependent patients. The REMOTE 2 study will yield further insight in a possible dose-dependency effect of RBC transfusions. Since we lack evidence to support transfusion-guidelines for chronically transfusion-dependent patients, we strongly encourage future trials to incorporate physiological outcomes as objective parameters together with subjective patient reported outcome measures.

PA05-L02 | What wins the final faint face off: Temperature patterns, muscles or video?

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Background: Most blood donors have difficulty self-reporting emotional and physical reactions, so-called vasovagal reactions, in a timely manner until it is too late to prevent them. There are multiple identified risk-factors such as younger age, lack of prior donation experience, elevated anxiety; however, there is a lack of solutions that are able to monitor in real-time to what extent patients are experiencing early signs and symptoms of VVR. In 2018 the FAINT project started, during which we collected both infrared thermal imaging data as well as regular video data, and now that we've reached our goal of including 300 donors, we are ready to make up the score.

Aims: The aim of this study is to investigate to what extent we can best predict who is going to experience low and high VVR symptoms during the blood donation from video data and then compare the predictive performance between the following features: (a) facial temperature data, (b) facial action unit data, and (c) a continuous video stream of overall facial information.

Methods: We filmed three groups of blood donors using thermal and RGB cameras: (1) a control group, who never experienced VVR in the past ($N = 83$), (2) a 'sensitive' group, who experienced VVR at their last donation ($N = 63$), and (3) new donors, who are at increased risk of experiencing VVR ($N = 164$). All participants self-reported their physiological and psychological vasovagal reactions at several stages during the blood donation ($N = 7$). We used video data collected prior to the blood donation to predict low ($N = 220$) and high VVR ($N = 90$) scores during the blood donation. The facial temperature and action units' datasets were split into a train (80%) and test (20%) set and four machine learning models—decision tree, random forest, XGboost and artificial neural network–were applied.

Results: The preliminary results seem to indicate that the highest achieved F1 score using continues video stream with overall facial information was 0.81 using GRU on 25 frames, the highest achieved F1 score using action units as an input was 0.82 and using facial temperature data F1 score was 0.86. The most important features in all models were areas around the eyes and nose.

Summary/Conclusions: We would like to present overall conclusions from our FAINT study, that demonstrates that it is possible to predict vasovagal responses during a blood donation based on the donors' face in the waiting room. We will present overall learnings on which sort of facial information prior to the blood donation seems to give the best indication of how the donor is doing, and to what extent we can predict it in time.

PA05-L03 | Effects of virtual reality on psycho-physiological responses during simulated blood donation among young first-time donors

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Background: Although virtual reality (VR) has been shown to effectively alleviate pain and reduce stress during painful procedures, its potential to mitigate vasovagal reactions (VVR) during blood donation has received limited attention. While numerous distraction strategies have been proposed, most studies have focused on psychological rather than physiological effects. This study is the first to examine the psycho-physiological changes associated with VR distraction during simulated blood donation.

Aims: To investigate the impact of VR on physiological and psychological responses during simulated blood donation.

Methods: With ethics approval, we recruited 24 university students with no prior donation experience, and randomly assigned them to either the Control or the VR group. During simulated blood donation, we measured heart rate variability (HRV) in low-/high-frequency (LF/HF) ratios using a mobile HRV Sensor (Polar H10, Polar Electro Oy, Finland), and haemodynamic indices such as cardiac output (CO), stroke volume (SV), and systemic vascular resistance (SVR) using the Ultrasonic Cardiac Output Monitor (USCOM). Subjective psychological measures, including the State-Trait Anxiety Inventory (STAI) and the Blood Donation Reactions Inventory (BDRI), were collected through self-evaluation questionnaires. Data are presented as mean (SD).

Results: Compared to their baseline LF/HF, the VR group showed a significant decrease in LF/HF ratios, indicating parasympathetic dominance, while the Control group only showed a modest reduction (VR −51% (39%) vs. Control −12% (48%), $p = 0.028$). The VR group reported lower pain levels (VR 39.6 (23.0) vs. Control 49.2 (15.8)), as well as reduced anxiety and increased relaxation levels (VR 73.3 (14.2) vs. Control 65.4 (21.3)), although these subjective measures did not reach statistical significance. In terms of haemodynamics, the VR

group showed significantly higher HR (VR +7% (7%) vs. Control −4% (10%), $p = 0.008$), CO (VR +6% (12%) vs. Control −11% (9%), $p = 0.001$), and lower SVR (VR −7% (18%) vs. Control +14% (17%), $p = 0.006$) compared to their respective baselines.

Summary/Conclusions: Our results suggest that VR distraction can reduce stress levels and optimise haemodynamics during simulated blood donation, even in the absence of actual blood loss. This highlights the significant potential of VR as a distraction tool to mitigate VVR during blood donation.

PA05-L04 | Investigation on the cognition of blood donation knowledge related to novel coronavirus (2019-nCov) infection among Zhejiang residents during the epidemic period of 2019-nCov

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Background: Since December 10, 2022, China have entered the pandemic stage of 2019-nCov infection, and blood collection and supply institutions are facing the dilemma of sudden decrease of blood donors who meeting the health requirements of blood donation . To keep up with the adjustment of national policies and changes in the characteristics of the epidemic, the National Health Commission released "the Guidelines on the Prevention and Control of 2019-nCov Infection in Blood Stations (Second Edition)" on December 17, which revised the health requirements related to 2019-nCov infection . Blood donors' knowledge of blood donation related to 2019-nCov infection will directly affect their behaviour of blood donation during the 2019-nCov pandemic. Therefore, in order to encourage the public to donate blood, the relevant department have invested a large amount of financial resources to carry out unprecedented large-scale publicity . After a publicity period of nearly two weeks, our centre urgently needs to investigate the current cognition of blood donation knowledge related to 2019-nCov infection among residents of Zhejiang Province, further verify the influencing factors of cognition, and provide theoretical basis for accurate publicity in the next step.

Aims: To investigate the cognition of blood donation knowledge related to 2019-nCov infection among residents of Zhejiang Province, and to provide research basis for accurate publicity in the later period.

Methods: From December 31, 2022 to January 3, 2023, stratified sampling was conducted among residents aged 18–60 in Zhejiang Province through China Mobile Communications Corporation. We sent 100,151 SMS messages of questionnaires to investigate their knowledge about blood donation related to 2019-nCov infection. Spss21.0 was used to collate and analyse the data, *n* (%) was used to represent the count data, Chi-square test was used to analyse the differences in cognitive status and $p < 0.05$ was considered statistically significant. Logistic regression was also used to analyse the influence on blood donation intention.

Results: 1254 valid questionnaires were collected, and the statistical data showed that the awareness rate of the delay time of blood donation after the 2019-nCov infection (except severe and critical cases) was 14.99%, the delay time of blood donation after the 2019-nCov infection (severe or critical) was 19.38%, the delay time of blood donation after the vaccine injection was 8.93%, and the recent blood supply was 33.73% . The results of comparative analysis showed that those who donated blood for three or more times had better cognition than those who donated blood for less than three times ($p < 0.001$).Those who had bachelor's degree or above had better cognition than other degree ($p = 0.001$).And those who had intention to donate blood had better cognition than those who had no intention to donate blood ($p = 0.001$). The analysis of influencing factors on blood donation willingness showed that women, older people, those who donated blood more often and knew the degree of blood supply shortage were more willing to donate blood. Further comparative analysis showed that it was more accurate to know the tension of blood supply through propaganda materials and family or friends.

Summary/Conclusions: Residents of Zhejiang Province have a low cognition level of blood donation knowledge related to 2019-nCov infection. The blood collection and supply institutions should pay attention to the emphasis of publicity costs in different media channels and strengthen publicity in new media so as to improve residents' attention to blood donation.

PA05-L05 | Mobile plasmapheresis drive as a strategy to increase plasma haemoderivatives needs

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Background: The Blood and Tissue Bank (BST) is a public agency in charge of managing the blood donation, issuing, transfusion and in charge to plan and distribute the haemoderivates' requirements in Catalonia, 32,114 km^2 and 7.5 million inhabitants always maintaining an unpaid blood or plasma donation model. The constant growth of immunoglobulin G (IgG) consumption has caused a global deficit of this haemoderivative and highlights the fragility of the IgG supply system throughout Europe, based on the import of external markets. Internal initiatives are essential to increase plasma donations for the manufacturing of haemoderivatives and, consequently, to reduce dependence from other markets.

Aims: The BST plans to reach 50,000 selective plasmapheresis donations by 2025, this would mean to reach 50% self-sufficiency of IgG, similar to the consumption of patients with immunodeficiencies in our influence area. To achieve this, a specific marketing plan has started to be developed introducing, with other actions, plasmapheresis mobile drives since 2020 with the aim of making visible and publicizing this type of donation and improve the loyalty of our donors.

Methods: The BST currently has a very solid territorial structure with 12 fixed donation centres (FDCs) and the annual organization of 4275 mobile drives. Since 2017, these 12 blood and plasma donation sites have had a theoretical operational capacity to perform an average of

5346 plasmapheresis/year. In 2020, coinciding with the pandemic and the need to collect convalescent plasma, plasma donations were systematically introduced in mobile drives in a mixed mode, possibility of giving blood or plasma in the same mobile drive, or donate only plasma in a specific mobile drive.

Results: In 2016, our FDCs sites performed 8881 plasmapheresis with an average plasma collection volume of 545 mL. By the end of 2022, the donation FDCs had reached 15,185 plasmapheresis, opening 9 h per day from Monday to Friday and 5 h on Saturdays, with an average volume per procedure of 646 mL. The real operating capacity of FDC were 23.6%, focused in afternoons and Saturday slots. In the last two years, 1925 mixed donation mobile drives have been carried out with the achievement of 12,278 plasmapheresis and 597 specific plasmapheresis mobile drives achieving 8.598 plasma units. The number of plasma donors has increased from 8140 in 2016 to 11,126 in 2022, reaching a loyalty index of 1.90, far from our 2.5 target. The knowledge of the donor height, weight and sex is used to determine the volume of plasma to be extracted, without exceeding 13% of its blood volume, with the aim to optimise the donated plasma volume. The implementation of plasmapheresis in the mobile drives entails to have enough blood cell separators, to cover the whole activity increase, the corresponding plasma donor chairs, more staff per campaign and the appropriate space where to place these elements.

Summary/Conclusions: The possibility of bringing plasma donation closer to the population through mixed or exclusive plasmapheresis mobile drives favoured to publicise this type of donation, increased the potential base of donors to whom we can address by making blood and plasma donation compatible in the same donors and made easier the transfer of blood donors with the most suitable profile to plasmapheresis donation, improving easily their loyalty. The availability of resources to carry out these campaigns on mobile drives are conditioned by the spaces where the campaigns are carried out. The schedule of FDCs donation slots needs to be improved, basically those in the morning. The donor customised use of the volume collection tables has allowed a noticeable increase in the performance of the processes; we consider that we have improved on this point.

Parallel Session 6: Challenging cases–Transfusion support in pregnancy

PA06-L01 | Complex immunohematological cases in pregnancy

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Maternal red blood cell (RBC) alloantibodies can cause haemolytic disease of the foetus and newborn (HDFN). The immunohematological management of pregnancies involves multiple challenges related to the continuous assessment of foetal anaemia risk, availability of RBC units for transfusion of the foetus or newborn if required, and anticipation of blood needs in case of a maternal haemorrhage.

Three backgrounds define immunohematological complexity in obstetrics: (i) an alloantibody to a high-prevalence antigen in a patient with a rare blood type; (ii) an alloantibody to a low-prevalence antigen of paternal origin, inherited by the foetus; (iii) a complex mixture of alloantibodies.

Pregnant women with a rare phenotype may form the antibody to the high-prevalence antigen they lack, after a previous pregnancy or transfusion. This creates a potential risk for the mother, foetus and neonate. It is even more complex when additional antibodies of common specificity exist. Many antibody specificities to a high-prevalence antigen are known to be clinically significant in terms of HDFN, mostly with a haemolytic mechanism (e.g., Rh system, such as anti-Hr and anti-Hr_0). In some cases, inhibition of foetal erythroid progenitors is the dominant feature, as described for anti-Ge3 and anti-Jr^a. A major challenge is to find compatible blood, that may be required for intrauterine transfusion, the baby or the mother. For exceptional phenotypes, national or even international requests may be unfilled. In such case, serial maternal autologous blood donations to be cryopreserved may be the only option.

HDFN can be challenging to diagnose during pregnancy in the seemingly absence of an antibody. Indeed, antibodies to a low-prevalence antigen almost always go undetected upon RBC antibody screening, due to the lack of appropriate positive reagent RBCs. It is noteworthy that such antibodies may be clinically relevant in obstetrics. This is typically the case for numerous specificities related to the MNS system (e.g., anti-Mi^a and anti-Vw). To mitigate this risk, some countries strive to include some of these antigens in commercial RBC panels (e.g., Mi^a in Taiwan, Di^a in Brazil). In the setting of a negative RBC antibody screen in the mother with a suspicion of foetal anaemia, a serological crossmatch with the paternal RBCs is required.

Another challenging background is the complex mixture of antibodies potentially found in pregnant women with multiple negative RBC phenotypes. Such a mixture may mimic an alloantibody to a high-prevalence antigen. Some cases were reported with more than 4 to 6 antibodies developed after only a few uneventful pregnancies and with no transfusion history (high immunological responder status). There is then a risk of cumulative antibody toxicity if the foetus inherits several of the target antigens.

All complex immunohematological backgrounds require a close medical and biological monitoring: Doppler ultrasonography, antibody titter/concentration, antibody-dependent cell-mediated cytotoxicity (ADCC) for the prediction of the antibody activity.

The most challenging cases, especially those with great difficulties in finding compatible blood, require a comprehensive care plan with a multidisciplinary approach, involving obstetric, blood bank, anaesthesia, and neonatology specialists.

PA06-L02 | Managing the mother with allo-antibodies against high prevalence antigens, the foetus and baby

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Background: A rare blood type is a phenotype with a prevalence of less than 1:1000 or a combination of antigens with a prevalence of 1:100 to 1:1000. Over 50 different allo-antibodies against high prevalence (HP) antigens have been reported to cause varying degrees of Haemolytic Disease of the Foetus and Newborn (HDFN). Some of these antibodies have been associated with recurrent abortions (anti-$PP1P^k$) or severe HDFN [anti-Jr^a, anti-Hr_0 (Rh17)] requiring intrauterine transfusion (IUT). Pregnancy is a unique situation in which transfused RBC must be compatible with both the mother and foetus.

Aim: To describe dilemmas and challenges associated with provision of rare blood to mothers with an allo-antibody (antibody) against a HP antigen, their foetuses and babies.

Methods: Present cases of pregnant women with an antibody against a HP antigen requiring transfusion and dilemmas regarding provision of rare blood for IUT.

Results: A pregnant woman can present with an antibody against a HP antigen during her pregnancy or prior to conception. In cases where an antibody has been detected before pregnancy, autologous donations can be frozen and stored by local blood services where a frozen rare blood bank is established. Iron stores, B12 and folic acid should be monitored and supplemented to prevent anaemia. Plasmapheresis or intravenous immunoglobulin (IVIG) administration may be considered based on the clinical significance of the antibody, previous history of abortions or HDFN. When a pregnant woman presents with a clinically significant antibody against a HP antigen during pregnancy, family screening for compatible donors should be undertaken and autologous donation considered based on maternal clinical features, haemoglobin level and obstetric considerations. Communication between providers including the blood bank laboratory, Immunohematology reference laboratories and blood services regarding expected transfusion needs is essential. If compatible blood is not available locally, a search of the ISBT International Rare Donor Program (ISBT-IRDP) may facilitate timely importation of precious rare blood units. British Committee for Standards in Haematology Guidelines (New, *BJH*, 2016) on Red Blood Cell (RBC) products for IUT require provision of irradiated, CMV negative, K-negative RBC units that are less than 5 days old. Some countries recommend compatibility with maternal CcEe phenotype. Accessibility of fresh or frozen rare blood which is in compliance with all the above requirements is extremely challenging. If not all the requirements for IUT RBC units can be met, clinical decisions should be made in order to provide the most suitable RBC unit in a timely manner. Another challenge relates to the provision of sufficient rare blood units, especially for anaemic pregnant women with obstetrical risk for post-partum haemorrhage.

Summary: Managing the mother with an antibody against a HP antigen, her foetus or baby requires close communication among all care providers in order to minimise maternal, foetal and neonatal anaemia and to ensure.

Parallel Session 7: TRALI with TACO spice

PA07-L01 | Update on the pathophysiology of TRALI

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Blood transfusions are frequently administered and a life-saving intervention in diverse clinical settings; however, life-threatening adverse transfusion reactions can unfortunately also occur. This includes Transfusion-related acute lung injury (TRALI), which remains amongst the leading causes of blood transfusion-related fatalities. The pathophysiology is complex and not fully elucidated, and consequently there are no specific therapeutic strategies available. Generally, TRALI can be viewed as a 2-hit model, where the first hit represents the underlying clinical condition of the patient (e.g., state of inflammation) and the second hit is delivered by the transfusion product which can contain anti-leukocyte/endothelial-reactive antibodies, or non-antibody components such as lipids. The combination of both hits results, within 6 h of administration of the blood transfusion, in multicellular immunological reactions which culminate in damage to the pulmonary endothelium, resulting in pulmonary edema, acute respiratory distress and TRALI. Protective recipient cells in TRALI are T regulatory cells and dendritic cells, and the key pathogenic recipient cells include neutrophils, monocytes and macrophages. In addition, recent research has highlighted the complement cascade as a critical driver of TRALI. The so far identified cellular signalling pathways are diverse, may overlap and are dependent on the type of the second hit. This talk will present an overview of the current understanding of the TRALI pathophysiology, with consideration of recent advances.

PA07-L02 | Characterization of platelet traffic through the splenic red pulp during platelet transfusion refractoriness in a murine model

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Background: Upon platelet transfusion, the recipient's anti-HLA-I alloantibodies can cause rapid elimination of transfused platelets from the bloodstream, resulting in transfusion inefficiency and a major risk of haemorrhage. This refractory state remains a serious transfusion complication especially in patients requiring heavy platelet transfusion support. During refractoriness, platelets are partly eliminated in the

spleen, but the exact mechanisms leading to their removal are poorly understood. The spleen has a unique vascular organization where blood cells transit through an open area lacking the continuous anti-thrombotic protection from endothelial lining. How do endogenous and transfused platelets transit through the spleen, under physiological conditions or during refractoriness, is still an open question.

Aims: To characterise platelet traffic through the red pulp of the murine spleen in physiological conditions or during refractoriness

Methods: Platelet transfusion refractoriness was mimicked by transfusing allogeneic $H2^b$-platelets into a recipient ($H2^d$) mouse previously immunised against $H2^b$-platelets. To track platelets *in vivo*, $H2^b$- or $H2^d$- platelets were stained with a fluorescent probe (CFDA-SE-Oregon green 488) and transfused into naïve or refractory mice; or $H2^b$-mice with tdTomato- or eGFP-expressing platelets. Interactions of endogenous or transfused platelets with the splenic microenvironment were monitored by electron microscopy, flow cytometry or intravital multi-photon microscopy (MP). F4/80 (clone BM8) monoclonal antibody was used as a marker to stain macrophages.

Results: In naive mice, MP microscopy revealed that both endogenous and transfused platelets slowed down once they reached the open circulation in the splenic red pulp collagen fibres. Furthermore, no sign of platelet activation, evidenced by the presence of granules and the discoid shape, suggests involvement of inhibitory signals. To mimic platelet transfusion refractoriness, we transfused labelled syngeneic-$H2^d$ or allogeneic-$H2^b$ platelets into alloimmunised $H2^d$-recipient mice. Flow cytometry showed syngeneic platelets circulated for at least 48 h in alloimmunised animals, whereas allogeneic platelets were eliminated from circulation within the first 15 min. Moreover, *in vivo* MP data revealed that transfused allogeneic platelets were not flowing through the red pulp in the same way as syngeneic platelets, but rather held back, in close interaction with $F4/80^+$ cells.

Summary/Conclusions: Multi-modal observations revealed different interactions between platelets and splenic macrophages in naïve or refractory mice. Altogether these results provide a glimpse into platelet traffic mechanisms within the spleen after transfusion.

PA07-L03 | Platelet-derived microparticles could trigger the onset of acute lung injure in a LPS-primed TRALI mouse model

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Background: Platelet-derived microparticles (PMPs) could be accumulated rapidly during the platelets were stored at 22°C. It was reported that PMPs could trigger the respiratory burst of poly-morphonuclear neutrophils (PMNs) and lead to the damage of Lipopolysaccharide (LPS)-activated human pulmonary microvascular endothelial cells (HMVECs) *in vitro*, which might be potentially linked to the onset of transfusion-related acute lung injury (TRALI). However, the pro-inflammatory/pro-coagulant effect of PMPs *in vivo* and the role of PMPs in the development of TRALI are still unclear.

Aims: To determine the pro-inflammatory/pro-coagulant effect of PMPs transfused in the LPS-primed TRALI mouse model.

Methods: The PMPs was isolated by ultracentrifugation from the plasma of apheresis platelet concentrate (A-PLT) which were sorted at 22°C for 5 days, and their number and size were determined by flow cytometric and nanoparticle tracking analyses, respectively. And the LPS-primed TRALI mouse model was established that the BALB/c mice (WT, male, 20 g) were primed with LPS (3 μg/g). After LPS primed 2 h, the different dosages of PMPs (1×10^7/mice, 2×10^7/mice, 3×10^7/mice) were transferred *i.v.* into this TRALI mouse model. And another group of mice was injected with the same volume of the PMPs-free plasma which was removed PMPs by filtered through 0.1 μm PVDF membrane. After PMPs or PMPs-free plasma transfused 2 h, the mice were euthanised. The lung tissue myeloperoxidase (MPO) assay, lung water measurement, bronchoalvelolar lavage fluid (BALF) protein assay, histological examination of the lung tissue were assessed .

Results: In this study, isolated PMPs were identified as the heterogeneous vesicle population with diameter of 0.1–1 μm. The number of PMPs isolated from 5-day-stored A-PLT were increased significantly from $0.73 \pm 0.42 \times 10^7$/mL to $1.36 \pm 0.40 \times 10^7$/mL, which reached approximate 2-fold compared to that isolated from 1-day-stored A-PLT. To determine the damage function of PMPs *in vivo*, the established LPS-primed TRALI mouse model was used. Compared with the control mice that were only treated with LPS, all groups of the mice transferred with PMPs, no matter low dosage or high dosage, developed acute lung injury. In PMPs-transferred mice, the ratio of wet/dry lung weight was significantly increased, meanwhile the total protein content in the BALF also show a sharp rise. A more remarkable pro-inflammatory activity, worsened clinical pneumonic scores and histological end-points in lung were found in the PMPs-transferred group than those in the LPS-treated group. Importantly, the extent of lung injury became more severe with increasing PMPs infusion dose. Nevertheless, the mice transferred with PMPs-free plasma did not show severe lung damage.

Summary/Conclusions: PMPs could accumulated during A-PLT storage. The transfusion of PMPs could trigger the onset of acute lung injure *in vivo*, and these effects were dose-dependent. Removing PMPs or reducing the PMP content in platelet products could prevent the occurrence of TRALI and reduce the risk of platelet transfusion in clinical transfusions.

PA07-L04 | A lung injury phenotype two-hit animal model for transfusion-associated circulatory overload

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Background: Transfusion-associated circulatory overload (TACO) is a life-threatening pulmonary transfusion complication, characterised by

respiratory distress or pulmonary edema combined with symptoms of fluid overload. A large part of the pathophysiology of TACO is still unknown and research in animal models could contribute to our understanding and aid to develop new therapies. In our laboratory a two-hit animal model for TACO was developed, where transfusion resulted in a rise in pulmonary capillary pressure, but not yet in respiratory symptoms "Klanderman, Transfusion, 2019".

Aims: To evaluate if transfusion will lead to pulmonary edema or respiratory distress combined with an elevated pulmonary capillary pressure in an adaptation of a two-hit TACO animal model.

Methods: For this experiment adult male Wistar rats were used. The first hit was to induce volume incompliance. This was achieved by ligating both renal vascular bundles and inducing heart failure by ligating the left anterior descending artery leading to a myocardial infarction. The second hit was to induce volume overload using a transfusion. Animals received either six units of red blood cells (RBCs), plasma or Ringer's Lactate ($n = 9$) or no transfusion (sham, $n = 3$). Left ventricular end-diastolic pressure (LVEDP) was used as a surrogate for pulmonary capillary pressure. The primary outcome was the pulmonary wet/dry ratio, a marker for pulmonary edema. PaO_2/FiO_2 ratio, histopathological lung injury, cardiovascular changes and biomarker levels were secondary outcomes.

Results: Results are expressed as medians and interquartile ranges. There were no significant differences in baseline characteristics, except for animal weight. Pulmonary capillary pressure increased significantly after RBC and plasma transfusion to a maximum LVEDP of 25.4 mmHg (23.5; 27.7) and 23.2 mmHg (18.9; 24.8) compared to Ringer's Lactate infusion or sham animals 7.7 mmHg (6.8; 12.4) and 5.2 mmHg (4.9; 7.4), MWU $p < 0.01$. The pulmonary wet/dry ratio did not differ significantly between groups, Kruskal Wallis $p = 0.24$. the PaO^2/FiO^2 ratio at the end of the experiment was significantly lower after plasma transfusion (273 mmHg, 183;323) compared to groups receiving either RBCs (380 mmHg, 354;452), Ringers Lactate (418 mmHg, 363;463) or sham animals (483 mmHg, 478;499), MWU $p = 0.02$, $p = 0.02$ and $p = 0.01$ respectively. We also saw three animals in the plasma group that had an elevated pulmonary wet/dry ratio combined with histopathological signs of pulmonary edema and a PaO^2/FiO^2 ratio <200 mmHg. One of these three animals had a large elevation in LVEDP, blood pressure and heart rate after transfusion. Another had signs of inflammation and endothelial glycocalyx injury with high IL-6 and syndecan-1 levels. The last animal had high atrial natriuretic peptide levels, a marker for cardiac fluid overload.

Summary/Conclusions: Transfusion of plasma but not RBCs or Ringers Lactate did result in a lung injury phenotype in this adapted two hit TACO model. The PaO^2/FiO^2 ratio dropped after plasma transfusion, indicating respiratory distress in those animals. Pulmonary edema did not increase in any group. Interestingly in the plasma transfusion group, three animals did develop pulmonary edema combined with histopathological lung injury. Cardiovascular symptoms and biomarker levels were heterogeneous, suggesting individual differences in pathways involved in pulmonary edema formation in these animals.

PA07-L05 | Protective effect of mTOR inhibitors treated dendritic cells on mortality in TRALI mice

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Background: Transfusion-related acute lung injury (TRALI), the main symptom of acute respiratory distress syndrome (ARDS), is the main post-transfusion complication leading to transfusion-related mortality in recent years. It has been reported that the presence of DC plays an important role in protecting the body from lung injury mortality. Our earlier study found that DCs treated with mTOR inhibitors (rapamycin and temsirolimus) have the characteristics of more stable tolerance to infection-associated pattern ligand stimulation.

Aims: To investigate the feasibility of the strategy of dendritic cells (DCs) treated by mTOR inhibitors (mTOR-DC) in transfusion-related acute lung injury (TRALI) mice after infection.

Methods: The TRALI mouse model was induced by lipopolysaccharide (LPS) combined with anti-H2KD antibody. The mice anal temperature and the wet/dry ratio of lung, kidney, spleen and brain tissues were measured. Mouse bone marrow-derived DC cells were induced in vitro and treated with mTOR inhibitors (rapamycin or temsirolimus) for 24 h. Mice were injected with or without mTOR-DCs, injected with LPS intraperitoneally 1 h later, and injected with anti-H2Kd antibody 24 h later to induce the onset of TRALI. The death situation of the mice was observed and recorded. The condition of mice after the onset of TRALI was analysed by mouse body temperature, lung wet-dry ratio and pleural effusion weight and lung histopathological sections.

Results: By comparing the induction effects of different concentrations and volumes of anti-H2Kd antibody solutions, the mouse model induced by 0.1 mg/kg LPS combined with 4.5 mg/kg anti-H2Kd antibody (infusion volume of 100 μL) was selected as the TRALI mouse model for this study. After the onset of TRALI, the model mice can significantly increase the wet/dry ratio of the lungs and significantly reduce the body temperature.

After the intervention of TRALI mice with mTOR-DC, the mortality rate was significantly reduced, and the lung tissue lesions of the mice were significantly improved, which the protection effect was better than that of the groups with the inhibitors intervention. Compared with the TRALI incidence group, the weight of pleural effusion in the intervention group was significantly reduced ($P < 0.05$), but there was no significant difference in lung wet/dry ratio and body temperature.

In the mTOR-tDC mice, a large number of DCs can appear in the alveolar area, which greatly reduces the infiltration of PMN in the inflammatory area. mTOR-DCs effectively controlled the protein mucus precipitation and alveolar wall thickening in the alveoli of TRALI mice. At the same time, FOXP3+ T cells did not appear in large numbers in areas of inflammation.

Summary/Conclusions: The combination of LPS and antibodies can effectively induce a stable and typical TRALI mouse model, suggesting that the presence of infectious inflammation and blood transfusion-related inflammatory substances is the decisive factor for the

pathogenesis of TRALI. Meanwhile, DCs treated with mTOR inhibitors have a protective effect on post-infection transfusion-related acute lung injury, which is expected to be a potential cell therapy strategy to intervene in the exacerbation of TRALI.

Parallel Session 8: Pathogen inactivation

PA08-L01 | Update on pathogen inactivation of whole blood, red blood cells, platelets and plasma

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Recurring pandemics, including the recent global pandemic of COVID-19, epidemics and local virus outbreaks have raised the awareness for emerging pathogens threatening the supply with blood components. Until recently, the approach of risk mitigation of pathogen transmission by blood components has relied almost exclusively on a combination of selection of low-risk donors and testing for selected pathogens. This strategy has its immanent gaps (e.g., bacteria and low-titre infections) and is increasingly challenged by changes in donor policies and occurrence of emerging pathogens. In addition, further donor-excluding measures introduced to increase blood safety may have the unintended consequence of decreasing blood availability. Pathogen inactivation (PI) technologies for blood components are crucial for closing safety gaps caused by pathogens not covered by regular blood donor screening programs as they provide a proactive approach for reducing the chances of transfusion-transmitted infection (TTI). PI for plasma and platelet concentrates is already used in clinical practice and based on photodynamical, photochemical or physical methods. However, methods for red blood cells and whole blood, which are needed to achieve the full potential of this proactive protection strategy for blood safety, are still under development. Technologies that use a purely physical method or generate three pathogen-reduced components in a single step by treatment of whole blood provide interesting new perspectives.

Loss of transfusion product efficacy post-PI treatment and potential side effects in transfused patients as well as significant expenses for blood services have been the major concerns for the use of PI technologies. However, clinical studies have shown that pathogen-reduced blood components have sufficient clinical efficacy and acceptable safety profiles, albeit the use of PI technologies for platelets may result in a greater number of transfusions. Indeed, PI technologies require additional working steps on-top of the established preparation methods, and their introduction therefore generally increases the workload and complexity of the existing blood manufacturing. However, technical advances and automation may facilitate the implementation of the PI manufacturing processes by reducing workload and costs. Recent risk-benefit studies and economic evaluations suggest that the use of pathogen-reduced plasma and platelets would significantly and cost-effectively reduce the risk of TTI in Western countries in the event a new blood-borne pathogen entered the blood supply. Increasing data on efficacy, practicability, and clinical performance of PI for red blood cells as the most commonly used blood component require regular re-evaluation of the risk/costs/benefit balance of PI treatment.

Considering the spread of vectors and infectious agents driven by climate change and globalisation, PI offers a proactive solution to help safeguard the blood supply in terms of safety and availability.

PA08-L02 | Implementation of 100% pathogen-reduced platelet concentrates in France: Impact on manufacturing and issuing of platelet concentrates as well as on the risk of transfusion-transmitted infections

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Background: Pathogen reduction (PR) technology reduces the risk of transfusion-transmitted infections (TTI), notably transfusion-transmitted bacterial infection (TTBI) associated with platelet concentrate (PC). Pathogen reduction (Intercept, Cerus Corporation) was introduced for all PC in France in 11/2017. No bacterial detection was in place before PR implementation, while ≈ 8% PC underwent PR.

Aims: To assess the impact of 100% PR PC in France on PC manufacturing and issuing as well as on the risk of PC mediated TTI.

Methods: PC manufacturing, characteristics, transfusion as well as related TTI were compared before and after implementation of 100% PR PC (study period: 2013–2022). Reporting to health authorities of blood donations found positive for pathogens as well of TTI is mandatory.

Results: All PC were in additive solution with an increasing % of whole blood-derived pooled PC (WBPC vs. apheresis PC (APC)) from 51% (2013) to 72% (2022). Extension of PC storage from 5 to 7 days (d) was introduced in 2018, generalized in 2019. Increased WBPC pool size from 5 to 8 donors before splitting in two was introduced in 2018 (30%) and progressively increased to 96.5% WBPC in 2022. Split APC increased from 0% (2017) to 11% (2022). In 2021, transfused PC comprised 22% d6 and 10% d7 PC, while expiration rates decreased: APC: 2.02% (2017) to 1.06% (2021); WBPC: 4.71% to 1.40%. A mean 301 233 (SD: 1160) PC and 329 059 (SD: 4129) were transfused annually before (2013–2016) and after (2018–2022) PR implementation ($p = 0.02$). Mean platelet content/PC, No. PC/patient, and total No. of transfused platelets/patient were 4.05×10^{11}/$4.56/18.31 \times 10^{11}$ and 3.67×10^{11}/$5.22/18.99 \times 10^{11}$ before and after PR implementation, respectively ($p < 0.001$,

3 comparisons). No PC-related transmission of HIV, HBV, HCV and HTLV were reported from 2013 to 2022. HEV and HAV PC-related transmission did occur, before and after PR implementation. PC-mediated TTBI occurrence prior to 100% PR PC implementation was $n = 3$ (SD:1)/year (non-PR PC, $n = 15$, 1/92 700 PC between 2013 and 2016). A fatal outcome occurred in two patients (2/15). Since implementation of 100% PR PC, only one TTBI has been reported, involving Bacillus cereus (D4 APC, grade 3, life-threatening)(frequency: 1/1 645 295 transfused PC between 2018 and 2022 vs. non PR PC: $p < 0.001$). Two PR PC quarantined because of a negative swirling test were found to harbour bacteria: A d6 WBPC in 2021 (Bacillus cereus and Staphylococcus epidermidis) and a d7 APC in 2022 (Staphylococcus aureus). Inspection of the containers did not reveal leaks. Such occurrences were seldomly reported previously as well (five occurrences between 2013 and 2017) despite a requirement for bacterial culture upon abnormal PC visual inspection. Underreporting may have occurred.

Summary/Conclusions: Manufacturing and process modifications in relation with the introduction of 100% PR PC have resulted in PC with a longer shelf life and smaller number of platelets/PC while decreasing expiration rates. Despite an increased number of PC transfused yearly and an increased number of PC transfused / patient, the total number of platelets transfused / patient increased only slightly. Importantly, the transfusion of 100% PR platelets resulted in a steep reduction in TTBI occurrence. TTBI may however still occur, as recently described in the United States as well. Potential mechanisms may involve resistance to PR (spore- and biofilm-forming bacterial species, very high bacterial load) as well as perhaps post-PR contamination by means of container leaks.

PA08-L03 | A unique cluster of HBV subgenotype A2 is responsible for the residual risk of transfusion-transmission after the implementation of individual donation NAT screening for HBV in Japan

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Background: In Japan, blood donated for transfusion is strictly screened for hepatitis B virus (HBV) using individual donation nucleic acid amplification testing (ID-NAT) for HBV DNA and serologically tested for HBs antigen and anti-HBs and anti-HBc antibodies. However, rare cases of transfusion-transmitted HBV infection (TT-HBV) do occur.

Aims: To examine the characteristics of causative blood donors (BDs) and identify donor populations with high risks of causing TT-HBV.

Methods: For TT-HBV cases that occurred over the past 7.7 years (from August 2014 to March 2022), HBV subgenotypes and serological markers in the causative blood units were analysed. HBV DNA levels were estimated based on HBV doubling time. HBV strains in TT-HBV-related cases were phylogenetically analysed and compared to those in TT-HBV-unrelated BDs.

Results: Eight cases of TT-HBV occurred during the study period (Table 1), and the TT-HBV incidence rate was 1 case/4.27 million blood donations. Case 6 was voluntarily reported by a medical institution, while the others were verified through lookback studies, based on positive NAT results. All the causative BDs were males with a median age of 43 years. The index and previous donations of the six BDs were all negative for HBV markers, including HBV DNA and anti-HBc, indicating that the index blood donations occurred during the early phase of acute HBV infection, within the ID-NAT window period. Five cases were caused by HBV/A2 strains with extremely high homology (99.6%–100%) to one another. In Japan, these strains first spread in urban areas among human immunodeficiency virus (HIV)-1-infected men who have sex with men (MSM), and then among heterosexual men and women as well, in both urban and rural areas. A phylogenetic analysis of 1005 HBV strains found in blood donated between August 2014 and July 2015 showed that 65 (6.1%) HBV strains were classified as HBV/A2, 26 of which were clustered into one group (Group A) and separated from the others (Group B, $n = 39$). All five HBV/A2 strains that caused TT-HBV were classified into Group A. HBV viral load (7.8 vs. 5.7 log IU/mL), alanine aminotransferase levels (247 vs. 28 IU/L), and the ratio of BDs who were in the early infection phase (69% vs. 5%) were higher in Group A than those in Group B, suggesting that BDs in Group A mostly donated blood soon after acquiring their infections. The mean age of BDs in Group A was 15 years younger than that in Group B. Furthermore, Group A included three HIV-1-positive BDs, two of whom were also positive for anti-*Treponema pallidum*, suggesting that Group A may have represented a high-risk group for sexually transmitted infections (STIs).

PA08-L03 - Table 1. Characteristics of the causative blood units related to TT-HBV cases in Japan

Case no.[a]	HBV DNA (IU/mL)	Total HBV infused (IU)	HBV subgenotype	Blood component[b]
1	0.06	9.10	A2	PC
2	0.11	20.36	C2	PC
3	0.11	22.13	A2	PC
4	2.04	400.53	A2	PC
5	0.02	4.02	A2	PC
6	No data	No data	A2	FFP
7, 8	0.002	0.36	B2	PC

[a] Split PCs were manufactured and were transfused in Cases 7 and 8.
[b] FFP, fresh frozen plasma; PC, platelet concentrate.

Summary/Conclusions: After the implementation of ID-NAT in Japan, the majority of TT-HBV cases were caused by blood products with large volumes of plasma that contained extremely low amounts of unique HBV/A2 strains, possibly derived from sexually active BDs in the early phase of acute HBV infection. Strict application of STI-related questionnaires and responsible donations are crucial. In addition, lookback studies should be performed to prevent TT-HBV infection and allow patients to undergo early and appropriate treatment.

PA08-L04 | Secretion of enterotoxins by staphylococcus aureus in platelet concentrates modulates release of anti-inflammatory and pro-inflammatory cytokines

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Background: Platelet concentrates (PCs) contaminated with *Staphylococcus aureus* have been involved in septic transfusion reactions worldwide. We have demonstrated that *S. aureus* secretes staphylococcal enterotoxins (SEs) during PC storage (e.g., SEG and SEH), resulting in delayed growth and enhanced biofilm formation of *S. aureus*, ultimately heightening the chances of missed detection during PC screening with culture methods. SEs induce cytokine production and septic shock symptoms in clinical settings; however, it is unknown how these virulence factors modulate platelet cytokine release during PC storage, which is the focus of this study.

Aims: To determine the effects of SE secretion on platelet cytokine release during storage of PCs contaminated with *S. aureus*.

Methods: Transfusion relevant *S. aureus* CBS2016-05 and its derivative SEs mutants (Δ*seg* and ΔΔ*segh*), were used for these experiments. PC units were either not spiked (control) or independently inoculated with wildtype, Δ*seg* and ΔΔ*segh* strains at a concentration of 30 CFU/PC bag. Control and spiked PCs were incubated under standard storage conditions for 48 h before samples were collected for cytokine detection using a dot-blot human cytokine antibody array C5 assay. Dot-blot membranes were analysed with a Gel Analyser software to determine differentially expressed (DE) cytokines between wildtype-spiked and control PCs, and between PCs inoculated with wildtype and mutant strains.

Results: Ten DE cytokines (≥1.0 fold change) were highly expressed in PCs contaminated with *S. aureus* compared to control units (3 anti-inflammatory and 7 pro-inflammatory cytokines, **Table 1**). Interestingly, comparison of cytokine expression in PCs inoculated with wildtype and mutant strains showed higher expression of 5 out of 7 proinflammatory cytokines and the 3 anti-inflammatory cytokines (1.8 to 8.4 fold change) in mutant-spiked PCs compared to the wildtype-inoculated counterparts (**Table 1**). Surprisingly, pro-inflammatory cytokines IL-6, TNF-α and IFN-g were only elevated in mutant inoculated PCs.

Summary/Conclusions: Our results indicate that the septic shock symptoms experienced by patients transfused with PCs contaminated with S. aureus are likely due to an increased content of proinflammatory cytokines in PCs such as CXCL9 and MIP-d1. These findings are further supported by the increased expression of anti-inflammatory cytokines in mutant-spiked PCs vs wildtype-spiked units. Enhanced expression of some pro-inflammatory cytokines in mutant inoculated PCs indicates a complex interaction between SEs and cytokine release that merits further analysis. Our study elucidates a novel approach with focus on SEs and consequent cytokine modulation when investigating septic transfusion reactions involving contaminated PCs with *S. aureus*.

PA08-L04 - Table 1: Cytokine expression in PCs contaminated with *S. Aureus*

Cytokines	Cytokine expression in control versus spiked PCs (fold change)	Wildtype	Δseg	ΔΔsegh
Antiinflammatory	IL-8	0.2	2.5	3.1
	EGF	0.5	2.3	2.4
	TGF-β1	1.0	9.9	9.4
Proinflammatory	IL-6	0.1	1.5	1.2
	1L-15	0.5	1.0	2.2
	CCL2	0.5	1.1	0.9
	CXCL9	1.1	1.0	1.6
	MIP-d1	1.1	0.8	1.0
	TNF-α	0.1	1.2	1.8
	IFN-g	0.0	1.3	1.5

Note: IL-6; IL-8; IL-15; Interleukin-6; -8; -15, EGF; Epidermal growth factor, TGF-β1; Transforming growth factor β1; CCL2; CC class chaemokine 2, CXCL9; C-X-C motif ligand 9; MIP-d1; Macrophage inflammatory protein-1d, TNF-α; Tumour necrosis factor α, IFN-g;

Parallel Session 9: Cell therapy by design

PA09-L01 | Restoring tumor immunogenicity with dendritic cell reprogramming

O Zimmermannova

Immune evasion is an important hallmark of cancer ensured by diverse strategies, including immunosuppression and downregulation of antigen presentation. Here, to restore immunogenicity of cancer cells, we employed the minimal gene regulatory network of highly immunogenic type 1 conventional dendritic cells (cDC1) to reprogram cancer cells into professional antigen presenting cells (APCs). We showed that enforced expression of PU.1, IRF8 and BATF3 (PIB) was sufficient to induce cDC1 phenotype in 33 cell lines derived from human and mouse hematological and solid tumors. PIB gradually modified the cancer cell transcriptional and epigenetic program imposing global antigen presentation and cDC1 gene signatures within 9 days. cDC1 reprogramming restored the expression of antigen presentation complexes as well as co-stimulatory molecules at cell surface, leading to the presentation of endogenous antigens on MHC-I, and to CD8+ T cell mediated killing. Functionally, tumor-APCs acquired the ability to uptake and process exogenous proteins and dead cells, secreted inflammatory cytokines and cross-presented antigens to naïve CD8+ T cells. Importantly, tumor-APCs were efficiently generated at the

single cell level from primary cancer cells of 7 solid tumors that presented antigens to memory and naïve T-cells as well activated patient-specific intra-tumoral lymphocytes. Alongside with antigen presentation, tumor-APCs harboring TP53, KRAS and PTEN mutations showed impaired tumorigenicity in vitro and in vivo. Finally, using in vivo mouse models of melanoma we showed that intratumoral injection of tumor-APCs promoted lymphoid infiltration, delayed tumor growth and increased survival. The antitumor immunity elicited by tumor-APCs was synergistic with immune checkpoint inhibitors enabling tumor eradication.

Our approach combines cDC1's antigen processing and presenting abilities with endogenous generation of tumor antigens and serves as a platform for the development of novel immunotherapies based on endowed antigen presentation in cancer cells.

PA09-L02 | Leukoreduction filters (LRFs) as a novel source of CAR-NK cell based immunotherapy

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Background: Providing a safe source of NK cells with maintained proliferative and cytotoxic capacity, regarding the time and cost-benefit, has challenged the Chimeric Antigen Receptor (CAR) NK cell immunotherapy. In addition, NK cell-based immunotherapy requires relatively large numbers of effector cells, which is not easily achievable.

Aims: Here, we proposed to isolate NK cells from leukoreduction filters (LRFs) to generate CAR NK cells against B-cell maturation antigen (BCMA) as the specific target in patients with multiple myeloma, in terms of returning the leukocytes to use, which are trapped in LRFs and are usually discarded.

Methods: Leukocytes were isolated from LRFs using the back flushing method, and NK cells were purified using the MACS column separation kit. The NK cells immunophenotype and proliferative capacity (treated with IL-2) were assessed using anti-CD16 and anti-CD56 antibodies and CFSE staining, respectively. In addition, the cytotoxic effect of NK cells following co-culture with the NK-sensitive K562 cell line was evaluated using 7AAD/ annexin V kit. NK cells were then successfully transduced with lentiviral vectors containing the *in-silico* confirmed the second generation of anti-BCMA CAR construct. Transduction rate and anti-BCMA construct expression on transduced NK cells using anti-IgG(Fab')2 were evaluated. Moreover, the expression of CD107a as a cytotoxic marker and the mRNA expression of IFN-γ and granzyme B as anti-tumour agents were measured following the co-culture of CAR-NK cells with $BCMA^{\pm}$ cell lines. All experiments were assessed using the flow cytometry method, except the expression of cytokines, which was checked with the real-time PCR method.

Results: High count of NK cells ($85 \times 10^6 \pm 5.7$ cells/ filter) were enriched from leukoreduction filters with two subsets, including; $CD56^{dim}$ $CD16^{high}$ phenotype (89% ± 2.8%) and $CD56^{bright}CD16^{low/neg}$ phenotype (10.5% ± 2.6%). Data were indicative of an IL-2 dose-dependent increase in NK cells proliferation capacity. In addition, the specific lysis of K562 was 26% ± 4.4% following co-culture with NK cells. A high transduction rate (MOI 10 = 51% ± 8%) was observed, and the highly significant expression of anti-BCMA CAR construct on transduced NK cells (96% ± 4%) was confirmed. In addition, following co-culture with the $BCMA^+$ cell line, anti-BCMA CAR NK cells revealed a specific and highly significant increase of CD107a expression (70.3% ± 4.78%) compared to those that were co-cultured with the $BCMA^-$cell line (31.3% ± 2.35%; $P < 0.001$). Also, anti-BCMA CAR NK cells revealed enhanced expression of IFN-γ and granzyme B following co-culture with $BCMA^+$ cell line in comparison to those were co-cultured with $BCMA^-$ cell line ($P < 0.001$) and non-transduced NK cells which were co-cultured with $BCMA^+$ cell line ($P < 0.001$).

Summary/Conclusions: LRF source keeps providing a high count of NK cells needed for CAR NK cell immunotherapy regarding its safety with cost-time benefit. Moreover, data demonstrated the preserved and highly significant proliferation and cytotoxic capacity of NK cells and the subsequent related engineered anti-BCMA CAR NK cells against $BCMA^+$ cells, suggesting the LRF as a reliable source for NK-based immunotherapy.

PA09-L03 | A protein corona around human platelet-derived EVS promotes regenerative functions

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Background: Platelets are essential for haemostasis, immune defense, wound healing and tissue regeneration, In addition to growth factors and cytokines stored in specific granules also platelet-derived extracellular vesicles (EVs) are important for platelet function. Whereas clinical efficiency of platelet derivatives, such as platelet-rich plasma, still lacks definitive evidence, human platelet lysate (hPL) is currently used as potent substitute for foetal bovine serum for clinical cell manufacturing. Notably, we have observed accelerated skin organoid formation and in vivo wound healing by hPL (Ebner-Peking P. et al., Theranostics 2021). As shown by others and us, EVs bear a biologically active protein corona (Toth E. et al., JEV 2021; Wolf M. et al., JEV 2022), depending on the mode of preparation and the protein milieu.

Aims: In this study we asked whether hPL-derived EVs or platelet-derived soluble factors mediate these trophic effects of hPL. We separated EVs from soluble factors of hPL to understand the mode of action during skin organoid formation and immunomodulation as model systems for tissue regeneration.

Methods: EVs were concentrated from hPL by tangential-flow filtration (TFF-EVs) and further purified by size-exclusion chromatography (TSEC-EVs) separating EVs from (lipo-) protein-rich soluble factors (TSEC-sol.F). Samples were characterised by tunable resistive pulse sensing, western blot, tandem mass-tag proteomics and super-resolution microscopy, and functionally tested during organoid formation and immunomodulation.

Results: We identified three major protein clusters by proteomic principle component analysis separating TSEC-EVs from hPL clustering with TFF-sol.F and TFF-EVs clustering with TSEC-sol.F. TFF-EVs induced significantly improved skin-organoid formation and inhibition of T-cell proliferation, compared to TSEC-EVs or to TSEC-sol.F. Reconstituting the corona on TSEC-EVs with TSEC-sol.F re-established functionality comparable to TFF-EVs. Zeta potential and super-resolution imaging confirmed corona formation.

Summary/Conclusions: TFF is a permissive technology enabling scalable enrichment and separation of functional corona-bearing EVs and soluble factors. Depletion of the TFF-EV corona by SEC or ultracentrifugation abrogated functionality indicating a novel mode of action. The corona could be artificially reconstituted in add-back of sol. F showing similar effects compared to TFF-EVs. This enables EV engineering with selected corona proteins for specific purposes and therapeutic applications.

PA09-L04 | Human bone marrow organoids as a model of blood cell generation

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Blood cell production is a carefully regulated process supported by specialised stroma and vasculature in the microenvironment of the bone marrow. There is a huge need for complex, *in vitro* models which emulate these niches, and therefore enable the study of both healthy and diseased haematopoiesis in the context of the bone marrow microenvironment. We recently developed a bone marrow 'organoid' model, derived from human induced pluripotent stem cells (hiPSC) that more faithfully recapitulates key features of human myelopoietic bone marrow. Using a combination of step-wise cytokine exposure and specialised hydrogel embedding, we generated 3D cultures including stromal cells, myeloid cells and their progenitors and lumen-forming vasculature. Haematopoietic cells generated include platelet producing megakaryocytes, erythroid cells, CD14+ monocytes and Eo/Baso/Mast progenitors, and the stroma includes a branched, 3D network of sinusoid endothelial-like cells supported by fibroblasts and mesenchymal stromal cells (MSC). As well as providing a model to test the impact of inflammatory stimuli on the bone marrow niche, the organoids also support engraftment and proliferation of cells from healthy donors and patients with blood cancers (e.g., multiple myeloma, myelofibrosis and acute lymphoblastic leukaemia), enabling a scalable, ex vivo system for the holistic study of healthy and diseased haematopoiesis.

Parallel Session 10: Bridging vein to vein with big data

PA10-L01 | Transfusion-related immune modulation and the use of big data analytics

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Background/aims: Transfusion-related immune modulation (TRIM) may lead to an increased risk of patient complications, none of which are currently monitored by post-transfusion surveillance systems. The impact of blood policy, collection and process changes on TRIM outcomes also remain unexplored.

Methods: All adult transfused and non-transfused hospitalized in-patients from 2002 to 2018 in Hamilton, Ontario Canada were included in this retrospective study. Data were analysed using IN-TRUST (an interactive interface to our large Transfusion Research Utilisation Surveillance and Tracking (TRUST) database); TRUST is a multihospital database with clinical, laboratory, and transfusion data. TRIM outcomes (sepsis, respiratory failure, venous thrombosis and organ dysfunction) were captured by International Statistical Classification of Diseases and Related Health Problems (ICD-10-CA) codes, Canadian Classification of Health Interventions (CCI) codes, and laboratory parameters where applicable with validation studies performed. Blood supplier provided data on changes made to blood policy, collection and processing, as well as their quality control impacts. Time series trend graphs using aggregate data were used to identify the CBS change(s) most correlated with changes in TRIM outcomes.

In the second phase of the study, we performed a traditional logistic regression analysis adjusting for key covariates to explore the association between consolidation of blood production in Brampton Ontario (consolidation) and TRIM outcomes. Admitted hospital in-patients who received 1 or more red blood cell (RBC) transfusions from Jan 2010 to Dec 2014 were included. Primary outcome was in-hospital mortality, and secondary outcomes included sepsis, respiratory failure and organ dysfunction.

Results: ICD-10 sepsis codes were validated using prospectively collected observational study from a published study (DYNAMICS), showing specificity of 94% and sensitivity of 42.2%. Respiratory failure CCI codes were validated using manual chart review with specificity of 89.4% and sensitivity of 71.6%. The blood supplier identified 10 key product policy, collection or production changes. A total of 32 time series trend graphs were generated comparing transfused with non-transfused patients—identifying consolidation as a key production change. In the second phase of the study, 9871 and 7871 patients with an index hospital admission receiving 1 or more RBC transfusion were identified pre- and post-consolidation, respectively. Multivariate analysis found no increase in in-hospital mortality when post-consolidation was compared to pre-consolidation (odds ratio [OR]1.003, 95% confidence interval [CI] 0.887–1.135, $p = 0.954$). Respiratory failure (OR 0.831, CI 0.650–1.062, $p = 0.139$) and organ dysfunction (OR 0.949, 95% CI 0.836–1.078, $p = 0.421$) similarly showed no harm following consolidation. There was a statistically significant reduction with sepsis following consolidation (OR 0.811, 95% CI 0.743–0.886, $p < 0.001$).

Conclusion: Using a hypothesis-generating analytics approach, consolidation of blood production was identified as a key change made by the blood supplier. Consolidation was not associated with changes in in-hospital mortality, respiratory failure and organ dysfunction but was associated with a reduced risk of sepsis.

PA10-L02 | Bloody big data: Haemovigilance using routinely collected healthcare data

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As blood transfusions are only possible due to the generosity of blood donors but also have an inherent risk for transmitting disease, the safety of both blood donation and transfusion is rigorously controlled. Haemovigilance is the systematic surveillance of adverse events in the entire blood supply chain, stretching from blood donation to transfusion and follow-up care. Although transfusion safety has dramatically improved in recent decades, especially in regard to transfusion-transmitted infections, there are still evidence gaps for many aspects of blood donation and transfusion safety. In a series of studies conducted using the most recent version of the Scandinavian Donations and Transfusions database (SCANDAT3), we piloted how large vein-to-vein databases could be used for large-scale data-driven blood donation and transfusion safety monitoring. In one study, we showed that frequent donation of blood platelets using a widely-used instrument, called the leukoreduction system chamber, is associated with fewer number of T-cells and an increased risk of infections for donors. In another study, we showed that patients transfused with blood from blood donors that subsequently developed multiple spontaneous intracerebral haemorrhages were more likely to develop spontaneous brain bleeds themselves, suggesting possible transfusion-transmission of an agent causing intracerebral haemorrhages. Furthermore, exploiting a natural experiment in blood allocation, we showed that the sex or parity of the blood donor does not affect survival in transfused patients. More recently, we have further developed these methods to study a wider range of possible deleterious effects of blood donation, as well as agnostic data-driven monitoring of possible transfusion-transmitted illnesses. Overall, these studies serve as a collective proof-of-concept that routinely collected health data from healthcare systems and blood banks can be used for haemovigilance.

PA10-L03 | Chemical individuality of the blood donor as gleaned by high-throughput metabolomics of over 13,000 end of storage red blood cell samples and multi-omics analyses of 643 recalled donors

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Background: Garrod's principle of chemical individuality posits that "each person is biochemically unique due to inherited differences in enzymes". Application of omics technologies to the field of transfusion medicine is shedding new light on this principle, to the extent that the metabolic phenotype of donated blood impacts the storage quality of blood products and, potentially, transfusion efficacy. Using metabolomics approaches, we introduced the concept of "metabolic age" of the stored red blood cell, which is impacted by (i) biological factors (e.g., donor sex, age, body mass index), (ii) genetic polymorphisms and (iii) non-genetic factors (e.g., dietary habits, nicotine, caffeine or alcohol exposure, exposure to drugs that are not grounds for blood donor deferral). However, technical limitations have constrained studies of stored red blood cell metabolism to small scale investigations.

Aims: To determine the metabolic signature of haemolytic propensity and intra-donor reproducibility of metabolic phenotypes across multiple donations in the largest cohort of blood donors investigated to date with omics approaches.

Methods: Here we leveraged a novel ultra-high throughput metabolomics method to investigate the metabolic phenotypes of end of storage packed red blood cell samples from 13,091 donor volunteers enrolled in four different blood centres across the United States as part of the Recipient Epidemiology and Donor Evaluation Study—REDS RBC Omics. A subset of these donors ($n = 643$) were identified as extreme haemolyzers, as they ranked either below the 5th or above the 95th percentile with respect to end of storage red cell haemolytic propensity (spontaneous or following oxidative or osmotic insults). These donors were asked to donate a second unit of packed red blood cells, which were stored for 10, 23 and 42, for a total of 1929 samples. These longitudinal samples from the recalled donor cohort underwent multi-omics characterization via metabolomics, proteomics and lipidomics. For all the index and recall donors, genomics data on 879,000 SNPs were screened through a precision transfusion medicine array.

Results: Combined analysis of the index and recalled cohort of over 13,000 and 643 donors, respectively, identified novel markers of red blood cell haemolytic propensity, while indicating that only hypoxanthine strongly (positively) correlated with storage and oxidative haemolysis, among the previously reported eight markers of the metabolic storage lesion. We also observed that kynurenine—a metabolite derived from tryptophan catabolism—was significantly positively correlated with osmotic fragility ($q = 5.56$ E-05 at storage day 42). Like osmotic fragility, end of storage kynurenine levels were found to be extremely reproducible for the same donor across multiple donations ($n = 643$; rho = 0.523; $p = 1.27$ E-46), suggesting a strong impact of donor biology on this metabolic pathway. Quantitative Trait Loci (mQTL) analyses were then performed to identify the genetic underpinnings of end of storage kynurenine levels on over 13,000 blood donors, analyses that identified polymorphisms in the tryptophan transporter (SLC7A5/LAT1), ATXN2 and IDO1 as contributors to inter-donor heterogeneity in kynurenine levels and osmotic fragility.

Summary/Conclusions: Donor metabolite levels are linked to haemolytic propensity and genetic factors. Further mQTL studies on the whole metabolome, and linkage of metabolomics data (including the exposome) to available recipient databases (which include haemoglobin increments in recipients of units from the same donors characterised here) promise to revolutionize our understanding of the genetic and non-genetic factors impacting red cell storage biology and transfusion efficacy.

PA10-L04 | Bridging the gap: Geospatial analysis to estimate demand and unmet need of blood products in rural Kenya

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Background: Blood products are an essential medicine, and understanding demand and transfusion patterns is crucial for resource allocation and healthcare delivery. In Sub-Saharan Africa and much of the world, there is a widely known shortage of blood available for transfusion. However, little is known about the actual demand for blood or its availability at the facility level, specifically for rural populations. We believe addressing these gaps in our knowledge of blood supply systems may be of value in Turkana, a rural county in North-western Kenya with a population of about one million, where hospitals have transfusion capacity but are forced to rely on distant centres for essential blood banking processes like screening for HIV, Hepatitis and Syphilis or blood components processing.

PA10-L04 – Table 1.

Drive time to hospital	Population served	Observed annualized demand in units	Expected annual need in units	Estimated unmet need in units
30 min	76.434	1.239	2.626	306 (196–416)
45 min	119.512	1.944	4.105	480 (308–651)
60 min	158.468	2.568	5.443	635 (407–863)
90 min	208.398	3.377	7.158	835 (535–1.135)

Aims: We aimed to measure the demand for blood products at a rural referral hospital in north-western Kenya and compare this with estimated blood needs based on the population served.

Methods: We used high-resolution population density maps for Turkana County, a rural region of north-western Kenya, and ArcPro software to count the population served in time-based facility service areas. We used a facility-based chart review to obtain data on blood products requested and transfused between April and August 2022. Lastly, we compared the actual blood demand with estimations of blood need based on modelled Kenya-specific blood need rates.

Results: We estimate this Kenyan referral hospital serves a population of 76,434–208,393, depending on the driving distance to the hospital between 30 and 90 min. During the study period, 486 requests for whole blood were placed, and 409 (84.15%) were fulfilled, representing an annualized demand of 1621 units. Based on an estimated county population of 926,976, we estimate 174.87 units of blood demand per 100,000 population less than the estimated national blood need of 3435 per 100,000 population. We also calculated the population catchment for 30-, 60- and 90-min drive areas (see Table). There were notable differences in population catchment across the facility service areas. The observed and estimated demand in the 60-min area was 2.07 times that in the 30-min area. Moreover, a 15-min difference represented a rise in the demand of 56.36%, while 30-min increments showed increments between 31.51% and 32.60%. Similarly, the estimated unmet demand varied widely across facility service areas. Going from a 30- to 45-min service area showed a rise in the unmet demand of 56.86%, from 30- to 60-min a rise of 107.52%, and from 30- to 90-min a rise of 172.88%.

Summary/Conclusions: Our study successfully used facility service areas to provide demand and unmet demand estimates. Moreover, we provided insights into blood product demand estimation, and population served using geospatial analysis. This approach may guide stakeholders in the blood banking system, healthcare managers and policymakers in making informed blood product allocation and management decisions. By improving the accuracy of blood product demand estimates, healthcare facilities can enhance their resource allocation, which could lead to improved patient outcomes and optimization.

Parallel Session 11: Recent technological advances in immunohematology

PA11-L01 | How high-throughput targeted enrichment for third-generation sequencing can improve blood group typing

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Blood group genotyping is becoming an increasingly attractive alternative to classical serological blood group typing. While genotyping by sequencing remains comparatively expensive, microarray-based genotyping already provides an inexpensive and scalable platform for blood group typing. The design of reliable microarrays for such purposes, however, requires sufficient knowledge about alleles and their distribution among populations. This can only be achieved with comprehensive and haplotype-resolved data generated by third-generation long read sequencing. We here present a scalable and affordable protocol for the enrichment of long genomic fragments of blood group loci for long read sequencing. We achieve high sequencing coverage for desired blood group loci, can reliably phase heterozygous SNPs and leverage the advantages of long reads for accurate SNP and structural variation detection. This protocol can be used in research to further refine ISBT reference alleles and, in addition, may be performed for diagnostic purposes in case of ambiguous test results. In summary, we propose a high-throughput capable and affordable protocol for the enrichment of long fragments of genomic blood group loci to be used in research and diagnostics.

PA11-L02 | Characterization of the genomic landscape of blood group antigens and alleles in the Indian population utilising whole genome sequencing data

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Background: Blood group antigens are genetically inherited macromolecular structures which form the underlying factor for inter individual variations in human blood. Currently there exists over 390 human blood group antigens corresponding to 44 blood group systems and

two erythroid specific transcription factors. Distribution of these blood group antigens have been found to differ significantly among various ethnic populations. Accurate knowledge of the population specific blood group profiles is vital for effectively managing alloimmunization and multi-transfusion cases. Owing to the extensive diversity of human blood group antigens, obtaining a complete blood group profile through traditional serological or molecular methods is challenging. However, with the aid of population scale high throughput genomic datasets, we are now able to investigate previously overlooked antigens. India being an abode of over 4500 diverse ethnic groups, demands the need to maintain population specific blood group profiles for effective regulation of transfusion practices. Our study aims to systematically assess large scale genomic data of the Indian population to explore complete blood group antigen profiles and to understand the distribution of their associated phenotypes.

Aims: To accurately characterise the genomic landscape of known and rare blood group alleles and antigens in the Indian population using the whole genome sequencing data of 1029 self-declared healthy individuals

To understand the distinct similarities and differences in blood group genotypes and phenotypes across diverse global populations through systematic comparison of genomic datasets.

Methods: Whole genome sequence data (hg38) of 1029 self-declared healthy Indian individuals generated as a part of the pilot phase IndiGen programme were used for the analysis. Variants spanning the genes of 44 blood group systems and two transcription factors KLF1, GATA1 were fetched and annotated for their functional consequences. Genotype and phenotype frequencies of each blood group system were determined and were duly compared with other global population datasets including the 1000 genomes project, gnomAD, Greater Middle Eastern Variome, Singapore Sequencing Malay Project (SSMP), Singapore Sequencing Indian Project (SSIP), China Metabolic Analytics Project (ChinaMAP) and Japanese genome variations (TogoVar).

Results: Our study reports a total of 40,712 blood group related variants of which 695 were identified as non-synonymous variants in the coding region. Of the total non-synonymous variants, 105 were found to have a known blood phenotype. A total of 45 variants were mapped back to ABO and RH blood group systems and the rest 60 variants belonged to other minor blood groups. Interestingly, on comparing the blood group variants identified in the Indian population with other global populations we found that ~30,000 variants were unique to the population. Notably, we also identified 127 variants which were computationally predicted to be deleterious by at least three tools. In addition, a list of 10, 15 and 10 variants belonging to ABO and RH blood groups were found significantly distinct between the Indian and global (1000 genomes project, gnomAD, GenomeAsia), Greater Middle Eastern and East Asian populations respectively. Almost 1 in 1000 Indians carry a weak D antigen (RHD weak) variant which results in RH (w) phenotype. In addition, 7 in 1000 (~9 million) Indians are found to be carriers of Bombay phenotype (Hh phenotype). Our study was also able to identify a few rare blood phenotypes including Au(a-b+), Js(a+b+), Di(a+b-), In(a+b-) and KANNO-.

Summary/Conclusions: This study is the first to use genomic data to understand the blood group antigen profiles of the Indian population, and it also systematically compares these profiles with those of other global populations.

PA11-L03 | Molecular *RHD* donor screening in Switzerland: Discovery of novel alleles by Nanopore-sequencing

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Background: Multiple *RHD* variants can substantially reduce RH1 expression on red blood cells and may be missed even by phenotyping methods including indirect antiglobulin test. To prevent alloimmunization due to such variants, molecular screening for the presence of *RHD* was implemented in Switzerland in 2012 for all serologically RH1 negative first-time donors, according to the guidelines of the Swiss Red Cross. This screening strategy revealed previously unknown *RHD* alleles, which we resolved by Sanger as well as third-generation Oxford Nanopore sequencing for performance comparison.

Aims: Herein we report on the complete data collection of the mandatory molecular *RHD* screening at Blood Transfusion Service Zurich over the last 6 years.

Methods: All RH1-negative donors were screened using the RBC-FluoGene D-Screen kit including primers for *RHD* exons 3, 5, and 10 (inno-train GmbH, Germany). When positive, genotypes and phenotypes were individually reassessed using commercial PCR-SSP (sequence-specific priming) kits as well as standard and extended serological methods, including adsorption-elution technique. Sanger-sequencing and newest long-read sequencing technology of Oxford Nanopore Technologies (ONT) were applied to resolve unknown *RHD* alleles. For ONT sequencing, the entire coding region of *RHD* (~57 kb, exon 1 to 10) was amplified in six overlapping long-range PCRs using previously published *RHD*-specific primers. The PCR-products (~10 kb) were sequenced on MinION flow cells.

Results: Since 2017, more than 12,000 serologically RH1-negative donor samples have been screened at the Blood Transfusion Service Zurich. Overall, 0.69% ($n = 85$) were genetically positive for at least one of the three typed *RHD* exons. The majority of these donors carried known *RHD* null or weak alleles ($n = 82$). Remarkably, in three samples our combined sequencing strategy uncovered novel *RHD* alleles. All were caused by frameshift mutations and serologically defined as null-alleles, also by adsorption/elution techniques, when applicable. One sample had a small duplication in exon 3 (c.395_396dup, p.K133Gfs10), one sample had a single basepair deletion in exon 2 (c.245delT, p.F82Sfs17), and the third donor carried

an allele with a 4-bp deletion (c.1199_1202del, p.K400Ifs*48) in exon 9 in addition to the DAU-specific SNV 1136C>T.

Summary/Conclusions: Molecular *RHD* screening of serologically RH1-negative first-time donors demonstrated efficacy to detect RH1 variants of very low expression. This cross-validation serves as an advantageous strategy for mitigating the potential hazard of alloimmunization in patients. Here we describe three novel *RHD* variants all defined as null alleles based on genetic and phenotypic data. Successful confirmation of all novel alleles by our *RHD* long-read sequencing strategy provides evidence that ONT is a reliable and emerging tool for routine diagnostics.

PA11-L04 | Implementation of a ddPCR method for non-invasive fetal blood group typing for alloimmunised pregnant women

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Background: When a mother has produced blood cell antibodies, maternal to foetal blood group incompatibility can result in haemolytic disease of the foetus and newborn or foetal and neonatal alloimmune thrombocytopenia, with possible severe consequences for the foetuses' health. To timely start treatment, foetuses need to be genotyped in the early second trimester. Traditional non-invasive foetal blood group typing is performed by RQ-PCR on cell free foetal DNA (cffDNA) extracted from maternal plasma. This technique has shown to have limitations, including background signal due to nonspecific amplification of maternal DNA and absence of a universal foetal DNA marker. Droplet digital PCR (ddPCR) is currently emerging as a valid alternative method.

Aims: To evaluate the performance of a ddPCR method for foetal blood group genotyping in alloimmunised women in clinical practice.

Methods: Between April 2022 and mid-February 2023, Sanquin Diagnostics performed a total of 235 foetal blood group genotyping tests (*RHD*: $n = 54$, *KEL*: $n = 50$, *RHE*: $n = 72$, *RHC*: $n = 15$, *RHc*: $n = 33$, *HPA1-a*: $n = 3$, *HPA5-b*: $n = 8$). Presence of foetal DNA was confirmed using the universal foetal marker methylated (m)*RASSF1a*.

Results: In 97 cases the foetal blood group assay result was positive, of which in 82 cases the presence of foetal DNA could also be confirmed by (m)*RASSF1a* ddPCR. In 123 cases the foetal blood group was negative, of which in 115 cases the inclusion of mRASSF1a enabled to issue a negative foetal blood group result. Overall, (m)*RASSF1a* ddPCR results indicated lower amounts of cffDNA when compared to the results obtained for the foetal target of interest. This property of our assay reduces the risk of false-negative blood typing results.

Summary/Conclusions: In conclusion, our ddPCR method is a valid diagnostic test to determine foetal blood group typing and aid clinical decision making for alloimmunised pregnant women.

PA11-L05 | Automated data analysis framework for next generation sequencing blood group testing

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Background: Next generation sequencing (NGS) techniques are capable of generating so-called "long reads" of single DNA strands. Such reads allow attributing variant information to specific haplotypes (phasing), circumventing problems of correct allele assignments in cases where two allelic variants are located several hundred base pairs apart.

However, obtaining phased haplotypes from amplicon-based NGS raw data remains a painstaking process that requires a high level of bioinformatic knowledge. While low sample numbers may be processed manually, this becomes less feasible with high throughput sample numbers required in testing blood donors. By conservative estimates, manual processing of raw data would require approximately 3–6 h per sample and NGS method. Processing results from multiple genes and high sample numbers thus becomes not only time-consuming but also error-prone.

While Oxford Nanopore Technology (ONT) currently offers the longest reads, which is advantageous for phasing distant variants, Illumina is thought to provide better accuracy in base-calling, particularly in areas where ONT has been known to fail, such as Poly-A sequences. We therefore developed an automated data analysis framework for raw data from ONT and Illumina to combine the strengths of both technologies.

Aims: To facilitate NGS data processing for amplicon-based ONT and Illumina raw data and allow for automated result comparison between the two methods in order to alleviate present limitations in applicability for high throughput blood group testing.

Methods: We constructed a data analysis framework with two principal analysis pathways, that allow for automated processing of high sample numbers of NGS raw data. For ONT, the pathway consists of the publicly available tools nanofilt, minimap2, bcftools and whatshap. For Illumina, the pathway comprises nanofilt, bwa, bcftools and whatshap. Both pathways yield .vcf files with quality and phasing information. This data is automatically read, merged and color-coded for agreement and discrepancies.

In addition, all haplotype combinations are counted and listed by frequency. For optimal performance in different applications, quality filters and read length can be adjusted as required. Further, parts of the framework can be used independently, for instance to process ONT data only. To test the framework, we designed long range PCRs for the partly homologous FUT1, FUT2 and FUT3 genes, that encode the human blood group systems "Lewis" and "H". Samples from 400 blood donors were collected and analysed, using both ONT and Illumina systems.

Results: With regard to coding sequences, a total of 357 SNPs were called in FUT1, 2439 SNPs in FUT2 and 1272 SNPs in FUT3. The ONT pathway achieved phasing of 99.9% of identified variants, whereas Illumina allowed for phasing of 95.4% of variants. Average quality scores and read depths for variants identified by the ONT-pathway were 165.5 ± 52.7 and 690.5 ± 175.4 for FUT1, 167 ± 46.7 and 790.6 ± 237.7 for FUT2 and 184.7 ± 61.0, and 569.1 ± 131.3 for FUT3. The Illumina-pathway yielded quality scores and read depths of 222.6 ± 1.2 and 880 ± 156.8 for FUT1, 223.3 ± 9.9 and 771.9 ± 214.2 for FUT2, and 223.9 ± 13.9 and 450.1 ± 214.0 for FUT3. Agreement in variant calling between the two methods was 98.9% for FUT1, 99.1% for FUT2 and 92.2% for FUT3.

Summary/Conclusions: We developed a data analysis framework for handling raw data from both Illumina and ONT. The framework is able to automatically compare results, show discrepancies and list haplotypes. The presented framework allows for simple and time-efficient, simultaneous processing of amplicon-based NGS raw data generated by Illumina and ONT systems. Information from both systems is merged and evaluated, combining the advantages of both technologies.

Parallel Session 12: Antigens and Thrombocytopenia

PA12-L01 | New therapies for ITP

J Semple

Abstract not available.

PA12-L02 | Laboratory approach and analysis of platelet-antibodies in clinically-suspected vaccine-induced thrombotic thrombocytopenia (VITT) patients in The Netherlands

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Background: Vaccines have been essential in managing the SARS-CoV-2 pandemic. In 2021, however, vaccine-induced thrombotic thrombocytopenia (VITT) emerged, which was clinically associated with the occurrence of thrombocytopenia and/or thrombosis at unusual sites. VITT is characterised by the presence of IgG-antibodies directed against platelet factor 4 (PF4), indicating a critical role for PF4. The pathophysiology of VITT, however, appears to be complex and incompletely understood, warranting further investigations.

Aims: We aimed to characterise the clinically-suspected VITT patient population in the Netherlands in terms of the presence of PF4-antibodies, PF4-dependent platelet-activation, the relevance of platelet-FcγRIIa in PF4-dependent platelet activation and the presence of antibodies directed against platelet-glycoproteins (GPs), in relation to the type of administered vaccine and the occurrence of thrombocytopenia and/or thrombosis.

Methods: We analysed and defined clinically-suspected VITT patients in The Netherlands ($N = 275$) from a diagnostic perspective using sera in an anti-PF4 IgG ELISA and a functional PF4-dependent platelet activation assay (PIPAA), for which a double-positive outcome was considered as probable VITT patients. Additionally, we blocked platelet-FcγRIIa using a monoclonal antibody (clone IV.3) in the PIPAA. We also collected clinical data regarding the type of vaccine which was administered and the presentation of thrombocytopenia and/or thrombosis. Subsequently, we investigated the presence of platelet-antibodies directed against GPIIb/IIIa, GPV and GPIb/IX in sera from 223 of the 275 clinically-suspected VITT patients using a monoclonal antibody immobilization of platelet antigens (MAIPA) assay.

Results: Out of the 275 clinically-suspected VITT patients, 21 (7.6%) tested positive, 243 (88.3%) tested negative, and eleven (4.0%) tested weakly positive in the anti-PF4 IgG ELISA. 19 patients (6.9%) tested positive in both the anti-PF4 IgG ELISA and the PIPAA. Thirteen of these clinically-suspected VITT patients presented with both thrombocytopenia and thrombosis. Furthermore, we observed that PF4-dependent platelet activation was inhibited when platelet-FcγRIIa was blocked. 46 of the 217 clinically-suspected but unconfirmed VITT patients, but none of the clinically-suspected and probable VITT patients ($N = 6$), tested positive for anti-platelet GPs. The development of platelet-antibodies may be associated with adenovirus-based COVID-19 vaccines. 87.5% of the patients who tested positive for platelet-antibodies were diagnosed with thrombocytopenia, only 7.4% presented with both thrombosis and thrombocytopenia.

Summary/Conclusions: Based on a positivity in both the anti-PF4 IgG ELISA and the PIPAA, 6.9% of our cohort was considered to be probable VITT patients. We confirmed the importance of platelet-FcγRIIa in PF4-dependent platelet activation. The development of platelet-GP antibodies may be related to adenovirus-based COVID-19 vaccines and with the occurrence of thrombocytopenia, but our data does not support an essential role for these platelet-antibodies in the VITT pathogenesis. To gain further insights, it is of vital importance that more research is performed on the dissection of pathophysiological VITT-mechanisms, including the functional role of platelet-antibodies, also in relation to the possible development of immune thrombocytopenia.

PA12-L03 | Blocking human FcγRIIIA as a novel therapeutic option for the treatment of platelet alloimmune and autoimmune conditions

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Background: Platelet-transfusion refractoriness (PTR), fetal and neonatal alloimmune thrombocytopenia (FNAIT), as well as immune thrombocytopenia (ITP), are conditions where IgG antibodies mediate platelet destruction by phagocytosis through the mononuclear phagocyte system. Macrophages recognise and phagocytize antibody-opsonized platelets via Fc gamma receptors (FcγR), and FcγRIIIA has been considered one of the main activating receptors associated with platelet destruction in ITP. Monoclonal antibodies can block the interaction of this FcγR with antibody-opsonized platelets, but conversely, provoke undesirable inflammatory responses due to FcγR crosslinking. Thus, our group has demonstrated that monovalent antibody constructs can ameliorate ITP and circumvent these adverse events. Whether this strategy could also work for PTR and FNAIT has not yet been evaluated.

Aims:

- Develop new monovalent FcγRIIIA-blocking constructs
- Assess the binding of these constructs to FcγRIIIA *in vitro*
- Evaluate the inhibition of IgG-opsonized platelet clearance using these anti-FcγRIIIA constructs.
- Assess the adverse event profile of these constructs in murine models.

Methods: Phage display was used to select molecules with high efficacy blocking of FcγRIIIA and minimal cross-reactivity to other Fc receptors. The best candidate was examined for its ability to inhibit the interaction between FcγRIIIA and human IgG. This candidate was expressed as a monovalent albumin fusion protein as well as a full-length mouse IgG2a antibody.

The capacity of this molecule to bind FcγRIIIA on cell lines and primary cells was evaluated by flow cytometry. Additionally, their *in vitro* capacity to inhibit the destruction of IgG-opsonized platelets using sera from patients with alloimmune or autoimmune conditions was assessed using THP-1-CD16A cells. A passive model of ITP was induced in FcγR-humanized mice by injecting a rabbit anti-mouse platelet serum. Animals were then treated with the anti-FcγRIIIA molecules to assess therapeutic potential as determined by platelet counts two hours after disease induction. Body temperature was measured in 15-min intervals post-treatment as a metric of adverse systemic inflammation.

Results: Both the full-length antibody and the albumin fusion protein possess the ability to bind FcγRIIIA on macrophages and NK cells from human donors. Furthermore, the antibody and albumin fusion protein inhibited FcγRIIIA-mediated phagocytosis of anti-HPA-1a- or anti-HLA-opsonized platelets. In a murine ITP model using FcγR-humanized mice, anti-FcγRIIIA blocking ameliorated thrombocytopenia.

Summary/Conclusions: These results suggest a potential use for blockade of human FcγRIIIA as a potential treatment for platelet alloimmune and autoimmune conditions. As a monovalent formulation, platelet counts were successfully increased in mice without associated adverse activity suggesting that these molecules may provide a novel safe, and effective therapeutic option for these conditions.

PA12-L04 | Trogocytosis as a mechanism of antibody-mediated immune suppression

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Background: Red blood cell (RBC) alloimmunization to paternal antigens during pregnancy can cause haemolytic disease of the foetus and newborn (HDFN), which is a severe and potentially life-threatening condition. However, the administration of polyclonal anti-D can prevent HDFN through a mechanism known as antibody-mediated immune suppression (AMIS). Despite the effectiveness of anti-D prophylaxis in preventing HDFN, the lack of mechanistic clarity has made it difficult to replace it with recombinant molecules. Although the major theories behind AMIS induction have mainly focused on erythrocyte clearance, recent studies have suggested that antigen (Ag)-loss may be a potential mechanism of AMIS induction. However, the significance of Ag loss as a reliable indicator of AMIS and the underlying mechanism driving this process remain undefined.

Aims: The present work aims to study the ability of eleven different antibodies and variants to induce (i) AMIS activity, (ii) RBC clearance, (iii) erythrocyte Ag loss, and (iv) RBC membrane loss and to determine if a relationship exists between AMIS activity and each of these mechanisms. In addition, this work has sought to elucidate the mechanism through AMIS-inducing antibodies induce erythrocyte Ag loss.

Methods: Transgenic HOD mice possess erythrocytes expressing an antigen composed of hen egg lysozyme (HEL), in sequence with ovalbumin (OVA) and the human Duffy transmembrane protein [HOD]. HOD-RBCs labelled with a fluorescent dye (PKH67) were transfused into C57BL/6 mice. After 24 h, AMIS-inducing HEL-, OVA- or Duffy-specific antibodies were administered. Mice were bled at 2 and 24, and 48 h and the percentage of HOD-RBC in circulation and HOD-Ag levels on the surviving RBC assessed by flow cytometry. HEL-specific IgM and IgG responses were measured by ELISA. Fluorescent HOD-RBCs sensitized vs non-sensitized were incubated *in vitro* with or without macrophages to explore *in vitro* mechanisms. HOD-RBCs were recovered, macrophages were washed and remaining RBCs were lysed. HOD-Ag detection on the recovered erythrocyte surface and the percentage of PKH67+ macrophages, and their PKH67 MFI, were evaluated by flow cytometry. Live-cell confocal microscopy was also performed.

Results: Strikingly, all antibodies capable of causing significant AMIS activity also caused significant *in vivo* Ag loss as well as loss of transmembrane lipids from the RBCs. In contrast, a number of antibodies that induced AMIS did not cause erythrocyte clearance. Further, *in vitro* studies demonstrated that AMIS-inducing antibodies provoked Ag loss in both a macrophage- and antibody-dependent manner. In addition, AMIS antibodies induced *in vitro* RBC membrane transfer to macrophages, as assessed by confocal microscopy. Further, live-cell confocal microscopy revealed that the membrane-fluorescence within macrophages came from RBC nibbling by the macrophages, that is, trogocytosis, rather than phagocytosis of the entire RBC.

Summary/Conclusions: Trogocytosis is a process where an acceptor cell removes or extracts cell surface molecules and pieces of transmembrane lipids from a donor cell and can modulate several biological response, including adaptive and innate immune response. The present work demonstrates the elements of the trogocytosis process under erythrocyte Ag loss induced under AMIS conditions using both *in vivo* and *in vitro* models. We, therefore, propose trogocytosis as a mechanism of antibody-mediated immune suppression.

PA12-L05 | Prophylactic platelet transfusion prior to central venous catheter placement in patients with severe thrombocytopenia: A multicenter randomised controlled trial

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Background: International transfusion guidelines recommend varying platelet transfusion thresholds (20–50 $\times$ 10^9/L) prior to central venous catheter (CVC) placement due to lack of good quality evidence. Ultrasound guidance has made CVC placement safer, while platelet transfusions are scarce, expensive and carry a risk of adverse reactions.

Aims: Is it non-inferior to withhold prophylactic platelet transfusion prior to CVC placement in patients with severe thrombocytopenia?

Methods: Adult haematology ward and intensive care unit patients in 10 hospitals in the Netherlands, with a platelet count of 10–50 $\times$ 10^9/L requiring CVC placement, were randomised to no prophylactic platelet transfusion (experimental group) or one unit of prophylactic platelet transfusion (control group) before CVC placement. CVCs were placed with ultrasound guidance by experienced physicians blinded for the treatment arm. The primary outcome was grade

PA12-L05 - Table 1

	Transfusion ($N = 188$)	No transfusion ($N = 185$)
Median age (IQR)–years	58 (47–65)	59 (50–65)
Female–No. (%)	63 (34)	70 (38)
Median platelet count (IQR)–$\times 10^9$/L	30 (20–38)	30 (20–37)
Median INR (IQR)	1.1 (1.0–1.3)	1.1 (1.0–1.2)
Median aPTT (IQR)–sec	29 (25–34)	31 (26–35)
Median haemoglobin (IQR)–g/dL	8.2 (7.4–9.2)	8.5 (7.7–9.5)
Haematology ward–No. (%)	108 (57)	104 (56)
Dialysis catheter–No. (%)	33 (18)	30 (16)
Tunnelled catheter–No. (%)	20 (11)	18 (10)

2–4 bleeding (i.e., requiring major or minor intervention to stop bleeding, not including <20 min of manual compression, and/or requiring red blood cell [RBC] transfusion). This was a non-inferiority trial, with an expected bleeding incidence of 1% in the control group and a relative risk (RR) non-inferiority margin of 3.5. Secondary outcomes included grade 3–4 bleeding (i.e., requiring major intervention and/or RBC transfusion), transfusion reactions, platelet transfusions within 24 h after CVC placement, and transfusion- and bleeding-related costs. Secondary outcomes were not adjusted for multiplicity and may not be used in place of hypothesis testing.

Results: We included 373 catheter placements in 338 patients. Baseline characteristics were balanced between the groups (Table 1). There was more grade 2–4 bleeding in the no transfusion group (22/185 [11.7%]) than in the transfusion group (9/188 [4.8%]); RR (90% confidence interval [CI]) = 2.5 (1.3–4.7). There was also more grade 3–4 bleeding in the no transfusion group (9/185 [4.9%]) than in the transfusion group (4/188 [2.1%]); RR (95% CI) = 2.4 (0.75–7.9). More platelet transfusions per patient were administered within 24 h after CVC placement in the no transfusion group (0.47) than in the transfusion group (0.14); rate ratio (95% CI) = 3.3 (2.2–5.0). Bleeding risk increased with lower platelet counts. Platelet transfusion rates after CVC placement increased with lower platelet counts, especially in haematology ward patients. There were three allergic transfusion reactions (2 in the transfusion group) and 1 case of transfusion-related acute lung injury (in the transfusion group). Transfusion- and bleeding-related costs were lower in the no transfusion group ($562) than in the transfusion group ($972); mean difference (95% CI) = $410 (285–545).

Summary/Conclusions: Withholding prophylactic platelet transfusion before CVC placement in patients with a platelet count between 10 and 50 $\times$ 10^9/L resulted in more CVC-related bleeding. Bleeding incidences were higher than previously reported in retrospective studies. Withholding prophylactic platelet transfusion resulted in a significant cost reduction. Bleeding risk, scarcity and cost reduction should be balanced in clinical practice.

Parallel Session 13: Platelets… but not as we know them

PA13-L01 | New platelet products - cold stored, cryopreserved and thrombosomes

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Platelets have a short shelf life, which can lead to shortages and wastage. Frozen or cold storage of platelets could extend platelet component shelf-life to weeks or even years, as well as reducing the risk of bacterial contamination. Other approaches, such as lyophilised and synthetic platelets are also being explored.

Pre-clinical *in vitro* studies have evaluated the impact of cold storage and cryopreservation on platelet quality and function, with comparisons to room temperature storage of platelets. Both cold storage and cryopreservation increase the procoagulant activity of platelets, due to increased phosphatidylserine externalisation, generation of more procoagulant microparticles and increased thrombin generation. These features suggest that they may be more haemostatically effective than conventional room temperature stored platelets, and therefore particularly useful for the treatment of haemorrhage and traumatic resuscitation. Similarly, lyophilised platelets, also known as thrombosomes, have high levels of externalised phosphatidylserine that facilitates thrombin generation, and they can adhere to collagen under flow. However, transfusion of these novel platelet products does not always lead to a platelet increment, and while they may be effective in stemming bleeding, they may not be suitable for prophylactic transfusions.

In order to achieve regulatory approval and more widespread availability of these products, clinical trials of cold-stored and cryopreserved platelets are currently in progress. Pilot studies have shown that frozen and cold-stored platelets are effective in reducing bleeding in a number of settings, including cardiac surgery and chaemotherapy-induced thrombocytopenia. Similarly, phase I trials of lyophilised platelets suggest they may be safe and effective. Larger, definitive non-inferiority studies of cold-stored, cryopreserved and lyophilised platelets are now underway.

In this presentation, the advantages and disadvantages of these novel platelet products, together with findings from *in vitro* and clinical studies will be reviewed.

PA13-L02 | Inhibition of cold-induced apoptosis of platelet concentrates better maintains platelet functionality and survival

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Background: Transfusion of platelet concentrates (PCs) is an essential medical approach to treat bleeding or to prevent it. Nevertheless, the standard storage at room temperature (RT) increases the risk of bacterial contamination. We reported that cold-stored PCs have better functionality but reduced survival due to cold-induced apoptosis.

Aims: In this study, we investigated the impact of apoptosis inhibition on platelet functions and half-life during cold storage.

Methods: PCs collected from healthy volunteers were stored for 1, 4, 7 and 10 days at 4°C with or without the apoptosis inhibitor G04 (RhoA GTPase inhibitor). The functionality was assessed: by flow cytometer, testing CD63 and CD62 upon TRAP; by aggregometry and performing an adhesion assay (on fibrinogen coated surfaces). The thrombus formation ability was analysed by thromboelastography and using an *ex vivo* system (Bioflux). Platelet survival was investigated using the NSG mouse model.

Results: We found that upon inhibition of RhoA GTPase the CD63 levels were significantly enhanced (CD63: day 7, $p = 0.035$; day 10, $p = 0.049$) and CD62 expression was comparable to cells stored in buffer. Similarly, a significantly higher aggregation ability in response to both TRAP ($p = 0.038$) and ristocetin ($p = 0.042$) was detected after incubation with G04 on day 10, compared to untreated cells. Moreover, we observed that the presence of G04 better maintained the adhesion ability of cold-stored platelets after 4 days, in comparison to buffer ($p = 0.0493$). Next, the *in vitro* thrombus formation capability was not affected by the apoptosis inhibitor. More importantly, a higher percentage of circulating human platelets, after 7 days of cold storage, was observed in the mouse circulation upon incubation with G04 compared to untreated cells (2 h post injection, $p = 0.0387$, 5 h post injection, $p = 0.0355$). We performed preliminary tests to verify the feasibility of using PCs for *ex vivo* thrombus formation assay. We first analysed thrombus formation from PCs after 24 h at RT under physiological shear stress. Increased thrombus formation upon TRAP stimulation compared to cells treated with buffer was observed suggesting that the *ex vivo* system is a suitable tool to test the contribution of PCs to thrombus formation *ex vivo*.

Summary/Conclusions: Our findings show that the inhibition of cold-induced apoptosis significantly reduces the clearance of cold-stored platelets without impairing the haemostatic functionality of the cells. Therefore, the use of apoptosis inhibitor/s may be a promising strategy to prolong the storage time, improve the platelet survival and reduce the risk of bacterial infection post transfusion.

PA13-L03 | The effect of N-acethyl cysteine on expression of apoptotic and antiapoptotic microRNAs in platelet concentrates during storage

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Background: Platelet apoptosis is one of the most influential agents in platelet storage lesion (PSL) and can affect platelet quality and platelet viability during storage. In this regard, MicroRNAs (miRNAs) are known as the main regulator of mRNA translation in platelets and have a vital role in process of apoptosis during storage.

Aims: This study aimed to evaluate the effect of N-acethyl cysteine (NAC) as an additive to expression of apoptotic and anti-apoptotic platelet miRNA during storage of platelet concentrate (PC).

Methods: In this experimental study, 10 PC was collected and each bag was divided into two equal parts, NAC-treated and NAC free PC. The expression of mir-16 and mir-7 respectively as apoptotic and anti-apototic miRNAs were determined using real-time PCR. Moreover, platelet count and platelet viability using WST-1 method were measured in all samples.

Results: Our findings indicates a lower expression of mir-16 in the NAC-treated PC than in the control group (without NAC), which was significant in terms of expression on days 5 and 7 of storage ($P < 0.05$). Also decreased expression on mir-7 on days 3, 5 and 7 are less in the NAC-treated PC than in the control group ($P < 0.05$). Increased level of platelet count and platelet viability were detected in the NAC-treated compared to the control PC ($P < 0.05$).

Summary/Conclusions: N-acethyl cysteine (NAC) at 1 mM concentration has a protective effect of platelet apoptosis, which reduces the expression of apoptotic associated miRNA and prevents the reduction of anti-apoptotic associated miRNA. For this reason, may be in the future it can be used as an additive in maintaining the quality of PC during storage.

PA13-L04 | Membrane molecular changes associated with platelet cryopreservation

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Background: It is well known that cryopreservation affects platelet quality and function by triggering platelet activation or apoptosis, thereby inducing the release of platelet extracellular vescicles (PEVs). The concept of plasma membrane blebbing leading to the shedding of PEVs is based on a transverse migration of anionic phospholipids such as Phosphatidilserine (PS) from the inner layer to the outer layer of the plasma membrane (Antwi-Baffour et al., 2015). Nevertheless, the biochemical events occurring in the plasma membrane over time after thawing concurring to these phenomena have still not been well investigated.

Aims: The present study aim (i) to evaluate the influence of cryopreservation on membrane phospholipids and protein folding; (ii) to elucidate the roles of molecular structure and conformation in damage to cryopreserved platelets over time after thawing leading to PS exposure and PEVs release.

Methods: We performed a comparative study between fresh-(T0) ($n = 5$), either unstimulated or stimulated with physiological agonist (Thrombin 62 mU/mL), and cryopreserved ($n = 5$) platelet-apheresis units. Fresh components were collected in plasma, analysed within 5 days from collection, whereas cryo-platelets were thawed after 6 months, and analysed at 1, 3 and 6 h after reconstitution in platelet additive solution. Phosphatidylserine externalization and PEVs release were assessed by means of flow cytometry, whereas FTIR spectroscopy (SISSI-Bio beamline, Elettra Synchrotron) was exploited to evaluate the influence of cryopreservation on membrane phospholipids and protein folding. FTIR data were analysed with a univariate approach by evaluating the ratios between the absorbance of significant peaks to obtain data on membrane stiffness (CH_2/CH_3 ratio); protein content (protein/lipid ratio); Peroxidation processes C═O/lipids and C═O/proteins. After that a Principal Component Analysis (PCA) was performed to understand the key grouping variables in the data and spot outliers. ANOVA with

multiple comparisons test was performed to assess statistical significance among different samples.

Results: Our data showed an increase of PEVs in cryopreserved units with a 38-fold increase of EV concentration after 1 h from thawing with respect to the fresh unstimulated samples (overall ANOVA $p < 0.05$). In accordance, 1 h thawed samples presented an increase of PS signal compared to fresh components (36.5 ± 4.2 vs. 77.0 ± 8.4%, $p < 0.0001$). Although the difference with fresh samples was still statistically significant, this increase tended to be reduced over time after thawing (3 and 6 h). Similar to PS exposure, PEVs release decreased over time. CH_2/CH_3 ratio increased as the time after the thawing grows; T0, 1 h and 3 h values are significantly different, whereas 3 and 6 h are not. Protein/lipid ration indicate a loss of protein whereas both C═O/lipids and C═O/proteins increase in time after thawing; again 3 and 6 h are no longer discriminable. Finally, PCA revealed an overlap of fresh and 1 h samples that can be assigned to proteins in α-helix conformation while data at 3 and 6 h after thawing are separated from those at T0 and 1 h and can be assigned to proteins in β-sheet conformation, (higher in samples at 3 and 6 h).

Summary/Conclusions: The data herein reported demonstrates how time after thawing affects membranes' functional characteristics and integrity. The decreases in PS-positive platelet counts and of the EVs release over time after thawing mirrors the increased peroxidation process and misfolding of phospholipid bilayer proteins seen by FTIR analysis and account for the alteration of membrane integrity and stiffness, which occur irreversibly 3 h after thawing.

PA13-L05 | Cryopreserved platelets in a novel non-toxic DMSO-free setting maintain haemostatic function in vitro

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Background: Cryopreserved platelets (CPs) that can be stored for several years may be important in the management of bleeding in remote hospitals and in preparedness plans. The original method containing dimethyl sulfoxide (DMSO, Me2SO) as a cryoprotectant agent (CPA) was developed in the 1970s and has become the standard routine for platelet cryopreservation. Various alternative CPAs have been tested without greater success including propane-1,2-diol, glycerol cocktails with hydroxyethyl starch and dextran, trehalose or trehalose combined with phosphate, along with other mixtures containing a second messenger like ThromboSol1 or epinephrine.

Aims: The aim of this study was to evaluate hypertonic saline as a non-toxic medium for cryopreservation of platelets, on *in vitro* parameters.

Methods: In a paired study design, double-dose buffy coat platelets were divided into Reference ($n = 10$) and TEST ($n = 10$). A mixture of 25% DMSO/NaCl (50 mL) was added to the Reference platelet concentrates, while DMSO was excluded and 100 mL NaCl (9 mg per mL) was added to TEST units. The final volume in each freezing bag was approximately 10 mL platelet suspension, with 5% DMSO in the Reference group. Both units were immediately frozen at −80°C, stored for 2–4 months and thereafter thawed and reconstituted in compatible fresh plasma to a total volume of 200 mL. Analyses, including cell count, metabolic, phenotypic, and functional properties of the platelets and viscoelastography to assess the haemostatic function of the final product, were done pre-freezing and 1 h after thawing.

Results: After thawing, all CPs showed several biochemical and ultrastructural changes as compared to the pre-freezing data. In DMSO-free units platelet recovery was 87.4% ± 8.6%. This was significantly better ($p < 0.001$) compared to cryopreservation with DMSO (recovery 69.6% ± 6.1%). Minor differences in metabolic and phenotypic parameters were found, but all were within acceptable values. With exception of glycoprotein Ib, IIIa, VI and PECAM-1 ($p < 0.01$), no significant differences were found in the phenotypic comparison between the DMSO-free group and conventional DMSO freezing. Thus, no significant difference in activation level or in the response to various agonists were observed. The mitochondrial membrane potential was significantly reduced ($p < 0.001$) in DMSO-free products compared to DMSO (JC-1+ 35.3% ± 8.5% vs. JC-1+ 61.2% ± 11.9%). However, in a new set of experiments using controlled freezing equipment ($n = 6$), the platelet viability was on average 16.3% ± 5.8% higher ($p < 0.001$). DMSO-free platelets showed no significant differences in haemostatic function regarding clot formation time (119.9 ± 24.6 vs. 168.8 ± 102.6, $p = 0.175$) and slight significance in clot firmness (28.0 ± 3.6 mm vs. 31.5 ± 3.5 mm, $p = 0.034$). By reducing the reconstituted plasma volume in the DMSO-free units resulted in 20.5 s ($p = 0.031$) faster clot formation time and 8.5 mm ($p < 0.001$) expansion in maximal clot firmness.

Summary/Conclusions: We have shown that platelets cryopreserved in hypertonic saline have high recovery and maintain haemostatic function in vitro. They display only minor differences compared to platelets frozen with DMSO, proposing a novel non-toxic freezing alternative. Controlled freezing equipment is required to optimise the product quality. A DMSO-free method may increase the use of cryopreserved platelets for transfusion.

Parallel Session 14: Transfusion meets Implementation Science

PA14-L01 | Implementation of a national non-invasive foetal DNA testing programme

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Non-invasive foetal DNA testing can be used to analyse foetal genetics just by using a standard blood sample from a pregnant woman. One application is predicting foetal RhD in RhD negative pregnant women, which serves as the first application of cell-free foetal DNA testing to be implemented for clinical use.

As a screening programme, antenatal *RHD* screening guides the rationale use of antenatal and postnatal anti-D prophylaxis, restricting the administration of anti-D immunoglobulin only to those women who will benefit from it, and thus avoiding unnecessary use of and exposure to anti-D immunoglobulin in up to 40% of the RhD negative women.

We implemented this programme in Denmark in 2010, running two years with detailed investigations of discrepancies between predictions based on foetal DNA and postnatal serology, followed by a large evaluation of all the five regions in Denmark and the performance of the antenatal *RHD* screening setup.

High performance data led to termination of postnatal cord blood testing, as well as a more simplified screening setup. Over the years, various minor programme adjustments have been made. Our programme is now ISO1589 certified.

An expert group formulated recommendations for validation and quality assurance of this method.

Implementing other assays, for example for immunised women immunised against other antigen targets than RhD, is less straightforward due to the limited availability of samples, restricting the options for large validation testing. Another route for such setups may be required.

PA14-L02 | Impact of the malware attack on serious adverse events in the Republic of Ireland

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Background: On the 14th of May 2021 the Health Service Executive (HSE) of Ireland suffered a major ransom-ware attack which forced its information technology (IT) systems nationwide to be shutdown. HSE Hospitals around Ireland reported being unable to access electronic files and electronic databases, such as the Laboratory Information Systems (LIS), and the REES temperature monitoring systems. The HSE uses the electronic identification systems 'Blood Track' (EBTS) and the Cerner Maternity Neonatal Clinical Management System (MNCMS) to aid the sampling process and reduce the occurrence of wrong blood in tube (WBIT) errors.

Aims: The aim of this study was to examine the impact that the 2021 ransom-ware attack had on adverse events in transfusion.

Methods: In Ireland transfusion related adverse events and reactions are reportable to the National Haemovigilance Office (NHO). Adverse event reports reported to the NHO and accepted for analysis from 14 May 2021 to December 2022 were retrospectively reviewed. Database searches were performed using search terms 'Malware' and 'cyber-attack'. Interpretation was subjective with a single reviewer determining whether the case met inclusion criteria. Qualitative analysis of the free text narrative in reports using thematic coding was used to identify error trends.

Results: The NHO received 10 SAE reports which cited the cyber-attack as a contributory factor in the error that occurred. Storage errors due to insufficient temperature monitoring as a result of the REES temperature monitoring system being unavailable was cited in 8 reports. Failure to give an irradiated component as a result of being unable to check patient's history on LIS was cited in 2 reports.

The NHO received 5 Near Miss reports associated with the cyber-attack. Deviations in Storage were cited on two reports; Issue on one report and other on the remaining two reports. Two near miss reports were caused when staff failed to verify labels on units correctly. EBTS is used routinely to alert staff of incompatibilities and expiration dates. In one case a sample was handwritten incorrectly because EBTS was unavailable.

The NHO received three WBIT reports which cited the cyber-attack. All three cases occurred at the sampling stage of the transfusion process and were a direct result of EBTS or MNCMS being unavailable due to the cyber-attack. In all three cases the sample was taken from the wrong patient and labelled as per the intended patient's details. The NHO also received a WBIT notification that details on a request form were incorrect as a result of EBTS being unavailable due to the cyber-attack. This case was not accepted as the NHO do not collect WBIT cases where details on the request form do not match the details on the sample

Summary/Conclusions: There is limited information available on the consequences and effects of ransom-ware attacks experienced by healthcare facilities and healthcare bodies. The NHO identified 18 adverse events associated with the May 2021 malware attack. Ten of these events were serious adverse events that led to inappropriate blood or blood components being issued/distributed for clinical use. The Near Miss and WBIT events led to the issue of inappropriate blood or blood components but not the subsequent transfusion of the units. Sampling processes where electronic identification systems are routinely used are at risk of an increase in WBIT events in the event of a malware attack. Laboratory information systems and REES temperature monitoring systems are also areas of weakness in the transfusion process during malware attacks.

PA14-L03 | Implementation of an electronic vein- to- vein reporting tool in Ethiopian blood and tissue bank service; 2021–2022 review

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Background: The blood transfusion service in Ethiopia is provided through the national blood bank service centre and its 42 regional blood banks and collection centres across the country. A total of 561 Health facilities receives safe blood and blood products from these blood services to save the lives of their patients.

The national centre receives regular monthly reports and generates periodic reviews to monitor the blood service and availability of blood

units and their use. Since almost all of the blood banks used manual or paper based reporting the quality and availability of timely data has been a great issue.

Aims: An online *electronic vein-to-vein reporting tool* was developed by the national centre in January 2021. The aim of the system was to make easily available online portal for the managers and data clerks working at regional blood banks in Ethiopia.

Methods: The blood bank conducted an analysis of the different reporting requirements based on the ministry of health and international blood safety data elements. A detailed requirements was prepared based on these data elements and a new web-based reporting portal was developed. A total of 105 staff from all blood banks (Blood bank Managers, Data clerks, regional Health Bureau focal points) were trained on the reporting tool in February 2021 in Four rounds. The tool was refined and validated based on the feedbacks during the trainings launched for use starting March 2021.

Results: After implementing the tool, the blood bank was able to receive reports from 95% of all functional sites within 5 days of closing of each month. The Blood bank also was able to analyse the reports for monitoring the performance of each site based on the forty seven key performance indicators for the strategic plan period of the service. These key performance indicators were more readily available now and can easily be shared with concerned stakeholders and the ministry of Health in a timely manner. Feedback from health facilities related to blood transfusion has also improved. Feedback on blood utilisation which was hard very low and hard to get was recorded at 45% in 2021 at the beginning of using the tool. In end of 2022 this number has increased to 66%. The new tool also allowed the service to monitor new indicators that were hard to track using the previous manual system. These include having regular data related to donor deferrals, adverse transfusion events, critical supplies stock status, post donation counselling services coverage and related data.

Summary/Conclusions: The implementation of the *electronic vein-to-vein reporting tool* has created a good opportunity to make available timely and quality data from all blood banks and health facilities. The national centre is utilising the system to enhance its national coordinating capacity and strengthening of the national monitoring evaluation system in blood transfusion in the country.

PA14-L04 | Usage and wastage of rare frozen red cells: An audit of outcomes of thawed and washed units from the UK's national frozen blood bank

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Background: The role of the UK's National Frozen Blood Bank (NFBB) is to provide rare blood when no compatible units can be sourced from the liquid inventory and clinical urgency does not allow donor call-up. The auditing of request and outcome data helps to ensure optimal and safe management of this limited and expensive resource.

Aims: All units issued by the NFBB over a two year period (2020/2021) were in scope for this audit to establish trends in requesting patterns, indications for transfusion, ultimate unit outcome, haemoglobin increments and adverse events.

Methods: This was done through collating and analysing data returned via a response form, and supplemented by information provided through direct contact with hospital transfusion laboratories.

Results: Over the 2-year period there were 78 requests for a total of 170 units. Response forms were received for 61.8% of requests with variably complete data. Overall, 73.5% of units were transfused to the intended recipient, with the rate being significantly higher in 2020 (83.9%) than 2021 (61.0%). 14.1% of units across the audit were discarded. Obstetric indications accounted for only 10% of units requested but 88.2% of these were not transfused to the intended recipient. Medical requests outnumbered other indication areas in both years (55% of all requests) with the lowest percentage of units not used for the intended recipient (17.3%). There were 29 cases where haemoglobin increment data were sufficient for analysis and deemed eligible for inclusion. In 69.0% of these cases a haemoglobin increment of at least 50% of expected (arbitrarily set at a rise of 10 g/L or more per unit transfused) was demonstrated. Adverse event data were provided infrequently, with three response forms reporting on increased haemolysis/hyperhaemolysis following transfusion, and one case of incompatible crossmatch at the hospital transfusion laboratory.

Summary/Conclusions: This audit provides an overview of demand and use of rare frozen units from the UK's National Frozen Blood Bank. The process for managing outcome information had been changed after a previous audit. Data suggests improvements compared to previous audits with regards to response forms received (43% of response forms were returned during 2016 to 2018), as well as usage of issued frozen blood for the intended patient (45% of units during 2016 to 2018). Wastage remains a significant area of concern, with the non-use of requested frozen blood for patients with obstetric indications particularly noteworthy. This data will help to inform ongoing service delivery and development of guidance for appropriate requesting and usage of rare frozen units.

PA14-L05 | Assessment of personal digital assistant usage in transfusion bedside checks

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Background: Human error is a factor in transfusion medicine that needs to be eliminated but remains challenging. Several processes and personnel are involved, and thus, errors can occur at any point. One critical checkpoint is patient identification when administering the blood. This step essentially ensures that the right blood is transfused

to the right patient. The bedside check is the last opportunity to stop an incorrect transfusion. When an error occurs during bedside checks, it can lead to disastrous outcomes. Many previous reports recommend using a personal digital assistant (PDA) during bedside checks to reduce human errors. Our institution has been using PDAs for pre-transfusion bedside checks since 2018. Medical staff scan the barcodes on a patient's wristband and blood product to ensure a match. Although PDAs may reduce the incidence of incompatible transfusion events, they are only safe when used accurately.

Aims: We aimed to check the status of PDA use in bedside check at our institution and analyse how much it prevented blood transfusion accidents. We analysed the PDA data from the hospital's electronic medical records (EMR) and monitored the bedside transfusion practices of the nursing staff, including their use of PDAs.

Methods: We analysed the PDA use for bedside checks and mismatch data from November 2021 to July 2022. PDA use rate was defined as the number of transfusions that used PDA for bedside checks divided by the number of transfusions. PDA mismatch rate was defined as the number of mismatches divided by the number of transfusions. The bedside transfusion practices of the nursing staff were monitored from January 24, 2022, to April 30, 2022, using a checklist.

Results: Among the 39,250 transfusions administered, 37,271 (94.96%) used PDAs during bedside checks. Sixty-three mismatches were detected over nine months. The PDA mismatch rate was highest in the paediatric haematology and oncology department. Among the 63 mismatches, 13 (20.63%) were ABO-incompatible red blood cell transfusions. A total of 201 bedside checks were observed during the 3-month monitoring period. Forty-seven cases failed to check full peri-transfusion vital signs. In nine cases, the transfusion was not started within 30 min of the issue from the blood bank.

Summary/Conclusions: More than 90% of transfusions used PDA and we identified 63 near-miss events that had not been previously reported in our transfusion management division. Reviewing PDA data allowed identification of the frequency of PDA use, recognition and analysis of underreported near-miss events in our institution; which will aid in preventing incompatible blood transfusions and implementing corrective measures.

Parallel Session 15: Factors affecting supply

PA15-L01 | Individual risk-based donor screening

A Lewin

Abstract not available.

PA15-L02 | Mixed-methods exploration of the knowledge of young adults about blood donation processes; a one-center cross-sectional study in Cape Coast, Ghana

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Background: Despite having a majority youthful population, sub-Saharan Africa is plagued with inadequate blood supply. To improve blood donor retention and blood supplies, understanding the perspectives of potential blood donors are important considerations.

Aims: To explore the perspectives, and experiences of young adults on blood donation processes.

Methods: This descriptive cross-sectional exploratory study employed the mixed-methods approach (semi-structured questionnaire and focus group discussion, FGD). A convenience sampling technique was used to recruit 382 young adults (aged 18–49 years). The data collection was sequential; the questionnaire distribution was completed before the FGD commenced; themes that emerged from the questionnaire responses guided FGDs. All statistical analyses were undertaken using the two-tail assumptions; $p > 0.05$ was considered statistically significant.

Results: Overall, whereas the majority (79.3%) of the participants were in their twenties, less than a third (31.7%; 127/382) had previously donated blood. Overall, less than one-third of participants could correctly identify the minimum weight (26.4%), or the inter-donation interval (14.7%); 37.4% and 58.1% could indicate the required donor age or ≥3 infectious agents screened for prior to blood collection. Among previous donors, 37.2%, 28.1% and 43.0% could identify the required weight, acceptable inter-donation period, and donor age respectively. Attitude-wise, although two-thirds of participants indicated willingness for voluntary unrelated donations, a-third preferred paid donations. Whereas 42.4% of participants indicated that blood donation has intrinsic health benefits, 17.0% suggested that blood donation was associated with disease risks. Thematic analysis of the FGDs found that both previous donors and non-donor groups consider lack of education, fear of post-donation health issues and lack of privacy as main hindrances to donor recruitment.

Summary/Conclusions: Targeted intentional intensive educational campaigns are warranted to address the knowledge gap on blood donation processes among young adults' study population.

PA15-L03 | Novel reagents for screening of haptoglobin-deficient or IgA-deficient donors

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Background: Haptoglobin (Hp)- and IgA-deficiency are reported in about 1 in 4000 and 1 in 14,000, respectively, in the Japanese population. The individuals with Hp- and IgA-deficiency may produce anti-Hp or anti-IgA through allo-immunization, leading to serious transfusion reactions such as anaphylaxis. Therefore, implementation of systematic supply of Hp- or IgA-deficient fresh frozen plasma (FFP) is an important issue.

Aims: We aimed to develop novel reagents for the easy and large-scale identification of individuals with Hp- and IgA-deficiency, as potential donors of FFP.

Methods: We established mouse monoclonal antibody-producing cell lines by the hybridoma method, by immunizing mice with Hp and IgA recombinant proteins. Mouse monoclonal antibodies were purified by protein-A Sepharose from ascitic fluid of mouse injected with each hybridoma cell line. Purified antibodies were conjugated with carboxylate-modified polystyrene latex beads, and applied for Hp and IgA measurements by the Latex agglutination method using an automatic analyser (LABOSPECT008). Samples with low protein concentrations were re-examined by ELISA for confirmation. To confirm Hp gene deletion, genomic DNA was extracted from peripheral blood leukocytes, and amplified the Hp gene by PCR using specific primers (H-del-U, H-del-L, H-Ex1-U and H-Ex1-L).

Results: From February 2022 to March 2023, in Kanto-Koshinetsu block blood centre, 7476 and 24,977 donors were screened for Hp and IgA, respectively. Two Hp-deficient, 21 low Hp expression, and 4 IgA low expression individuals were identified. Genomic analysis of the two Hp-deficient donors revealed Hp gene deficiency.

Summary/Conclusions: Supply of Hp- and IgA-deficient FFP is essential for the safety of blood transfusion to this group of deficient individuals, which can be achieved by a registry of blood donors. Our reagents proved be effective for the easy and large-scale screening of the donor population, and they will be also of interest to other countries, especially in Asia, where Hp-deficiency is present in relatively high rate.

PA15-L04 | Sustainability impact as a motivator of unremunerated blood donor recruitment

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Background: There are immediate, pressing issues endemic across the majority of US blood banks. Staff shortages, diminishing retention, critical product disruption from fragile supply chain and increasingly endangered donor base (−47.7% in donors under 30, last 10 years—internal data). Regarding the latter, the low rate of voluntary, unremunerated donors under the age of 40 (U40) is particularly worrisome. The need for a consistent, age-diverse donor population and the impact of a lack there of have also been well-characterised in the international literature. Like blood donation, climate change and its impacts on the global environment, economy, and public health landscape create an inherently social issue. Given these circumstances, there is an opportunity to build on literature regarding social impact subsets such as sustainability impact and its role as a motivator of voluntary, unremunerated blood donation. Here we present comparison data from a sustainability-themed blood donor recruitment campaign.

Aims: The aims of this comparison were: (1) to evaluate the impact of a sustainability-themed blood donor recruitment campaign ("One Donation, One Tree") in a cooperative of independent BCOs and (2) gain more visibility into future expansion of this and other sustainability impact efforts globally.

Methods: The cooperative and an ecosystem restoration start-up company executed an agreement under which one tree would be planted in Uganda for one whole blood collection from participating donors. To minimise seasonal influences and donor base variation a participating BCO (PBCO) was matched to a peer (size, footprint, population) nonparticipating BCO (NBCO). Standard donor recruitment initiatives were employed at both BCOs with the PBCO having access to recruitment materials in support of the sustainability campaign. Deidentified data was compiled over the course of 1 month (1–31.10.22). Data was grouped by age group, donors and first-time donors, respectively. Statistical analysis was performed.

Results: During the study period PBCO received 6125 donors (1222 or 20% first-time donors). In the PBCO U40 donor category, there were 1709 donors (28%); 46% (793/1709) of these were first time donors. The NBCO received 3202 donors (633 or 20% first-time donors). In the NBCO U40 donor category, 36% or 370 / 1032 total U40 donors were first-time donors. The differences between PBCO and NBCO U40 first-time donors as proportions of U40 donors in total ($\Delta = 10\%$) and all first-time donors ($\Delta = 7\%$), respectively were significant ($p < 0.01$). The difference in proportions of PBCO 20–24 years old first-time donors (57%; 183/320) and NBCO 20–24 years old first-time donors (37%; 64/175) was significant ($p < 0.01$). As proportions of all first-time donors at their respective sites, the difference between PBCO 20–24 years old donors (183/1222) and NBCO 20–24 years old donors (64/633) was also significant ($p < 0.01$).

Summary/Conclusions: These results demonstrate significant impact of a novel, month-long, sustainability-themed recruitment campaign on U40 unremunerated blood donor behaviour in a cooperative of independent BCOs. Within the U40 age group, significant impact was seen specifically in the donor age group of 20–24 years old. The overall project resulted in a total of 212,491 trees being planted across three districts in northern Uganda, employed over 300 Ugandan households (60% female), and achieved a total offset of 13,451 tons of CO_2 emissions over the next 5 years. Taken together, wider data analysis over a longer time period may more fully assess.

PA15-L05 | Crisis donors as a short-term relief or long-term solution? A follow-up of blood donors who registered to donate during the COVID-19 pandemic

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Background: In times of crises the blood supply still requires donors, and individuals are often more willing to become donors. Despite the lockdown measures during the beginning of the COVID-19 pandemic when people were urged to minimise travelling and encouraged to stay at home, there was a huge influx of newly registered donors in the Netherlands (Spekman et al., Transfusion, 2021). However, it is unclear whether such 'crisis donors' are willing to become regular donors; it is often suggested that these are one-time donors.

Aims: The aim of this study was to compare the donation behaviour in the first 2 years after registration of the newly registered 'crisis donors' to newly registered donors in previous years.

Methods: We followed up all donors that registered within the first phase of the pandemic (week 11–20 in 2020) and retrieved their donation information from eProgesa (the blood bank database) for a follow-up period of two years. For all donors, we checked whether they visited a donation centre for the new donor examination (i.e., an exam to determine the eligibility of new donors), and the first-time donation (which is typically a whole blood donation) and retrieved information about their complete donation history including deferrals in the follow-up period. These data were analysed and compared with data from donors that had registered in the same period in the previous 3 years.

Results: In week 11 to 20 of 2020 almost 27,000 new donors were registered, almost as many as in the preceding three years together in that same period. Of the newly registered donors in 2020, 80% of donors came to a blood bank location for the new donor examination and 60% made a first donation (attempt) within the two-year follow-up period, which is comparable to the previous three years. Of the donors that did come in for a new donor examination, 74% also visited a blood bank for their first-time donation. More than half (56%) of the donors who came in for a new donor examination but did not return in the follow-up period were deferred at least once; 52% of them were deferred for a year or longer, which is also in line with the preceding years. At the end of the follow-up period, donors that had a new donor examination visited the donation centre on average 3.7 times (including the new donor examination). This did not significantly differ from any of the preceding 3 years (3.8 in 2017 and 2018, and 3.6 in 2019).

Summary/Conclusions: In 2020, more donors registered than prior to the pandemic, probably due to a message of shortage of blood products that was picked up by many media. Newly registered donors in 2020 had comparable show rates for their new donor examination and first-time donation as those in previous years. The call for (COVID convalescent) plasma donors may have influenced the results for 2019 and 2020. However, these results suggest that people who respond to donation appeals in (temporary) crisis situations are not more likely to drop out than newly registered donors in the preceding years.

Parallel Session 16: Diving deep into blood groups

PA16-L01 | The new Er blood group system: *PIEZO1* and its role in red blood cell physiology

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The high prevalence red cell antigen Er^a and antithetical antigen Er^b, were identified in the 1980s, and together with Er3, were recognised as the Er collection (208) by ISBT in 1990. However, the molecular background of these antigens was yet to be discovered until now.

At IBGRL, Bristol, UK, we used whole exome and Sanger sequencing of individuals with serologically defined Er alloantibodies and identified several missense mutations within *PIEZO1*, a large gene comprised of 51 exons and located at 16q24.3. All the observed mutations encoded amino acid substitutions within the extracellular domain of the Piezo1 mechanosensor ion channel, a large transmembrane protein known to translate mechanical forces into the electrochemical signals through cell membranes, linked to red blood cell volume regulation and a variety of somatosensory cellular events. The importance of Piezo proteins in human physiology was recently recognised by the award of the 2021 Nobel Prize in Medicine and Physiology for their discovery.

In our study, we confirmed that Piezo1 was the carrier molecule for the Er blood group antigens through immunoprecipitation experiments, CRISPR/Cas9-mediated gene knockout and expression studies in an erythroblast cell line BEL-A. *PIEZO1* mutant human erythroid BEL-A cell lines carrying individual Er-related mutations were tested by flow cytometry using different human anti-Er sera. In total, we have identified and confirmed the molecular bases of five Er blood group antigens: the already recognised Er^a (ER1), Er^b (ER2) and Er3 (ER3) and two novel high incidence antigens ERSA (ER4) and ERAMA (ER5). The antigens were associated with the following changes in Piezo1: p.Gly2394Ser (Er^a/Er^b), p.Glu2392Lys (Er(a-), Er3-),

p.Glu2407Gln (ERSA-), p.Glu2407Lys (ERSA-), p.Arg2245Gln (ERAMA-). These five antigens with their fully described molecular bases were ratified by the ISBT as the new Er blood group system (043) in 2022.

The lack of the high prevalence Er3 antigen, originally thought to be a potential Er null phenotype, did not appear to be associated with a true null phenotype, but represented lack of both Er^a and Er^b antigen expression, thus a question remains whether a true Piezo1 null is compatible with life. The protein is linked to a number of hereditary diseases and *PIEZO1* is highly polymorphic, bearing rare loss-of-function mutations (encoding Generalised Lymphatic Dysplasia of Fotiou), more common gain-of-function mutations (encoding Dehydrated Hereditary Stomatocytosis), and a number of additional non-pathogenic single nucleotide variants. Interestingly, two Er-associated mutations in this study, Gly2394Ser, the antigenic site of Er^a/Er^b, and Glu2407Gln, associated with ERSA antigen expression, have been previously reported as pathogenic. However, apart from anti-ERSA and anti-ERAMA being implicated in severe haemolytic disease of the foetus and newborn, we found no evidence from Er literature or the patients tested in our study, of any RBC pathology or morphological abnormalities. To investigate this further, we showed that in vitro patch clamping, on mutant BEL-A cells, indicated no differences in Piezo1 channel activity.

The polymorphic nature of *PIEZO1* will inevitably result in more variants being discovered and the overlap between the known hereditary diseases and the blood group antigens will need to be investigated further.

PA16-L02 | Elucidating the blood group regulome

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Identifying blood groups correctly is essential in matching for blood transfusion and organ transplantation. Antigens are generated by variation in the blood group genes and expression levels of antigens on red blood cells (RBCs) are modulated by regulatory elements and the associated transcription factors (TFs). Antigen expression may be altered via mutations in the TF genes themselves or through changes in the TF motifs, the DNA sequences that TFs recognise and bind to. Variations in these motifs have previously been found to abolish or reduce expression levels of certain blood groups. A well-known example of abolishment is the Duffy blood group system where disruption of the GATA1 motif in the promoter region of the *ACKR1* gene results in the Fy(a–b–) phenotype. Another example of weakened expression can be found in the ABO system where mutations in the GATA1 and RUNX1 motifs of *ABO* intron 1 lead to weak A or B antigen expression.

High throughput sequencing technology has facilitated genome-wide studies, enabling us to utilise public datasets and process those datasets through bioinformatic pipelines. We have developed and used a pipeline to identify erythroid TF motifs in blood group genes, and to validate predictions of gene regulatory elements in *in vitro* experiments to better understand the roles of TFs in the blood group regulome that lead to variable antigen expression levels.

Four GATA1 ChIP-seq datasets from adult primary erythroid cells were analysed using known GATA1 motifs in *ACKR1*, *ABO* and *XG* as positive controls. We identified 193 GATA1 binding motifs associated to blood group genes, including a motif in the *RHD* promoter which has been reported in a weak D case. In the *CR1* gene of the Knops blood group system, multiple GATA1 motifs were predicted. Their potential association with the extremely reduced level of CR1 protein, resulting in the Helgeson phenotype, was further investigated since the underlying genetic mechanism was unclear. For the two GATA1 motifs in *CR1* intron 4, *in vitro* assays confirmed that GATA1 enhances transcript levels in both wild-type sequences, compared to disrupted motifs where GATA1 binding was abolished. The minor allele of rs11117991:C disrupts the motif and, in Swedish and Thai rs11117991:C/C samples, CR1 mRNA and antigen expression levels were reduced in quantitative PCR and flow cytometry. These findings led to a proposed regulatory mechanism for the Helgeson phenotype.

Nevertheless, the 193 predicted GATA1 targets remain daunting to validate *in vitro*. To increase the credibility of predicted targets, we further expanded the datasets used to include: (1) other major erythroid TFs such as KLF1, RUNX1, NFE2; (2) histone markers such as H3K4me3, H3K4me1, H3K27ac which provide promoter and enhancer location indicators; and (3) chromatin accessibility (assay for transposase-accessible chromatin with sequencing; ATAC-seq). The inclusion of multiple TFs also allows us to study the interactions and relationships between TFs.

In conclusion, layering sequencing data for TFs, histone markers and open chromatin regions allows us to study the regulome in a systematic manner. For blood groups with observed quantitative polymorphism, these predicted targets may facilitate the search for the mechanisms underlying antigen expression variability.

PA16-L03 | A deep learning approach to the genetic prediction of blood group antigens

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Background: Deep Learning (DL) techniques have made great advances in recent years by utilising very large training sets. Next generation sequencing techniques and digitalization of biological data

have revolutionized the field of biology with access to large datasets. The next logical step is the marriage of the latest DL techniques and large biological datasets.

Since the discovery of ABO over a century ago, 43 blood groups were discovered, covering 349 antigens. Due to their clinical significance in blood transfusion, testing for certain blood types, such as ABO and RhD, is ubiquitous. However, this leaves the remaining blood groups as either not consistently tested everywhere, or not tested at all unless special needs arise.

The cost and time associated with performing serological or custom-made PCR testing for every phenotype in each blood group hinders widespread testing. However, a comprehensive dataset of blood phenotypes is invaluable in circumstances that require blood from donors with rare blood types or rare combinations. This can be particularly challenging if the blood group of interest is not routinely tested.

Comprehensive phenotypes are also useful for general improvement of matching donors to recipients to avoid immunization and delayed transfusion reactions, especially for patients with chronic transfusion needs.

Aims: In this study, we aim to apply such techniques to develop blood type prediction models based on cheap-to-analyse and easily scalable screening array genotyping platforms.

Methods: Combining existing serological or genotyped blood types from blood banks and imputed screening array genotypes for ~111,000 Danish and 1168 Finnish blood donors, we used Deep Learning techniques to train and validate blood type prediction models for 32 antigens in 13 blood group systems, HPA-1a/b and secretor status. To account for missing genotypes a Denoising Autoencoder initial step was utilised, followed by a Convolutional Neural Network blood type classifier.

Results: All (A, B, A1, Coa, Doa/Dob, Fya, HPA1a, HPA1b, K/k, Jka/Jkb, Lua/Lub, M/N, S/s, C/c, D/D-weak, E/e, Se, Vel, Yta, Ytb) but seven prediction models demonstrated an overall prediction accuracy above 99%, with two coming close with accuracies of 98.8% (P1) and 98.7% (Fyb). The Lewis blood group coming next with an accuracy of 97.8% (Lea) and 97.9% (Leb), and the bottom scoring three antigens (Kpa, Cw, Cob) achieving 92.9%, 92.8% and 92.6% respectively. Models for antigens with low or high frequencies like for example, Cw, small training cohorts like for example, Cob, or very complicated genetic underpinning like for example, RhD proved to be more challenging for high accuracy (>99%) DL modelling. However, in the Danish cohort only 3 of 36 models (Cob, Cw, Kpa) failed to achieve a balanced overall prediction accuracy above 97%. The high predictive performance in the Danish training cohort was replicated in the Finnish cohort.

Summary/Conclusions: High accuracy in a variety of blood groups proves viability of applying Deep Learning to genetic blood type prediction using routine phenotypic and array chip genotypic training sets, even in blood groups with nontrivial genetic underpinnings. These techniques are suitable for aiding in identifying blood donors with rare blood types by greatly narrowing down the potential pool of candidate donors before clinical grade confirmation. Replication of high predictive performance in an external genetic cohort other than the Danish training cohort proved the viability of DL models in external genetic datasets.

PA16-L04 | Intriguing outcomes from Nanopore sequencing of two cryptic A3 samples: A case of blood group mosaicism and a novel regulatory variant in the ABO system

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Background: Mixed-field agglutination (MFA) in ABO phenotyping (A_3,B_3), that is, when two separable cell populations are present in agglutination tests with anti-A or anti-B, is mostly linked to rare variants in *ABO* exon 7 and regulatory regions. Alternatively, MFA could be observed because of chimerism (cells derived from different zygotes, e.g., due to bone marrow transplantation) or, in exceedingly rare cases, true mosaicism (genetically different cell lines derived from the same zygote, e.g., due to somatic mutations in stem cells). Elucidating the genetic cause of MFA is technologically challenging. Incomplete knowledge about regulatory regions further hinders resolving unexplained cryptic ABO phenotypes. Latest long-read sequencing has great potential in this regard by enabling complete gene haplotype sequencing to a high read depth, even allowing the detection of subclonal variants at low variant allele frequencies (VAFs).

Aims: We used long-read sequencing by Oxford Nanopore Technologies (ONT) to resolve two cases with cryptic ABO phenotypes (A_3 and A_3B, respectively).

Methods: ABO phenotypes were determined by standard serological methods, including anti-A1 and anti-H specific agglutination. PCR-SSP kits (inno-train, Germany) were used for *ABO* genotyping. Expression of A-, B- and H-antigen was measured by flow cytometry. The entire *ABO* gene was amplified by two overlapping long-range PCR fragments of ~13 kb each. PCR-products were sequenced with ONT on a MinION flow cell to a read depth >1000×. Result confirmation was derived from multiple lines of evidence, including analysis of presence of chimerism and mosaicism by digital PCR (STILLA, France), Sanger sequencing of the region of interest, and family analysis where applicable.

Results: Blood cells of the first case, genotyped AO_1 and presenting A-antigen on ~80% of erythrocytes, showed strong reactions with anti-A_1 and anti-H, pointing to very distinct cell populations. Nanopore sequencing depicted a subclonal 3-bp insertion in exon 6 (VAF ~10%). The variant was only present on *ABO*A* haplotypes and translated into a frameshift (Tyr126Ilefs*69) causing a null phenotype in the affected cells. Allele-specific Sanger sequencing confirmed the insertion and no chimerism was detected. Instead, we suggest the uncommon case of mosaicism as a digital PCR approach quantified VAF at ~14% and a blood sample donated 10 months later pointed to a robustly affected cell proportion (VAF ~17%). The second case, a A_3B sample, showed weak and absent agglutination with anti-H and anti-A_1, respectively. Almost 60% of erythrocytes carried only B

antigen. Nanopore sequencing revealed a novel heterozygous g.10924C>A variant phased to the *ABO*A*-allele in a known transcription factor binding site for RUNX1 in intron 1 (+5.8 kb site). Sanger sequencing confirmed this variant, and its inheritance was proven by analysing the donor's mother, who shared the anti-A MFA.

Summary/Conclusions: Here, we disclose an exceptional case of genetic ABO mosaicism presumably arising from a somatic indel mutation in a hematopoietic stem cell before its division into myeloid and lymphoid progenitor cells. The second reported case of MFA was resolved by the discovery of a regulatory variant in the 8-bp RUNX1 motif of *ABO*, extending current knowledge of four other variants affecting the same motif and also leading to A_3 or B_3 phenotypes. Overall, long-range PCR combined with ONT sequencing proved powerful for the resolution of both ABO MFA cases.

Parallel Session 17: Paediatric transfusion medicine

PA17-L01 | Paediatric transfusion medicine has a healthy growth curve

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Paediatric transfusion medicine has matured over the last 10–15 years, going through a growth spurt. Over that time multiple randomised control trials have been accomplished, especially in neonatal transfusion. These studies have nourished and provided the foundation for many current international neonatal/paediatric transfusion guidelines which will be presented during this session. Guideline rationale and evidenced-based studies will be emphasized as well as gaps and controversies in this coming of age field.

PA17-L02 | Randomised controlled trial on the use of darbepoetin to reduce transfusion episodes in infants with red blood cell alloimmunisation treated with intrauterine transfusion

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Background: Up to 80% of infants with haemolytic disease of the foetus and newborn (HDFN) treated with intrauterine transfusion (IUT), require at least one red blood cell (RBC) transfusion for anaemia during the first three months of life. Erythropoietin deficiency is considered a possible contributing factor to postnatal anaemia in HDFN.

Aims: The objective of this study was to evaluate the effect of exogenous erythropoietin in the form of darbepoetin on the need for RBC transfusion in infants with HDFN due to red cell alloimmunisation treated with IUT.

Methods: We conducted an open label single centre randomised controlled trial. All (near) term infants (gestational age ≥ 35 weeks) with HDFN (due to D, C, c, E, K or other red blood cell alloimmunisation) treated with IUT and admitted to our centre after October 2017 were eligible. Patients were randomised to treatment with darbepoetin subcutaneously at a dosage of 10 µg/kg once a week for a period of 8 weeks (intervention), or "standard care", without darbepoetin. The primary outcome was the number of RBC transfusion episodes required per infant after birth. Secondary end points were: the percentage of infants requiring a RBC transfusion up to 3 months of life; time from birth to first RBC transfusion (days); number of days of hospitalisation and readmission(s) associated with RBC transfusion.

Results: A total of 44 (near)-term infants with HDFN and treated with IUT were included. An overall 50% reduction in RBC transfusion episodes was shown with darbepoetin treatment compared to standard care (median 1·0 vs. 2·0 transfusion episodes, $p = 008$). Overall, in the darbepoetin group, 15/19 (79%) of infants were treated with RBC transfusions after birth; compared to 22/24 (92%) in the standard care group ($p = 0.232$). With regard to the time between birth and first transfusion, there was no statistically significant difference between the groups (median of 29 vs. 22 days, $p = 0.143$). The total duration of hospitalisation after birth did not differ significantly between the groups: infants were discharged home after a median of 8 versus 12 days ($p = 291$).

Summary/Conclusions: There is a clear effect of darbepoetin on the number of transfusion episodes after IUT treatment for HDFN. Cost-effectiveness analysis and evaluation of long term follow-up are necessary to decide upon implementation of treatment with darbepoetin or other types of erythropoietin as part of the postnatal treatment of severe HDFN.

PA17-L03 | Increase of cerebral blood oxygenation in preterm neonates after transfusion: Distinctive effects of RBC units from umbilical blood

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Background: Repeated red blood cell (RBC) transfusions in highly preterm neonates cause the progressive decrease of foetal haemoglobin (HbF) levels. Noteworthy, HbF is endowed with a higher affinity for oxygen than adult Hb, whilst preterm neonates are especially exposed to oxidative challenges since they lack an efficient antioxidant

defense. It is therefore conceivable that HbF depletion might have detrimental consequences for these fragile patients. The Italian trial "BORN" (NCT05100212) is currently addressing this issue by exploring the eventual favourable role of transfusing allogeneic umbilical RBC products (enriched with HbF) instead of RBC units from adult donors.

Aims: This study investigates whether the type of transfused products influences oxygenation changes occurring in cerebral tissue after RBC transfusions, either HbF-enriched umbilical RBC concentrates or standard RBC concentrates.

Methods: As per standard procedure at our neonatology intensive care unit, patients are routinely monitored by Near Infrared Spectroscopy (NIRS) to assess regional oxygen saturation (rSO_2) of blood in the brain and whenever possible in the splanchnic region (INVOS Oximeter, Somanetics), with a sampling interval of 30 seconds. Cerebral NIRS records of patients enrolled in the BORN study were then retrieved by attending neonatologists (blinded to the type of transfused RBC product), verified to exclude artefactual and non-reliable data due to changes in oxygen demand or respiratory support, aligned with transfusion times, and sent to haematologists. Each record was then tagged according to the RBC type (umbilical cord or adult) and analysis was accordingly performed. The following 3-h-monitoring time points of cerebral rSO_2 were evaluated at baseline (i.e., before transfusion) and at the end of transfusion. Oxygenation changes were expressed as the percentage of the respective baseline rSO_2 value.

Results: Data relative to 8 patients receiving 19 RBC transfusions (10 from umbilical RBC and 9 from adult donors) were analysed. Patients receiving adult or cord blood transfusions were comparable for characteristics at birth (gestational age, birth weight, Hb concentration). At comparison of single transfusion events, no significant differences were detected between adult and cord RBC groups regarding postmenstrual age, Htc, ventilatory support and oxygen supplementation. In all cases, transfusion resulted in a stepwise increase of cerebral rSO_2. The increase was significantly more pronounced after adult RBC transfusions (14.7 ± 1.7 vs. $8.9 + 1.8$, $p < 0.001$). To exclude that this finding could be partially due to a lower basal rSO_2 value in the adult group, we repeated the analysis by including only transfusion events with basal rSO_2 values >55% (Seidel D, J Perinatol, 2013). We could confirm that, despite comparable rSO_2 pre-transfusion values, cerebral rSO_2 increased by 12.7% $\pm$ 0.7% after adult donor RBC transfusions and 8.9% $\pm$ 1.8% after cord blood transfusions ($p < 0.001$).

Summary/Conclusions: Transfusing preterm neonates with adult or cord RBC products may elicit different effects on cerebral tissue oxygenation. Considering that oxidative stress is a major challenge in this population, our observations further support the rationale of exploring allogeneic umbilical cord RBC concentrates as transfusion therapy in this setting.

PA17-L04 | Rheological, metabolic, and morphological characterization of umbilical cord blood for potential transfusion use

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Background: Currently, less than 10% of cord blood (CB) units donated for haemopoietin stem cell transplant have an adequate volume and cell content for banking. The high rate of unsuitable units has encouraged the production of new blood components from CB, including red blood cells (CB-RBC), which have great transfusion potential for extremely low gestational age neonates (neonates born before 28 weeks of gestation).

Aims: The purpose of this work was to evaluate the morphological, rheological and metabolic properties of fresh CB, before and after irradiation and after storage in the same conditions for potential transfusion use.

Methods: CB-RBC agreeability and deformability, were analysed by means of Laser Assisted Optical Rotation Cell Analyser (LoRRca MaxSis) a laser diffraction viscometer, which measures the deformability of red blood cells subjected to a shear stress and analysed in a viscous solution with increasing osmolarity. Metabolic analysis was performed by determination of ATP by spectrophotometric assay. An extracted from a whole CB sample was prepared and a measure was performed according to E. Beutler's method. For analysis of the figured elements of CB, slides preparation were set-up for visualisation under a optical microscope. All analyses were performed on a total of 10 samples at day zero of storage, before and after irradiation and at day ten of storage under the same experimental conditions

Results: Echocytometric analysis showed that 90% of the samples at day zero, pre and post irradiation, follow a trend comparable to the control area. At day 10, all samples show a decrease in Ohyper value, reflecting the cellular hydration state, consistent with a significant increase in echinocytes, an event attributable to cellular ageing. No significant alterations in ATP were observed. Considering irradiation, no effects were observed on deformability or cellular metabolism.

Summary/Conclusions: Preliminary data concerning the characterisation of whole CB, seem to show that irradiation and storage do not lead to cell-responsive deformability, and that the use of CB-RBC is a viable alternative for transfusions in premature infants and newborns.

PA17-L05 | Association of platelet counts with bleeding in preterm neonates: An observational cohort study

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Background: In preterm neonates, the occurrence of major bleeding, especially severe intraventricular haemorrhage (IVH), is a serious concern. Most studies focus on neonates with thrombocytopenia to improve platelet transfusion practices, while it remains unclear how many neonates with normal platelet counts develop a major bleeding.

Aims: We aimed to describe the incidence of major bleeding according to different platelet count levels in both thrombocytopenic and non-thrombocytopenic preterm neonates. Second, we aimed to investigate the extent to which the association between different platelet count levels and major bleeding is independent of other factors.

Methods: In this observational cohort study, we included all consecutive neonates with a gestational age at birth <32 weeks admitted to the neonatal intensive care unit (NICU) of the Leiden University Medical Center (LUMC), the Netherlands, between January 2004 and July 2022. Neonates were classified into 9 groups based on their lowest (i.e., nadir) platelet count (in $\times 10^9$/L) during NICU admission and measured before any platelet transfusion was administered: ≤9, 10–24, 25–49, 50–99, 100–149, 150–199, 200–249, 250–299 and ≥300. The primary outcome was major bleeding during NICU admission. We used logistic regression analysis to explore the causal relationship between nadir platelet count levels and the risk of major bleeding.

Results: Of 2986 included neonates, with a median gestational age of 29 weeks (IQR, 27–30 weeks), 247 (8.3%) developed a major bleed (Table 1). These bleedings occurred in 58 (23%) neonates with a nadir platelet count $<100 \times 10^9$/L and 189 (77%) neonates with a nadir platelet count $\geq 100 \times 10^9$/L. The incidence of major bleeding in these groups was 9.0% (58/644) and 8.1% (189/2342), respectively. Multivariable logistic regression confirmed the absence of an association between nadir platelet count and major bleeding.

Summary/Conclusions: Although the incidence of major bleeding was between 8% and 9% at all platelet count levels, more than three-quarters of the total number of major bleeds occurred in neonates with nadir platelet counts $\geq 100 \times 10^9$/L.

PA17-L05 - Table 1. Incidence of major bleeding according to different platelet count levels

Nadir platelet count levels ($\times 10^9$/L)	Major bleeding[a], *n* (%)	Major intracranial bleeding[b], *n* (%)	Major pulmonary bleeding[c], *n* (%)	Major rectal bleeding[d], *n* (%)
≤9 (*n* = 8)	2 (25.0)	2 (25.0)	1 (12.5)	0 (0)
10–24 (*n* = 61)	3 (5.0)	4 (6.7)	1 (1.6)	1 (1.6)
25–49 (*n* = 178)	16 (9.0)	17 (9.6)	5 (2.8)	1 (0.6)
50–99 (*n* = 397)	37 (9.3)	41 (10.3)	7 (1.8)	2 (0.5)
100–149 (*n* = 573)	54 (9.5)	60 (10.5)	12 (2.1)	0 (0)
150–199 (*n* = 675)	55 (8.1)	57 (8.4)	7 (1.0)	1 (0.2)
200–249 (*n* = 592)	41 (6.9)	44 (7.4)	10 (1.7)	2 (0.3)
250–299 (*n* = 324)	22 (6.8)	23 (7.1)	3 (0.9)	0 (0)
≥300 (*n* = 178)	17 (9.4)	17 (9.6)	1 (0.6)	1 (0.6)

[a] Major bleeding: only the first major bleed is counted, multiple types of major bleeds may have occurred in one patient.
[b] Major intracranial bleeding: intraventricular haemorrhage (IVH) ≥grade 3, IVH of any grade complicated by parenchymal haemorrhagic infarction, or major intracranial haemorrhage other than IVH.
[c] Major pulmonary bleeding: an acute fresh bleed through the endotracheal tube associated with the need for intubation or ventilation or increased ventilatory requirements.
[d] Major rectal bleeding: fresh visible rectal bleeding except for mild bleeding caused by necrotizing enterocolitis.

Parallel Session 18: Epidemiology goes viral

PA18-L01 | The impact of babesia testing on transfusion-transmitted babesiosis

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Background: The intraerythrocytic parasite *Babesia microti*, endemic in the north-eastern and mid-western United States, has been considered the leading cause of transfusion-transmitted infection in the US for decades.

Aims: Provide an update on the impact of babesia testing on transfusion-transmitted babesiosis (TTB).

Methods: American Red Cross (ARC) implemented babesia blood donation screening in May 2020 in 14 states on the East Coast (Northern and Mid-Atlantic), plus Washington DC and the Upper Midwest, as required by the US Food and Drug Administration. Screening is performed using a transcription-mediated amplification nucleic acid assay (NAT) in pools of 16 whole blood samples. Reactive samples are tested for *B. microti* antibody (Ab) by immunoglobulin G immunofluorescence (IFA). One follow-up donation is collected from consenting donors a minimum of four weeks after the index donation.

Results: From May 2020 to December 2022, the ARC screened over 4.3 million donations and identified 1073 babesia reactives (0.02%). Higher rates of reactive donations were collected in the babesia-endemic states of Connecticut, Massachusetts, Maine, New Hampshire, New Jersey, New York and Pennsylvania. Follow-up donations were collected from 345 donors, and 298 (86%) remained NAT reactive up to 171 days after index. Ab-positive samples represented 78% of the total NAT-reactive donations. Ab-negative donations (window-period, $n = 85$) corresponded to 22% of the total reactives and were collected mainly between May and July (77%). When followed, 61 of 85 (72%) donors seroconverted.

Since babesia testing implementation, the ARC investigated seven potential cases of TTB, but positive donors were identified only in two occurrences. In both cases, the implicated donation was not tested by NAT for babesia because it was collected in a state where screening is not required (Ohio and West Virginia), whereas both recipients resided in states where babesia testing is performed (Maryland and Virginia, respectively).

Conclusions: The implementation of babesia testing in endemic areas in the United States has been highly successful in reducing TTB cases, virtually eliminating them. However, as demonstrated by the two cases that occurred post-implementation, a regional testing approach leaves room for potential TTB cases by infected blood collected in areas considered not endemic.

PA18-L02 | Ten-year experience of mini-pool nucleic acid testing in blood donors in Taiwan

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Background: Both mini-pool (MP) and individual donor (ID) nucleic acid tests (NAT) are widely used in detecting donor infection. Even though MP-NAT is less sensitive than ID-NAT, the testing cost of MP-NAT would be significantly lower and affordable by many blood collection centres. A universal NAT screening has been implemented in blood donors since 2013.

Aims: To determine the risk of transfusion-transmitted infection (TTI) for the human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) and to investigate the suspected TTIs during 10-year MP-NAT screening.

Methods: All donations were tested for triplex NAT for HIV, HCV, and HBV in pools of eight donations using the Procleix Ultrio Plus Assay or Procleix Ultrio Elite Assay. Positive individual donations were resolved from the positive pools and confirmed by discriminatory assays of HIV, HCV and HBV. Each donation was also tested for anti-HIV, anti-HCV and HBsAg. Western blot was used as a confirmatory test for anti-HIV and anti-HCV, while HBsAg was confirmed by a neutralization test. The residual risk (RR) of window period TTI in blood donations was estimated according to the World Health Organization guideline. Investigation of HIV TTI was conducted by Taiwan Centers for Disease Control (TCDC). Every HIV case was scrutinized for blood donation history, and the associated recipients were investigated. Donors associated with recipients who were suspected of HIV TTI would also be investigated. An expert committee has been established since 2015 to determine the immutability of HCV and HBV TTI for each suspected case according to their laboratory tests before and after transfusion, procedures received and the associated blood donors' follow-up testing results. If the donor does not return or has a positive result in a subsequent donation, the donors' and recipients' index blood specimens will be re-examined by TCDC.

Results: A total of 450 HIV, 3118 HCV and 20,009 HBV NAT-positive donations were detected in 17,855,971 donations collected from Jan 15, 2013 to Dec 31, 2022. The number of NAT yield donations were 12, 83, and 4236 for HIV, HCV, and HBV; the yield rates (95% CI) per 10^5 were 0.07 (0.04–0.12), 0.46 (0.37–0.58), and 23.72 (23.02–24.45). The overall NAT yield rates (95% CI) of HCV and HBV significantly declined, which decreased from 1.30 (0.85–1.97) and 33.45 (30.81–36.32) per 10^5 in 2013 to 0.21 (0.08–0.57) and 17.77 (15.96–19.78) per 10^5 in 2022 (both p for trend < 0.05). These rates were 1.5 to 5.7-fold in first-time donors than in repeat donors. Male donors had higher HIV ($p = 0.07$) and HBV NAT ($p < 0.05$) yield rates than female donors. The HCV ($p = 0.07$) and HBV NAT ($p < 0.05$) yield rates both increased with age. In contrast, donors younger than 30 had the highest HIV NAT yield rate ($p = 0.01$). The estimated RRs (95% CI) were 1.05 (0.90–1.23), 8.98 (8.22–9.82), and 49.17 (46.98–51.46) per million donations for HIV, HCV, and HBV in the case without NAT, and decreased to 0.35 (0.30–

0.41), 0.75 (0.68–0.82), and 31.61 (30.20–33.08) after NAT. A total of 3902 HIV cases who had donated blood and their associated recipients were actively investigated, and no TTI case was identified. A total of 37 and 5 suspected HCV and HBV TTI cases subjected to review and none of their infections were associated with blood transfusion.

Summary/Conclusions: MP-NAT effectively detects infectious blood units in Taiwan, and no HIV, HCV, or HBV TTI case has been identified so far.

PA18-L03 | Prevalence, incidence, and residual risk of HIV, HBV, and HCV in the US blood supply 2015–2021, on behalf of the US TTIMS program

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Background: Monitoring the results of infectious disease testing of the blood supply is important to assess safety and to evaluate the impact of changes in policies for assessing donor suitability.

Aims: To estimate the prevalence, incidence and residual infection risk for HIV, HBV, and HCV using the first 6 years (September 2015–September 2021) of the Transfusion-Transmitted Infections Monitoring System (TTIMS), which accounts for more than 50% of the US blood supply.

Methods: Four major blood collection organizations (American Red Cross, New York Blood Center Enterprises, One Blood and Vitalant) participated. Serologic and nucleic acid test (NAT) results for HIV, HBV and HCV were used with consensus criteria developed for positive results. Donor information, including basic demographics, was collected from routine records and data were assembled and managed centrally. Prevalences of positive results are presented per 100,000 donations; incidence was calculated per 100,000 person-years over 2-year periods among repeat donors. The likelihood of issuing a donation in the window period (residual risk, RR) was estimated from repeat donor incidence plus the assumed incidence for first-time donors, based upon the ratio of NAT yield among first-time and repeat donors. Residual risk from repeat donations and overall weighted donations are presented separately. A subgroup analysis of prevalence for the final year of the study (October 2020 to September 2021) was also performed.

Results: Overall, 41.7 million donations were reviewed and prevalence per 100,000 donations were between 1.6 and 3.0 for HIV, 5.3 to 6.6 for HBV and 9.1 to 20.2 for HCV; these were essentially stable for HIV and HBV but declined rapidly over the last 2 years for HCV. A total of 7,577,010 donations was evaluated for the final year of the study period with prevalence and 95% confidence intervals per 100,000 of 1.6 (1.4–1.9) for HIV, 5.3 (4.7–5.8) for HBV, and 9.1 (8.4–9.8) for HCV. Prevalence was higher among donations from males by a factor of 4.5 for HIV, 2.7 for HBV and 2.3 for HCV. HIV was most frequent among the 18–24 age group and for HBV and HCV, among the 25–39 age group. The lowest prevalence for HIV was seen among white donors, > than 1 race for HBV and Asian for HCV. Prevalence for all agents was highest among donations from the south US Census region. Incidence and RR declined for HIV and HCV but increased slightly for HBV. Overall incidence and RR estimates for the final 2-year period are shown in the Table; notably, the risk was less than 1 in 5 million for HIV and HCV and remained approximately 1 per million for HBV. Prior published TTIMS RR (per million) for HIV, HBV and HCV (Dec, 2017–Jul, 2019) were approximately 1:1.6, 1:1.0, and 1:2.0, respectively (Steele et al., Transfusion 2021).

PA18-L03 – Table 1

	Repeat donor incidence and residual risk method (Oct 2020–Sep 2021)		Overall weighted incidence and residual risk method (Oct 2020–Sep 2021)	
Agent	**Incidence**	**Residual risk**	**Incidence**	**Residual risk**
HIV	0.53	1: 7,626,524	0.83	1: 5,618,116
HBV	1.65	1: 1,193,146	2.77	1: 842,864
HCV	0.60	1: 8,206,441	1.12	1: 5,347,120

Summary/Conclusions: The prevalence and incidence of markers of transfusion-transmissible infections for more than 50% of the US blood supply are low, as is the risk of exposure for blood recipients. Prevalence over a 6-year period has been relatively stable or declining. This is particularly significant as the study covers the timeframe when deferral periods for HIV risk were reduced from permanent to 12 months, and then 3 months, and the early stages of the COVID-19 pandemic. These events were not associated with any apparent increase in risk to blood safety.

PA18-L04 | HIV incidence in US first-time blood donors during 12-month and 3-month MSM deferral policy periods on behalf of the US TTIMS program

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Background: Following FDA guidance, in 2016 US blood collection organizations implemented a 12-month time-limited deferral of male donors who reported sex with another man (MSM12m), and in 2020, further reduced to 3 months since last MSM sex (MSM3m). Here, we evaluate the incidence of HIV in first-time donors (FTD) using a recent infection testing algorithm (RITA) applied to HIV-infected donors at four large US blood collectors using data on 8.9 million donations.

PA18-L04 – Table 1

	Observed HIV incidence /10^5 PY (95% CI)	Standardised to MSM12m demographics /10^5 PY (95% CI)
MSM12m period	2.74 (2.05, 3.56)	2.74 (2.05, 3.56)
MSM3m period	1.85 (0.96, 2.85)	2.36 (1.34, 3.50)
Incidence difference	−0.89 (−2.00, 0.20)	−0.38 (−1.57, 0.81)

Aims: The aims are to assess changes in HIV incidence in US FTD following eligibility policy changes and to inform assessment of HIV transfusion-transmission risk in the US blood supply.

Methods: We estimated HIV incidence and corresponding rate differences in FTD during the MSM12m and MSM3m periods, using cross-sectional incidence estimation techniques, based on application of a RITA to HIV-positive donations, as follows. NAT-yield donations (seronegative) were considered recent; Ab+/NAT- were considered long-term and concordant Ab+/NAT+ were classified as recent or long-term using quantitative Limiting Antigen (LAg) Avidity enzyme immunoassay (EIA) and viral load tests. HIV prevalence, a key input to the incidence calculation, was estimated through imputation to account for any missing confirmatory serology and/or NAT results. Prevalence and incidence confidence intervals were derived from parametric bootstrapping. Using a previously described Bayesian method, we estimated a context-specific mean duration of recent infection for the RITA using repeat donor data (243 days) and obtained a subtype B-specific false-recent rate estimate from external data. Given a shift in donor demographics associated with the COVID-19 pandemic, we assessed the impact of this shift on HIV incidence through a counterfactual age, sex and race/ethnicity standardised MSM3m estimate; that is, we weighted donors according to the number of donors in each age/sex/race-ethnicity stratum who donated during the MSM12m period. We used Poisson regression to assess demographic correlates of incident infection.

Results: FTD HIV prevalence during the MSM12m period was 8.59 cases/10^5 (95% CI: 7.79, 9.43) and during the MSM3m period was 9.13 cases/10^5 (8.01, 10.28). FTD HIV incidence during the two periods was 2.74 cases/10^5 person-years [PY] (2.05, 3.56) and 1.85 cases/10^5 PY (0.96, 2.85), respectively. The incidence point estimate declined substantially, but the difference was not statistically significant. The demographically standardised analysis did not demonstrate a similar decline (see Table). Multivariable regression indicated that male sex, younger age, Black or African American race, Hispanic ethnicity and residing in the Southern US were significantly associated with FTD incident infection.

Summary/Conclusions: The decline in FTD HIV incidence from the MSM12m to MSM3m periods did not reach statistical significance (95% CI upper bound, increase of 0.2/10^5PY). The standardised analysis provides evidence that the decline may be partially explained by demographic shifts in the donor population. Similar demographic factors were associated with incident infection as in previous analyses. These results provide no indication of increased risk in FTD and are reassuring in that reduced deferral periods have not led to increased risk.

PA18-L05 | Ten-years insight into the HBV molecular epidemiology in infected blood donors in Dalian, Northeast China

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Background: Hepatitis B virus (HBV) remains a high priority for Chinese blood banks due to the high prevalence of infection. The nine HBV genotypes (A-I) identified differ in their geographic distributions, transmission, disease progression, and clinical outcomes. HBV genotypes B (HBV_B) and C (HBV_C) are the major genotypes endemic in Mainland China, with HBV_B being prevalent in the southern part and HBV_C in the northern part of the country. However, these figures are mainly from patients and data on blood donors are still scarce.

Aims: To study retrospectively HBV genotype distribution over the past decade in a subset of HBV-infected blood donors in the Dalian blood center, Liaoning province, Northeast China.

Methods: HBV-infected donors were randomly selected according to sample availability: donors tested HBsAg+ pre-donation (group 1), donors tested HBsAg+ post-donation (irrespective of HBV DNA status; group 2), and donors with confirmed HBsAg-/HBV DNA+ occult HBV infection (OBI; group 3). Pre-donation testing was performed using a HBsAg rapid test (95% LoD: 5 IU/mL). HBsAg and HBV DNA were tested post-donation using two different ELISAs (95% LoD: 0.2 IU/mL), and mini-pool or individual-donation multiplex nucleic acid test (95% LoD: 3-10 IU/mL). The whole HBV genome or PreS/S and PreCore/Core regions were sequenced and HBV genotypes were identified by sequence phylogeny.

Results: Between 2011 and 2022, 5,299 of 844,594 (0.63%) candidate donors tested HBsAg+ pre-donation. Of 942,874 collected donations, 584 (0.06%) tested HBsAg+/HBV DNA + or -, and 563 (0.06%) donations were confirmed HBsAg-/HBV DNA +. The pre-donation HBsAg+ rate decreased 3-fold over the years (1.05% in 2013 to 0.35% in 2022), whereas no significant change was observed for post-donation HBsAg+ and OBI rates. HBV genotypes were 26 (25%) HBV_B, 73 (71%) HBV_C, 2 (2%) $HBV_{B/C}$, and 2 (2%) HBV_D in group 1; 53 (55%) HBV_B, 42 (44%) HBV_C, and 1 (1%) $HBV_{B/C}$ in group 2; and 23 (15%) HBV_B, 125 (83%) HBV_C, and 3 (2%) HBV_D in group 3. HBV_B and HBV_C rates were not significantly different between group 1 and group 3. HBV_B rate was significantly higher in group 2 compared to the other groups. Median HBV DNA load was significantly higher ($p < 0.0001$) in group 1 (8.7E+03 IU/mL [5.0-1.9E+10]) than in group 2 (2.9E+02 IU/mL [5.0-8.3E+06]) and group 3 (5.0 IU/mL [5.0-2.6E+03]). Viral loads were not significantly different between genotypes in all groups. Overall, the median HBsAg level was higher in group 1 (168 IU/mL [0.05-2.6E+06]) than in group 2 (0.6 [0.05-1.2E+04]) ($p < 0.0001$). However, HBV_B samples showed lower HBsAg level than HBV_C samples in group 1 (54.6 IU/mL [0.05-3.2E+03] vs 9.1E+02 IU/mL [0.05-3.4E+05]; $p = 0.0004$), whereas the opposite was observed in group 2 (HBV_B: 1.12 IU/mL [0.05-2.5E+01] and HBV_C: 0.3 IU/mL [0.05-1.2E+04]; $p = 0.002$).

HBV strains from group 2 showed higher S protein amino acid diversity (HBV_B: 5.8% [1.8-12.0%] and HBV_C: 2.7% [0.0-7.5%]) than strains of corresponding genotypes from group 1 (HBV_B: 2.7% [0.4-3.5%] and HBV_C: 1.8% [0.0-9.4%]) ($p < 0.0001$). Potential immune escape mutations in S may be responsible for failure of pre-donation rapid testing (i.e. G130N+T131N and D144E+G145R).

Summary/Conclusions: A continuous decline in the yield of HBsAg pre-donation screening of blood donors has been observed in Dalian over the last decade. HBV_C was prevalent in HBV-infected blood donors in Northeast China, especially in OBI carriers. Pre-donation rapid testing was less efficient in detecting HBV_B infections. This might be related to higher antigenic variation in HBV_B strains and lower HBsAg production. These data may prompt debate about whether to adapt pre-donation testing in Dalian.

Parallel Session 19: Protecting donor health

PA19-L01 | Impact of ultra-high frequent apheresis platelet donation on donor health

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Apheresis platelet donors who donate at an extremely high frequency (up to 24 times per year) can develop severe T-cell lymphopenia. Some of these donors have CD4+ T-cell counts below 200 cells/μL, the threshold that defines AIDS in individuals who are HIV-positive. This session will review our current understanding of platelet pheresis-associated lymphopenia (PAL), including risk factors, natural history, mitigation and potential consequences for donor health and immunity.

PA19-L02 | Beyond the iron gate: An exploratory study to understand the experiences of haemochromatosis (HHC) patients who undergo therapeutic venesection at Lifeblood

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Background: Hereditary Haemochromatosis (HHC) is one of the most common genetic disorders in Australia. Since 1998 Australian Red Cross Lifeblood (Lifeblood) has offered a therapeutic venesection service for HHC patients referred by their treating physician who prescribes a suitable treatment schedule. In 2022 Lifeblood collected 32,143 clinically suitable whole-blood donations from HHC therapeutic (T) donors.

Research has identified benefits of therapeutic venesection for HHC patients with the HFE genetic variants that cause iron overload. However, little research has explored the barriers and enablers for donating and maintaining treatment schedules at blood collection agencies. Lifeblood recently introduced changes to allow plasma donations from eligible HHC T-donors when they reach target ferritin levels during their maintenance phase. In preparation for this we also explored HHC T-donors' views on donating plasma

Aims: The aim of this study was to understand how HHC T-donors view donating at Lifeblood. In particular, we wanted to investigate barriers and enablers to donating and maintaining treatment schedules, willingness to donate other products if eligible, and to improve understanding of how HHC T-donors can be supported by Lifeblood.

Methods: We used a mixed methods approach starting with interviews with 15 HHC T-donorsto gain insights into their experiences donating at Lifeblood. These insights informed the development of an online survey, which was distributed to 17,000 HHC T-donors who had donated in the last 5 years. Participants were asked about their HHC knowledge, adherence to treatment schedules, experience donating at Lifeblood and other locations, barriers and enablers to donating, how they view their role at Lifeblood, their knowledge about donating plasma and whether they would consider donating plasma if eligible.

Results: Overall, 4354 (RR: 24.5%) HHC T-donors completed the survey. Participants self-identified as blood donors (Med:6 [4–7]) more than they identified as patients (Med:4 [2–6]).

Participants reported good knowledge of their condition (Med:5 [4–7]) and self-reported compliance with treatment schedules was very high, with 53% reporting they donate exactly as prescribed, with a diverse range of barriers including distance to travel (4%) and difficulty getting an appointment (4%) noted. A third of participants reported having donated at other locations prior to Lifeblood. Blood being used to help others (89%), ease of access to donor centres (72%) and ease of managing their condition (58%) were the top three enablers for donating at Lifeblood. Key barriers were the inability to manage own appointments (23%), deferrals (13%) and difficult veins (12%). Donors reported little knowledge about donating plasma (Med:2 [1–3]) but indicated some interest in donating plasma if able (Med:4 [2–5]). Knowledge was weakly positively related to interest in donating plasma ($r = 0.14$, $p < 0.05$).

Summary/Conclusions: HHC donors are an under-researched cohort who value knowing their necessary donations will be used to help others. This study provides valuable insights into the donation experience for HHC T-donors, and provides evidence to support offering additional donation options, like plasma, for those willing and able. While increasing knowledge of plasma may increase uptake of this donation option, education on its own is insufficient to overcome barriers reported by HHC T-donors. Measures to address these barriers are required. In Australia, approximately 80,000 therapeutic

venesections were conducted external to Lifeblood in 2022. As such, reducing barriers to donation and building awareness of opportunities for therapeutic venesection at Lifeblood, and additional plasma donations by HHC T-donors offers clear opportunities to benefit the blood supply.

PA19-L03 | Blood donation and mental health: What is the prevalence of major depression disorder among Danish blood donors and which symptoms should blood bank staff be mindful of when assessing a donor?

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Background: Major Depressive disorder (MDD) is the most common psychiatric disorder globally. In Denmark, MDD has an estimated prevalence of 3% among the general population. Blood donors report better mental and physical health-related quality of life than the general population, but the exact prevalence of MDD in healthy donors is unknown. In Denmark, un-medicated donors with MDD are allowed to donate based on an individual assessment of their health and current tolerance of common effects of donation including fatigue.

Aims: We aimed to investigate the point prevalence of MDD among healthy Danish blood donors eligible for donation. Furthermore, we wished to elucidate potential sex differences in symptomatology to characterise which symptoms to look for in women and men, respectively.

Methods: The study was based on the questionnaire data from the Danish Blood Donor Study (DBDS). Donors were included in DBDS if they met the general donor health criteria and were eligible for donation. Depressive symptoms were assessed with the Major Depression Inventory (MDI), a validated self-rating scale. The MDI was included in the DBDS inclusion-questionnaire between 2015 and 2018. MDD was defined according to the ICD-10 classification as having two or more core symptoms of MDD and two or more accompanying symptoms. Individual-level data for the participants were retrieved from the Danish registries. These included medical prescriptions and socioeconomic status. Descriptive, Logistic and Cox regression analyses were conducted.

Results: In total, 51,658 blood donors were included in the analysis. Of these, 1.15% ($n = 596$) met the criteria for MDD (217 participants with mild MDD, 264 with moderate MDD and 115 with severe MDD). The baseline characterises differed significantly between donors with or without MDD: Donors with MDD displayed a higher proportion of women, smokers, higher bmi and lower education ($p < 0.001$). When assessing the specific MDD symptoms, women were more likely to report increased appetite ($p < 0.001$), concentration problems ($p < 0.01$) and less likely to report a feeling of "life not worth living" ($p < 0.01$), compared to men. Donors with MDD had an increased hazard of subsequently receiving a prescription of antidepressive medication (women, HR = 4.90 (95% CI: 3.85–6.23), $p < 0.001$; men, HR = 4.44 (95% CI: 3.00–6.56), $p < 0.001$). The hazard increased proportionally with MDD severity.

Summary/Conclusions: Danish blood donors have a low point prevalence of MDD compared to the general Danish population. This confirms previous findings of an improved mental health among blood donors. Our findings also support previous research reporting differences in MDD symptomatology between women and men. From a blood bank perspective, it was surprising to find that 0.7% of the donors deemed eligible to donate classified with moderate to severe MDD. Staff members in the blood banks should be aware that despite this high level of symptoms, donors might still present and objectively be eligible for donation. From a public health perspective, this study showed that among this generally healthy population group, more than 1% appear to suffer from MDD requiring treatment. This could indicate that diagnostic MDD outreach strategies are warranted.

PA19-L04 | For the love of "Kattankappi", a randomised control trial to study the effect of regional caffeinated beverage "Kattankappi" in blood donors before donation

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Background: Good habits are hard to start and even more difficult to keep. This simple RCT aims to find out the influence of a celebrated and much-loved caffeinated drink "Kattankappi" on blood donors. It is cheap, easy to make, and distribute. An altruistic donor is the first and the most important step when we talk about blood safety. To foster a habit of blood donation in young donors, we should be able to connect with them more. Donor reaction will demotivate the donor from becoming a regular voluntary blood donor which will affect the donor pool.

Aims: Primary objective:

- To find out the influence of regional caffeinated beverage "Kattankappi" in blood donors with regard to adverse donor reaction
- To find out the behavioural changes "Kattankappi" can cause in blood donors.

Secondary objective:

- To find out the relationship between cumulative waiting time from last meal to phlebotomy and donor reactions

Methods: This is a randomised control trial that took place in the Department of Transfusion Medicine, Government TD medical college Alappuzha, Kerala, India. The study had two arms. The donors willing for the study were randomly allocated to both groups using dice, all odd numbers were in the intervention group and all even numbers were in the control group. The control arm received 150 mL of hot water as a placebo. The intervention arm received 150 mL of "Kattankappi".

Preparation procedure: "Kattankappi" was prepared in our blood centre by our cook. Prepared by adding 1 L of water, five tablespoons of coffee powder of single brand, and five tablespoons of sugar

Sample Size:

We have around 14,000 donors every year, so the sample size was decided to be 1000 for each arm, a total of 2000.

Inclusion criteria:

- all donors willing to be part of the study

Exclusion criteria:

- all donors not willing
- donors who have had caffeinated beverage or snack before coming to the blood centre
- donors who did not finish the drinks

Results: The control and intervention group was homogenous for various characteristics like gender, age distribution, first-time or multiple donors, number of donations, history of donor reactions, elapsed time from last meal till registration, and elapsed time from last meal till phlebotomy.

Percentage of donor reactions in control and intervention group

PA19-L04 – Table 1

Donor reaction	Control group	Intervention group
No	89.5%	99%
Yes	10.5%	1%

Note: *p*-value < 0.001

Frequency of donor reactions in relation to cumulative time from last meal till phlebotomy in control and intervention group

PA19-L04 – Table 2

Time elapsed from last meal till phlebotomy (min)	Control group	Intervention group
121–150	20	0
151–180	15	0
181–210	30	0
211–240	25	5
>240	15	5

Note: *p*-value 0.001

Pre donation Anxiety signs was found to be significant for developing donor reaction later, *p* value < 0.001.

Percentage distribution of donors willing to donate blood again: 1.25% of donors who had donor reactions were not willing to donate again, while all donors without donor reactions were willing to donate again.

Binary logistic regression: Nagelkerke R square = 68.6%.

Summary/Conclusions: Through analysis "Kattankappi" was found to be an effective tool to reduce donor reactions. The researcher would like to acknowledge the positive change of the environment that she noticed during the period of study. Cumulative time from the last meal till phlebotomy was found to have a positive correlation with donor reactions. We have to take the necessary steps to actively reduce this delay. There was a sense of excitement among donors in all age groups on receiving Kattankappi. This reduced the donor reactions. Even after the study ended researcher was left in awe when two nursing assistants pointed out that donor reactions were less when there was "Kattankappi".

PA19-L05 | The hidden impact of repeated donation: Haemoglobin and ferritin iron levels in Irish blood donors

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Background: Blood donors are at risk of iron deficiency as donation results in loss of haemoglobin (Hb)-bound iron. Hb levels recover using iron from the storage protein ferritin, which in turn replenish slowly over time. At present, all European donors must have a minimum pre-donation Hb. However, measuring the donor ferritin level may be a more accurate measure of blood iron stores. Ferritin-guided iron donation policies have recently been implemented in several European countries but there is currently no consensus on the optimum donor iron management policy.

Aims: This study aimed to provide baseline data on ferritin iron stores in Irish whole blood donors and determine the demographic and haematological factors impacting this.

Methods: A selection of residual donor plasma samples, received during June and July 2022 for donor infectious disease screening, were included in the study ($n = 1882$). Limited donor demographic, donation and blood antigen information was collated prior to irrevocable anonymisation and subsequent ferritin testing. Quantitative plasma ferritin levels were measured using the Abbot Architect Ferritin assay according to the manufacturer's instructions.

Results: A total of 35% and 11% of female and male whole blood donors had ferritin levels below 30 μg/mL, respectively. Iron deficiency disproportionally impacted female donors, and 7.9% of women, compared to 0.8% of men, had ferritin levels <15 μg/mL. Repeated donation significantly reduced ferritin; however this reduction in iron levels was not detected by pre-donation Hb screening. Female donors that donated more than three donations in the previous 12 months were more likely to have ferritin levels indicative of iron deficiency ($p < 0.05$).

Summary/Conclusions: Repeated donation depleted iron stores below the recommended ferritin level and longer donation intervals, especially for female donors, may be an effective measure to mitigate donor iron deficiency. Crucially, this iron deficiency is not detectable by pre-donation haemoglobin, the levels of which remain stable regardless of number donations in the previous 12 months. A combination of Hb and ferritin testing of donors may provide a more accurate measure of blood iron stores, thereby improving our capacity to diagnose iron insufficiency amongst donors. Ferritin testing should be included in future blood donor iron management policies and practices.

Parallel Session 20: Peer review: A community approach to quality science

PA20-L01 | Peer review: A community approach to quality science

J Acker

Abstract not available.

Parallel Session 21: Crossmatching 2.0

PA21-L01 | CD47 biotherapy: A new incompatibility threat

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Pretransfusion (pretx) testing, including the crossmatch, is the mainstay of safe, effective transfusion. Transfusion services (TS) are skilled in investigating and resolving pretx incompatibilities due to allo- or autoantibodies. Biotherapy using monoclonal antibodies, particularly anti-CD38 and more recently anti-CD47 has presented new challenges to the timely sourcing of compatible blood due to their interference in pretx testing. Many anti-CD47 and CD47-blocking agents are in development or in clinical trials to treat various conditions including cancer, autoimmune diseases and recently in prevention of organ transplant rejection. Techniques to mitigate interference from anti-CD38, which weakly binds to red blood cells (RBCs) and platelets, have been developed and implemented. CD47 glycoprotein is expressed on all body cells, with high expression on RBCs as CD47 is part of the membrane bound Rh complex. CD47 levels are related to the Rhce phenotype in the order of rr > R1r/R2r > R2R2> -D- with weakest expression on Rhnull. CD47 is a marker of self and regulates macrophage mediated phagocytosis by sending a "don't eat me" signal to the signal regulatory protein alpha (SIRPα) receptor. Anti-CD47 drugs block SIRPα interaction, enhancing cancer cell clearance by macrophages. The type of anti-CD47 molecule, e.g., an active or inactive IgG molecule, a fusion protein or a biphasic antibody impacts the potential to bind to RBCs and cause interference. Transient hemolytic anemia has been associated with some anti-CD47 drugs, e.g., Magrolimab (Hu5F9-G4) and Evorpacept (ALX148). In pretx testing, plasma from patients receiving Magrolimab reacts in all phases of testing (4+ IAT), often with 3+ to 4+ immediate spin reactivity and can impact compatibility testing as soon as 1 hour post infusion. Magrolimab can cause spontaneous agglutination and interfere with RBC and plasma ABO typing; it may be impossible to obtain a serological ABO type or perform antigen typing. Magrolimab is subtype IgG4 and compatibility can likely be obtained by using antiglobulin reagents lacking anti-IgG4. If carry-over agglutination is observed, plasma adsorption using enzyme treated RBCs (or platelet products) may be required. For ALX148, a high titer IgG1 antibody, use of IgG4-deficient anti-IgG will not mitigate plasma IAT interference or compatibility tests. The high titer impedes the success of adsorptions and eluate preparations. ALX148 does not hamper ABO or antigen typing. Additional CD47 drugs are in clinical trials. Some, (Trillium TTI-621) do not bind to RBCs, others [Lemzoparlimab (TJ011133), Trillium (TTI-622)] may cause incompatibility that can often be overcome. Treatment of RBCs used for antibody investigation with proteolytic enzymes or 0.2M dithiothreitol (DTT) has no effect on CD47 or drug reactivity. Soluble CD47 or CD47 blocking agents, with the potential to inhibit plasma reactivity, are in development. Although it is anticipated that future CD47 drug development will prioritize those that do not interfere with pretx testing, for the foreseeable future, TS must devise protocols to overcome the interference and incompatible crossmatch. As the DAT and autologous control are usually negative, inclusion of CD47 therapy in the patient history is essential to avoid even more extensive investigation. CD47 targeting agents have unique serological profiles, interference will be drug specific and will require individual, often complex strategies to mitigate. As patients have some degree of anemia and thrombocytopenia following CD47 targeting therapies the possibility for repeat transfusions is increased, further complicating serologic studies. Communication between clinicians and the TS, obtaining a pretherapy sample for baseline ABO and antibody screening and RBC antigen extended pheno- or genotype is key to transfusion safety. The inability to determine crossmatch compatibility and the labor involved in workup of samples from these patients is likely to drive the need for cost-effective extended antigen-matched units for optimal patient care.

PA21-L02 | The effects of IMC-002, a novel anti-CD47 monoclonal antibody, on pretransfusion compatibility testing

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Background: Some therapeutic monoclonal antibodies currently used in clinical practice or trials target proteins expressed not only in cancer cells but also in normal cells, such as red blood cells (RBCs), raising concerns about their impact on pre-transfusion compatibility testing. CD47 is a glycosylated transmembrane protein with ubiquitous expression, including on RBCs. As tumour cells have been shown to highly express CD47 to evade phagocytosis by macrophages, many anti-CD47 monoclonal antibodies are being developed to treat cancers and hematologic malignancies. However, the evaluation of anti-CD47 monoclonal antibodies previously used in clinical trials showed variable interferences in pre-transfusion compatibility testing.

PA21-L02 – Table 1

	IMC-002 (μg/mL)			
	100	10	1	0.1
Tube–RT	1+	±	Neg	Neg
Tube–37°C albumin	2+	1+	Neg	Neg
Tube–AHG	±	Neg	Neg	Neg
CAT–AHG (Bio-Rad)	$3+^{mf}$	Neg	Neg	Neg
CAT–P/AHG (Bio-Rad)	4+	4+	$3+^{mf}$	Neg
CAT–AHG (Grifols)	$2+^{mf}$	Neg	Neg	Neg
CAT–P/AHG (Grifols)	4+	3+	$1+^{mf}$	Neg

Abbreviations: RT, room temperature; AHG, antihuman globulin; P, papain-treated RBCs; ^{mf}, mixed field (double population); Neg, negative.

Aims: This study aims to investigate the effect of IMC-002, a novel anti-CD47 monoclonal antibody in clinical trials, on pre-transfusion compatibility testing.

Methods: Group O/RhD positive, AB/RhD positive, and AB/RhD negative EDTA whole blood samples were incubated with IMC-002 at final concentrations of 1000 and 2000 μg/mL at 37°C for 30 min. ABO/RhD typing was performed using the tube method, column agglutination technique (CAT) (ABO/D+ Reverse Grouping ID-Card, Bio-Rad), and an automated immunohematology analyser (Qwalys 3, Diagast). A1, H and extended blood group antigen testing were performed by the tube method. Direct antiglobulin testing (DAT) was performed using CAT (LISS/Coombs ID-Card, Bio-Rad). To simulate the plasma of patients receiving IMC-002, the drug was spiked into group AB/RhD positive plasma with no unexpected antibodies at concentrations of 0.1, 1, 10, 100, 500, 1000, and 2000 μg/mL. Antibody screening tests were performed using the tube method and CAT (LISS/Coombs ID-Card, Bio-Rad; DG Gel Card, Grifols) with R_1R_1, R_2R_2 and rr RBCs.

Results: Qwalys 3 showed false-positive results for ABO forward typing and RhD typing. Although manual tube and CAT methods for ABO forward typing and RhD typing using washed RBCs showed no interference, false-positive results were identified in ABO reverse typing. Saline replacement resolved the interference in ABO reverse typing for the tube method. DAT and A1, H, and extended blood group antigen testing using washed RBCs showed no interference. Panreactive interference was observed in antibody screening in both manual tube and CAT methods at plasma IMC-002 concentrations of ≥10 and ≥1 μg/mL, respectively (Table 1).

Summary/Conclusions: Although IMC-002 showed interference in pre-transfusion testing the characteristics were distinct compared to other anti-CD47 monoclonal antibody drugs. The interference in ABO/RhD typing and antigen testing could be mitigated using washed RBCs and saline replacement. Panreactive results were observed in antibody screening at RT, 37°C and AHG phases with double populations in gel cards. However, the interference was not seen at relatively low drug concentrations.

PA21-L03 | $CD71^+$ RBCs mediate increased erythrophagocytosis and reduced monocytes in human suspension assay

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Background: Circulating immature red blood cells ($CD71^+$ RBCs) have been shown to modulate the immune system, potentially through their enriched intracellular reactive oxygen species (ROS). As $CD71^+$ RBCs are differentially present in blood products from male and female donors, they may play an important biological role in adverse donor-recipient sex-mismatched transfusion reactions. The erythrophagocytosis of RBCs by monocytes can be used in vitro to assess the risk of intravascular haemolysis from incompatible blood transfusions.

Aims: To investigate the effect of $CD71^+$ RBCs on the erythrophagocytosis and RBC lysis and how $CD71^+$ RBCs may affect these processes.

Methods: Enriched $CD71^+$ RBCs (32.3% ± 3.9, $n = 3$) and $CD71^-$ RBCs were isolated from donated whole blood using Percoll gradient density separation. We incubated enriched $CD71^+$ RBCs and $CD71^-$ RBCs with allogenic peripheral mononuclear cells (PBMCs) for 4 hours (37°C, 5% CO_2). $CD71^+$ RBCs were incubated in three ratios with anti-D sensitized $CD71^-$ RBCs in the presence of PBMCs. The phagocytosis index (RBC PI, the percentage of monocytes that phagocytose $CD71^-$ RBCs and $CD71^+$ RBCs) was determined using image flow cytometry by surface staining monocytes with CD14 and then intracellularly staining the RBCs with CD235a and CD71. RBC lysis was examined spectrophotometrically. Linear regression was performed to explore the relationship between the RBC PI, RBC lysis and $CD71^+$ RBC proportion. ROS antioxidant N-acetyl cysteine (NAC) was used to investigate how $CD71^+$ RBCs affect erythrophagocytosis. ROS was determined using dihydrodichlorofluorescein diacetate (H_2DCF-DA).

Results: The enriched $CD71^+$ RBC group showed a significantly higher RBC PI (11.3% ± 2.1%) compared to the $CD71^-$RBC group (5.9% ± 0.9%; $n = 3$; $p < 0.05$). There were positive correlations between the dose of $CD71^+$ RBC and $CD71^+$ RBC PI ($R^2 = 0.77$, $p < 0.0001$), and RBC lysis ($R^2 = 0.68$, $p < 0.01$). The high $CD71^+$ RBC group exhibited a significant reduction in the normalized monocyte number (46.8% ± 2.0%, $p < 0.01$), compared to the $CD71^-$ RBC group. Compared to the non-treated group (48.8% ± 3.0%), the reduction of normalized monocyte number was partially reversed in the NAC-treated group (46.6% ± 3.8%; $p > 0.05$). There was lower ROS expression in NAC-treated $CD71^+$ RBCs than in untreated $CD71^+$ RBCs ($p > 0.05$). There was no significant difference in RBC PI between the NAC-treated and untreated groups ($p > 0.05$).

Summary/Conclusions: Donated $CD71^+$ RBCs enhance erythrophagocytosis of $CD71^+$ RBCs in a dose-dependent manner and suppress monocytes. Treatment with NAC reduced ROS but did not affect $CD71^+$ RBC PI. This work contributes to understanding immature $CD71^+$ RBCs' role in post-transfusion immunobiology.

PA21-L04 | Evaluating the clinical significance of alloantibodies against GP.Mur using flow cytometry phagocytosis assay

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Background: Mur glycophorin expresses five kinds of low-frequency antigens including Mi[a], Mur, Hil, MINY and MUT. Alloantibodies against GP. Mur commonly are the mixed antibodies against multiple antigens carried by GP. Mur, which can be termed anti-Mi(a), and sometimes the specific antibody against single antigen. But the antibody identification for specificity is hard to be conducted since lacking a panel of cells with the rare glycophorin phenotype. In the Southeast Asia population with the high-frequency distribution of GP. Mur, anti-Mi(a) is commonly encountered, which could lead to haemolytic transfusion reaction and haemolytic disease of the foetus and newborn (HDFN). Usually, it causes only mild clinical symptoms, but in some cases, it can cause severe outcomes such as hydrops fetalis or even foetal death. The FCM phagocytosis assay has been established in previous studies to successfully predict the severity of clinical outcomes of alloanti-D. However, whether the assay could also be used for the prediction of the severity of clinical outcomes of anti-Mi(a) is unknown.

Aims: To attempt to use a flow cytometry phagocytosis assay to predict the clinical significance of anti-Mi(a).

Methods: Plasma of patients with anti-Mi(a) was collected. DTT was used to destroy the IgM of anti-Mi(a) in the plasma and retain the IgG anti-Mi(a). RBCs with GP. Mur phenotype were sensitized by the treated plasma containing IgG anti-Mi(a) respectively. A new Flow cytometry (FCM) two-colour phagocytosis assay was performed to analyse the phagocytic efficiency of monocytes on the sensitized RBCs. The titration of the anti-Mi(a) was also conducted. And the relationship between titters and phagocytic efficiency was analysed. The plasma from a special case of severe HDFN identified with anti-E combined with anti-Mur was also investigated by FCM phagocytosis assay to determine which antibody lead to the clinical outcomes.

Results: The phagocytic efficiency of monocytes was around 50%∼80% when the RBCs are sensitized by anti-Mi(a) from severe HDFN or hydrops fetalis patients ($n = 3$), while were less than 13% when the anti-Mi(a) are from patients with no related clinical outcomes ($n = 7$). The phagocytic activity of monocytes mediated by anti-Mi(a) was correlated with the antibody titters. The phagocytic efficiency was less than 30% when antibody titters were lower than 8 and increased sharply when titters ranged from 8 to 64, then, plateaued around 80% when titters were above 64. Furthermore, the FCM phagocytosis assay revealed similar phagocytosis activities when using E+Mur− and E−Mur+ RBCs respectively monocytes mediated by anti-E and anti-Mur mixed antibodies in the severe HDFN case.

Summary/Conclusions: FCM phagocytosis assay seems to be an effective method for predicting the clinical significance of anti-Mi(a). Prenatal monitoring should be conducted in pregnant women with anti-Mi(a) with higher phagocytic efficiency than 30%.

PA21-L05 | Detection of erythrocyte alloantibodies in patient sera using a novel flow cytometry approach

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Background: Accurate assessment of the presence of erythrocyte alloantibodies in a patients' blood is crucial for ensuring matched transfusions and avoiding immune reactions against the donor product. Currently, agglutination-based assays such as manual tube-based methods and gel card systems are used to identify erythrocyte-directed antibodies. However, these methods are semi-quantitative, consume a large amount of reagents and are not multiplex compatible.

Aims: The aim of this study is to develop a novel flow cytometry test that can detect erythrocyte-reactive antibodies in serum and plasma in a single tube assay and will result in increased sensitivity and specificity of alloantibody detection. The test is designed to convert the semi-quantitative agglutination assay into a quantitative flow cytometry assay, reduce assay costs by lowering reagents consumption and make it multiplex compatible.

Methods: To detect erythrocyte antibodies present in plasma/serum, the indirect antiglobulin test was modified to be compatible with a flow cytometry readout. Using 3–4 incrementing concentrations of NHS (succinimidyl ester)-fluorescein isothiocyanate (FITC) and pacific blue (PB), reference erythrocytes are differentially labelled to identify 12 different erythrocyte populations in a single flow cytometry tube. Alloantibodies within a patient sample bind to specific implicated antigen-positive erythrocytes in the 2D flow cytometry matrix. A fluorescent secondary F(ab)-fragment recognizing human IgG is used to identify the reference erythrocytes to which the antibody has bound. We composed a test set of 500 samples including samples with anti-c, C, E, e, D, K, Jk[a], Jk[b], M, N, Kp[a], Le[a], Le[b], Lu[a] Cw, Wr[a] and some single samples with other specificities, with different titters of antibodies. The clinical background of the patients was either screening during pregnancy or expected transfusion/surgery.

Results: The 2D flow cytometry matrix has been tested for the detection of IgG antibodies within samples from patients ($n = 500$). The required amount of erythrocytes can be reduced by 50–100 times, and the total volume of plasma/serum needed can be limited to 10 µL in total. This represents a significant reduction in volume compared to the agglutination test. A 94% concordance to conventional agglutination techniques was observed for IgG antibodies. Non-concordant samples contained alloantibodies that could potentially be of IgM class, which was confirmed in a

selection of samples by retesting using IgM specific secondary antibodies. The flow cytometry test showed an increased sensitivity in sample dilution experiments compared to the agglutination test ($n = 16$). Samples were diluted until they yielded negative results in the agglutination test (100–2000× dilutions). However, the flow cytometry test results for these samples remained positive. We observed that the 2D matrix can be freeze-thawed without loss of functionality. Additionally, the expiration time of the 2D matrix was found to be similar to that of conventional reference erythrocytes. Further validation is currently ongoing for samples that contain IgM antibodies.

Summary/Conclusions: We have developed a multiplex system through differential labelling of reference panel erythrocytes generating a 2D matrix that can be used for erythrocyte alloantibody specification, whereby antibody binding is detected by flow cytometry (patent pending). The flow cytometry test is fast, reduces costs due to reduced reagents requirements, generates objective readouts and can discriminate between IgG and IgM alloantibodies.

Parallel Session 22: When to transfuse?

PA22-L01 | Sickle cell disease update

C Eckhardt-de Groot

Abstract not available.

PA22-L02 | 2021 National comparative audit of NICE quality standard QS138

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Background: The UK's National Institute for Clinical Excellence (NICE) supports the improvement of outcomes for people using NHS and other public health and social care services by producing evidence-based guidance and advice, developing quality standards and performance metrics and providing a range of information services. NICE has developed Quality Standard 138 (QS138), which covers the general principles of blood transfusion in adults, young people and children over 1 year old. It describes high-quality care in priority areas for improvement. This audit evaluates compliance with the four quality statements set out in QS138 to highlight where practice is deviating from guidelines and identify opportunities for improvement.

Aims:

- Provide the opportunity to evaluate local evidence of compliance with NICE QS138
- Provide data to hospital teams to allow their understanding of what steps they can take to implement Patient Blood Management (PBM) and to measure its effectiveness in improving patient care
- Allow the transfusion community to benchmark the progress of PBM and improvements in patient care

Methods: All UK National Health Service providers (Trusts) were invited to take part in the audit. Trusts were allowed to enrol one or more hospitals, so we use the term "sites" to describe those that contributed data. Each participating site was issued with a stationery pack that allowed them to audit up to 40 patients. The audit standards were derived from the statements in QS138, and the audit was divided into four sections, A, B, C & D, and a patient's record could be used for more than one section. Data were collected on cases seen during October, November and December 2021, on transfusions that occurred during August to December 2021.

Results: 153 sites contributed data, representing approximately 64% (107/167) of UK Trusts

Data from 4679 patients were analysed

- 665/1131 (59%) of the patients who were known to have iron deficiency anaemia prior to being admitted for surgery were treated with iron before surgery
- 1079/1599 (67.5%) patients undergoing surgery with expected moderate blood loss received tranexamic acid
- 893/1534 (58%) patients receiving elective red blood cell transfusions had both their Hb checked and a clinical re-assessment after a unit of red cells was transfused
- 1032/1622 (64%) of transfused patients had evidence of receiving written or verbal information about the risks, benefits and alternatives to transfusion
- Only 422/1622 (26%) received both written and verbal information

Summary/Conclusions: The audit found evidence of significant compliance with elements of the four NICE Quality Statements for Blood Transfusion, but with some way to go to achieve uniformly good practice. There was and remains opportunities to improve patient care, with the potential to reduce length of stay and reduce costs, as well as protecting the blood supply by reducing unnecessary use of red blood cells.

Four out of every ten patients known to be iron deficient are not being given iron to combat anaemia, and a third of patients undergoing surgery are not receiving tranexamic acid. Both of these provide opportunities to reduce use of blood loss and the need for transfusion, a potential reduction in both cost and risk to patients.

In April 2023 a re-audit will be undertaken to determine if improvement is being achieved. Data collection will be repeated and further expanded to begin to understand the reasons for any continued non-compliance and highlight opportunities for further improvement. Headline results will be available allowing comparison between the audits cycles.

PA22-L03 | Predictors of the platelet corrected count increment, a secondary analysis of the PACER randomised controlled trial

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Background: Platelet transfusion effectiveness is measured with the corrected count increment (CCI; i.e., the difference between pre- and post-transfusion platelet counts, divided by body surface area). Several patient- and product-related factors have been associated with worse CCI. Different studies found different subsets of these factors to be predictive of CCI, which means their relative importance remains unclear.

Aims: To create a CCI-prediction model using augmented backwards elimination, and assessing model stability and variance-inflation and bias caused by variable selection, validating factors associated with lower CCI.

Methods: This is a pre-specified secondary analysis of the PACER study, a randomised controlled trial to assess the value of prophylactic platelet transfusion before central venous catheter (CVC) placement. Haematology ward and intensive care unit patients with platelet count 10–50 $\times$ 10^9/L were randomised to either one unit of prophylactic platelet transfusion or no transfusion before CVC placement. For this analysis, transfusion-arm patients who did not receive additional platelet transfusions in the 24 h after CVC placement were included. Pre-transfusion platelet counts were measured within 24 h before transfusion, and post-transfusion platelet counts were measured 24 h after transfusion. Variables were selected using augmented backwards elimination ($\alpha = 0.157$; $\tau = 0.05$), model stability, variance-inflation and bias were estimated using bootstrap resampling, and the influence of missing data was assessed using a multiple imputation sensitivity analysis (10 imputations).

Results: We included 131 complete cases in the primary analysis, and 166 patients in the multiple imputation sensitivity analysis. Sepsis, DIC, bone marrow depression, kidney failure and platelet concentrate (PC) irradiation were significantly associated with lower CCI (Table 1). The intercept CCI was 24.8 $\times$ 10^9/L. The bootstrap resampling and multiple imputation procedures arrived at the same final model (Table 2). Variable selection led to >10% bias for bone marrow depression and kidney failure. Additionally, the presence of allo-antibodies was included in 3 of 10 imputed models and storage length and storage medium in 4 and 7 of 10, respectively.

Summary/Conclusions: We found strong evidence for sepsis, disseminated intravascular coagulation, bone marrow depression, kidney failure and PC irradiation as negative predictors of platelet CCI. We found weak evidence for allo-antibodies as negative, and PC storage length and 100% plasma storage medium as positive predictors of CCI, possibly due to few events and missing data. We found no evidence for the influence of gender, age, body mass index, liver failure, fever and steroid use on CCI. This work is presented here on behalf of the PACER study group.

PA22-L03 – Table 1

	Global model		Selected model	
	Estimate	Std. error	Estimate	Std. error
Sepsis	−13.6	3.8	−13.7	3.4
Disseminated intravascular coagulation	−11.6	3.9	−11.6	3.6
Irradiated platelets	−6.4	2.5	−6.1	2.4
Bone marrow depression	−11.2	3.8	−11.3	3.4
Kidney failure	−7.2	3.7	−6.8	3.4
Storage medium 100% plasma	2.2	2.8	—	—
Storage length (days)	0.7	0.8	—	—
Allo-antibodies	−12.0	13.8	—	—

PA22-L03 – Table 2

	Bootstrap model			
	Inclusion frequency (%)	Median estimate	Variance inflation	Bias (%)
Sepsis	99.6	−13.8	0.87	2.0
Disseminated intravascular coagulation	99.2	−11.2	0.69	−1.6
Irradiated platelets	93.6	−6.2	0.97	1.8
Bone marrow depression	89.0	−10.8	1.79	12.0
Kidney failure	75.9	−7.2	1.37	25.8
Storage medium 100% plasma	31.9	0.0	1.14	111
Storage length (days)	30.8	0.0	0.96	83.0
Allo-antibodies	25.2	0.0	0.76	10.9

PA22-L04 | Profiling of RBC utilisation among RBC recipients in Singapore in 2015–2019

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Background: Red blood cell (RBC) transfusions are commonly used in the management of anaemia and other conditions. However, RBC use in clinical practice is not well studied, especially from a national context in Singapore. In order to improve patient outcomes and reduce transfusion-related risks, it is important to first understand RBC utilisation patterns in Singapore hospitals.

Aims: This study aims to profile the utilisation of RBC transfusions among hospital recipients in Singapore from 2015 to 2019, using national blood bank transfusion records and national medical insurance data (Mediclaims).

Methods: This retrospective cohort study used deidentified data from national blood bank transfusion records and national medical insurance data (Mediclaims). The study population consisted of all Singapore public hospital inpatients and outpatients who received at least one RBC transfusion between 2015 and 2019. Patient demographics, final diagnosis code (ICD-10), and transfusion information were extracted and merged from the two datasets. Final diagnosis codes were mapped to 15 disease categories associated with transfusions, and descriptive statistics were performed to characterise the patterns of RBC utilisation in Singapore.

Results: 183,045 visits and 133,872 patients were recorded, with a total of 470,500 RBC units used between 2015 and 2019. Yearly RBC use trends remained relatively stable between 2015 and 2019. Male patients used 243,366 RBC units while female patients used 226,323 RBC units. Patients in the age group 60–69 years old used the most RBC units ($n = 111,265$ RBC units). Overall, inpatients ($n = 112,632$ using 455,165 RBC units) utilised far more RBC units compared to outpatients and other admission types ($n = 2622$ using 15,335 RBC units). The median RBC units per visit was 2.0 (IQR 1.0–3.0), while the mean number of RBC units transfused per visit was 2.6.

Male patients had a higher mean RBC utilisation per visit (2.9 RBC units per visit) compared to female patients (2.3 RBC units per visit). Both male and female patients used a median of 2.0 RBC units per visit, but males had a larger IQR range (1.0–3.0) compared to females (IQR 1.0–2.0). Although inpatient visits required more RBC units overall compared to outpatient visits, outpatient visits required more RBC per recipient (mean = 6.8 RBC units per recipient) than inpatient visits (mean = 4.0 RBC units per recipient). Visits resulting in death had a higher RBC per visit (mean = 4.7 RBC units per visit), compared to other visit outcomes (mean = 2.4 RBC units per visit). Visits requiring ICU also utilise higher RBC per visit (mean = 4.5 RBC units per visit), compared to non-ICU visits (mean = 2.1 RBC units per visit). There were also significant variation in RBC use across different disease categories, with neoplasms requiring the most RBC units.

Summary/Conclusions: This study provides a comprehensive profile of RBC utilisation among hospital recipients in Singapore from 2015 to 2019, for example, visits with neoplasm-related diagnoses utilised the most RBC units among transfusion-associated disease categories. The findings quantifies the variations that exist in RBC utilisation in clinical practice in Singapore, which provides valuable insights for further optimisation of patient blood management and hospital clinical practice in Singapore. These results can inform clinical practice and resource allocation decisions, ultimately leading to improved patient outcomes.

PA22-L05 | Pulmonary and thrombotic outcomes in plasma and platelet transfusion recipients prior to and following periods of blood donor SARS-CoV-2 infection and vaccination

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Background: With ongoing SARS-CoV-2 infections and primary and booster vaccinations, there has been a significant rise in the prevalence and levels of anti-SARS-CoV-2 antibodies in the blood donor population. Without specific evidence, some patient advocacy groups have raised concern about the receipt of transfusions from COVID-19 vaccinated and antibody positive blood donors. While the efficacy of COVID-19 convalescent plasma has been studied in clinical trials, the safety of transfusion of SARS-CoV-2 antibodies in high plasma volume blood components to recipients without ongoing SARS-CoV-2 infection is not established.

Aims: We assessed whether transfusion of plasma or platelet blood components during periods of varying prevalence of blood donor SARS-CoV-2 infection and vaccination were associated with differences in thrombotic events or oxygen requirements in recipients without ongoing SARS-CoV-2 infection.

Methods: We performed a retrospective cohort study of adults who tested negative for SARS-CoV-2 by PCR and were transfused platelet or plasma blood products during hospitalization between 3/1/2018 and 2/28/2022. Based on donor SARS-CoV-2 epidemiology and antibody testing results, we defined the "pre-COVID-19 period" from 3/1/2018 to 2/28/2020, the "pre-vaccine COVID-19 period" from 3/1/2020 to 2/28/2021 and "post-vaccine COVID-19 period" from 3/1/2021 to 2/28/2022. We assessed the maximal oxygen requirements prior to and daily for seven days following transfusion. We also assessed the incidence of arterial and venous thromboses after transfusion using a combination of diagnosis codes and new anticoagulant administration during hospitalization. Patients with a history of thrombosis, prior anticoagulation, or who already required increased levels of oxygen support prior to transfusion were excluded. Multivariable logistic regression adjusting for demographics and comorbidities was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for outcomes of thromboses and increased oxygen requirements separately.

Results: Of 20,417 hospitalized patients transfused plasma or platelet products, thromboses occurred in 4.5% and oxygen requirements were increased in 24.5%. In plasma transfusion recipients, there were no trends for outcomes of thrombosis ($p = 0.65$) or increased oxygen requirements ($p = 0.99$) across study periods. In platelet transfusion recipients, there were no trends for outcomes of thrombosis ($p = 0.81$) or increased oxygen requirements ($p = 0.25$) across study periods. Compared to the pre-COVID study

period, the adjusted OR for thromboses was 0.9 (95% CI 0.7–1.2; $p = 0.55$) during the pre-vaccine COVID-19 period and 0.8 (95% CI 0.7–1.1; $p = 0.17$) during the post-vaccine COVID-19 period. Compared to the pre-COVID study period, the adjusted OR for increased oxygen requirements was 0.9 (95% CI 0.8–1.0; $p = 0.15$) during the pre-vaccine COVID-19 period and 1.0 (95% CI 0.9–1.1; $p = 0.97$) during the post-vaccine COVID-19. Among unvaccinated transfusion recipients, there were no trends for outcomes of thromboses ($p = 0.97$) or increased oxygen requirements ($p = 0.99$) across study periods.

Summary/Conclusions: Transfusion of plasma and platelet blood components collected during the pre-vaccine and post-vaccine periods of the COVID-19 pandemic was not associated with increased thrombotic events or oxygen requirements in recipients without ongoing SARS-CoV-2 infection.

Parallel Session 23: CD36 (and beyond)–Jack of all trades

PA23-L01 | Shared antigen carriers

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Different cell carriers express various shared antigens, which may cause alloimmunization or isoimmunization and consequently destruction of the carrier cells, but not necessarily the immunizing cells. In transfusion medicine, red blood cells (RBC), platelets and leukocytes, including leukocyte subsets, are the main antigen carriers of interest. When antibodies targeting RBCs are produced, haemolytic reactions, including haemolytic disease of the newborn (HDN), may occur; when platelets are targeted, conditions of immune-mediated thrombocytopenia, such as foetal neonatal alloimmune thrombocytopenia (FNAIT) and platelet transfusion refractoriness (PTR) may develop, and when neutrophils are the target, conditions of immune-mediated neutropenia, such as neonatal alloimmune neutropenia (NAN), may occur. HLA class II alloantibody binding to monocytes is one of the patho-mechanisms of transfusion-related acute lung injury (TRALI), a severe transfusion reaction. Once triggered by these antibodies, monocytes release soluble mediators that increase permeability of alveolar capillary endothelium. Similarly, allo-/isoantibodies targeting other antigen types expressed on monocytes, such as CD36 (GPIV), also results in TRALI. Thus, antibodies reactive with antigens expressed on monocytes may, theoretically, cause TRALI through a similar mechanism. In addition, alloantibodies to human neutrophil antigen (HNA)-3a have been implicated in TRALI, with pulmonary endothelium damage due to antibody-activated neutrophils as well as the direct antibody biding to endothelium. In addition, antibodies produced through blood transfusion may target other carrier cells, such as endothelial cells in transplanted organs or present on progenitor stem cells, causing transplantation rejection reactions. It is important to realize that allo-/isoimmunization may occur through exposure to one type of carrier, but the reaction/disease may occur through targeting of a different one.

The balance between antibody titters and antigen expression level on the carrier cells or the antigen distribution in different carriers determine the severity of immune-mediated conditions. When HDN caused by Rh(D) incompatibility is compared with that caused by ABO blood group type incompatibility, the disorder tends to be more severe with Rh(D), which is specifically expressed on RBC, whereas ABO blood group type is widely expressed in different cell types. ABO blood group incompatibility may also cause PTR and FNAIT, but usually without an evident clinical picture due to its wide distribution of ABO blood group antigens, in addition to the low/moderate expression on platelets; however, a small proportion of high ABO expressers is reported, and in such cases, the evident clinical picture may develop.

Therefore, it is essential to understand on the shared antigen carriers, the level of antigen expression in each type of carrier, and the antigen distribution, in addition to the antibody titters, to realize on the potential risks associated with allo-/isoimmunization to these antigens.

PA23-L02 | Challenging the definition of a blood group: Proposal to make CD36 a novel blood group system based on a case report and pre-existing genetic, proteomic and clinical data

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Background: Polymorphic molecules expressed on certain blood cells are traditionally categorized as blood groups, human platelet antigens (HPA) or human neutrophil antigens (HNA). Whilst some blood groups like Rh are mostly erythro-specific, others like ABO have wide tissue distribution and are termed histo-blood groups. Furthermore, some polymorphic systems are shared between blood cell lineages, for example, the ABO and MAM blood groups are mainly present on red blood cells (RBCs) but also found on platelets. Similarly, Choline Transporter-Like Protein 2 (CTL2), both underlies a blood group system and carries the HNA-3a antigen on neutrophils.

Aims: To evaluate a blood donor lacking a molecule normally expressed on both erythroid cells, platelets and selected leucocytes.

Methods: Surface markers were monitored on developing cells during *in vitro* erythroid culture of CD34+ cells, obtained from left-over material in leucocyte waste bags produced during automated blood component preparation. Serological, flow cytometric and proteomic analyses were performed on RBCs, platelets and cultured cells. A literature review of proteomic analyses of RBCs, as well as clinical case reports involving both haemolytic disease of the foetus and newborn (HDFN) and foetal/neonatal alloimmune thrombocytopenia (FNAIT) was performed.

Results: When culturing CD34+ cells towards erythroid differentiation, we noted that one donor appeared to produce burst-forming units erythroid (BFU-E) characterised as CD34+/CD36-/CD71low/IL3R-/GPA- by flow cytometry while failing to progress to colony-forming units erythroid (CFU-E). The latter is expected to become CD34-/CD36+/CD71+/IL3R-/GPA- but in contrast to cells from other donors, this donor's cells remained CD36- throughout culture. Apart from being expressed during erythropoiesis, CD36 also carries Nak(a), a platelet antigen. However, CD36 was undetectable on platelets from this donor. Sequencing of *CD36* identified homozygosity for c.1133G>T (rs146027667:T), which has a frequency of 0.1% globally (gnomAD, v.3.1.2). This variant encodes p.Gly378Val, and is known to abolish CD36 expression. By flow cytometry, we saw marginal staining of RBCs with anti-CD36 in control donors. Literature review indicated that CD36- phenotype is not uncommon in Asia and that anti-CD36 can lead to both FNAIT and HDFN [Okajima, Thromb Haemost 2006; Xu, Int J Hematol 2018; Flesch, Transfusion 2021]. These are clinical features normally attributed to HPA and blood groups, respectively. Whilst CD36 is considered absent from mature RBCs, we mined datasets from a recent proteomic study of RBC membranes and found evidence for CD36-derived peptides in the absence of HPA-related peptides, suggesting CD36 peptides were not due to platelet contamination [Bryk, J Proteome Res, 2017]. Also, low-level CD36-mRNA is found in reticulocyte datasets [Goh, Physiol Genomics 2007; Skulski, J Cell Mol Med 2019].

Summary/Conclusions: A growing number of polymorphic molecules are realized to exist on both RBCs and platelets. Based on the case reported here and our literature review, we conclude that CD36 appears to fulfil criteria for becoming a new blood group system. However, it is not clear if a blood group molecule needs to be detectable on mature RBCs or if reticulocyte and/or proteomic evidence suffices, especially in a case like CD36 where earlier erythroid cell stages express it at high levels. There is also convincing evidence that anti-CD36 cannot only cause FNAIT but also HDFN.

PA23-L03 | Transcriptome sequencing analysis identifies MMP-9 as a potential key gene in CD36 antibody-mediated transfusion-associated acute lung injury

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Background: Transfusion-related acute lung injury (TRALI) is a severe complication of blood transfusion, which can be fatal in severe cases. Immediate recognition and treatment are critical to prevent serious complications. The mechanism behind TRALI is largely unknown, and no specific treatment exists to explain the TRALI mechanism.

Aims: This study aimed to explore the pathophysiological mechanisms of TRALI through transcriptome sequencing and bioinformatics analysis and to identify potential key genes.

Methods: In this study, C57BL/6J mice were divided into three groups: the Normal group, the LPS group, and the TRALI group. A TRALI mouse model was established through the intraperitoneal injection of lipopolysaccharide and Anti-CD36 monoclonal antibody (32–106). The efficacy of the model was evaluated through indicators of acute lung injury, and mRNA transcriptome sequencing was conducted on mouse lung tissue, followed by bioinformatics analysis to pinpoint key genes and pathways. Analysis of Gene Ontology, KEGG, and Reactome enrichment, protein-protein interaction network, hub-gene and hub-gene pathway enrichment are all included. The results were further validated using RT-qPCR, Western Blot, serum ELISA assay, and fluorescence micrographs.

Results: The study successfully established a mouse model of CD36 antibody-mediated transfusion-associated acute lung injury. Transcriptome sequencing on lung tissue, which were validated are credible, from the LPS, and TRALI groups identified 879 differentially expressed genes (DEGs), which underwent GO, KEGG, and Reactome enrichment analysis. The results indicated that the cytokine-cytokine receptor interactions, IL-17 pathway and MAPK pathway play essential roles in TRALI. The PPI network diagram and Cytoscape 3.9.1 identified 10 potential hub-genes, including TNF, FOS, TLR2, CXCL1, CXCL2, IL-6, IL-10, CCL2, CCL4, and MMP-9. By conducting a literature review, MMP-9 was identified as a potential key gene for further investigation. The RT-qPCR, Western Blot, ELISA and fluorescence results all revealed a significant rise in MMP-9 expression in TRALI mice.

Summary/Conclusions: This study identified MMP-9 as a potential key gene involved in TRALI. Further investigation is needed to fully understand the role of MMP-9 in TRALI and its potential as a therapeutic target for TRALI treatment. This study provides a foundation for future research aimed at developing effective treatments for TRALI and improving patient outcomes.

PA23-L04 | The platelet-activating factor pathway can mediate the adverse inflammatory reactions associated with anti-erythrocyte antibody therapy in a murine model

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Background: Anti-D is a highly effective polyclonal anti-erythrocyte antibody used to treat patients with immune thrombocytopenia (ITP). Unfortunately, adverse effects and an FDA black box warning have limited its clinical use, highlighting the need to better understand the adverse event profile of anti-erythrocyte antibody-based therapy. Analogous to anti-D, the murine erythrocyte-specific antibody, TER119, has disease ameliorative activity and is also an antibody with an inflammatory signature. Therefore, TER119 has been used as a surrogate antibody to help understand the mechanism of adverse

inflammatory reactions of anti-erythrocyte antibodies. We hypothesize that inflammation caused by TER119 occurs via the platelet-activating factor (PAF) pathway.

Aims:

- Verify the therapeutic ability of TER119 in antibody-mediated murine ITP
- Assess the inflammatory profile of TER119 through changes in body temperature
- Investigate the role of the PAF pathway in TER119-induced inflammation
- Determine the role of the IgG Fc region and macrophages in this inflammation

Methods: Murine ITP was induced by injection of an anti-platelet antibody (MwReg30). Body temperature was measured in 15-min intervals post-treatment as a metric of systemic inflammation. The therapeutic activity of TER119 was assessed by enumerating platelets 24 h post-MwReg30 treatment. Select animals received an additional pre-treatment with PAF-receptor antagonists (ABT-491 and WEB2086) to examine the role of the PAF pathway in TER119-induced body temperature changes. To assess the involvement of macrophages in causing these inflammatory reactions, mice received clodronate liposomes to deplete macrophages 72 h prior to the administration of TER119, after which body temperature was assessed. To evaluate the functional role of the Fc domain in causing inflammation, deglycosylated TER119 was administered in vivo and examined for its ability to induce body temperature changes.

Results: TER119 significantly increased platelet counts in murine ITP with an associated decrease in body temperature. Temperature changes were ameliorated with two structurally different PAF-receptor antagonists, which did not interfere with the ability of TER119 to increase platelets counts. Macrophage depletion and antibody Fc deglycosylation were both independently sufficient to resolve changes in body temperature caused by wildtype TER119. Thus, our work demonstrates a novel role of the PAF pathway in inflammatory reactions caused by TER119 and provides a strategy to mitigate it.

Summary/Conclusions: TER119 has been well-known to improve platelet counts in murine ITP but also to have important ameliorative activity in a number of other autoimmune and inflammatory diseases. Like anti-D however, it suffers from an inflammatory profile. This work demonstrates that TER119-induced inflammatory decreases in body temperature are driven through the PAF pathway, which can be mitigated by PAF-receptor antagonist pre-treatment. Advancing our understanding of the inflammatory profile of anti-erythrocyte antibody therapies in murine models may assist in identifying safer and more accessible treatments for ITP patients.

PA23-L05 | A case of platelet transfusion refractoriness due to anti-CD36 with a successful treatment outcome in a patient with aplastic anaemia and allogeneic stem cell transplantation

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Background: The most frequent cause immune-mediated platelet refractoriness (PR) and of secondary thrombocytopenia is the production of anti-human leukocyte (HLA) and anti- human platelet antigen (HPA) antibodies. In some ethnic groups (African and Asian), the lack of CD36 platelet-monocyte antigen (glycoprotein 4, GPIV) enables the development of complementary antibodies when non-compatible platelets (CD36+) are transfused. In Caucasians, the prevalence of the CD36 negative phenotype is only 0.4%. The difficulty to find compatible donors, despite trying international search make the management and treatment in these patients a multidisciplinary challenge.

Aims: We present the case of a 7 years old female patient, with a post-hepatitis aplastic anaemia (AA) and PR that needed an HLA bone marrow identical related donor transplant and Identify the PR cause in order to provide the best transfusion support to the patient.

Methods: The patient received systemic corticosteroid therapy, remitting the hepatitis but not the cytopenias, She needed the transfusion of packed red blood cells and platelets concentrates. The very low pos-transfusion platelet count was compatible with PR. Patient´s samples were sent to the Blood Transfusion Center of Madrid. Platelets antibodies identification (PkLxAssay, Immucor and Luminex method) and CD36 phenotyping (Navios EX,Beckman by Flow Cytometry) were performed. The patient was treated with a thrombopoyetin receptor agonist (Eltrombopag) and therapeutic apheresis, using immunoabsortion columns (IC) (LIFE21®apheresis, Therasorb TM) to adsorb anti-GPIV antibodies present in her plasma .

Results: Antibodies against GPIV were detected (mean fluorescence intensity (MFI:1394). No CD36 negative familiar/unrelated donor was found. The patient received pre-transfusional Human Nonspecific Immunoglobulin intravenous (IVIG) (0.8 g/kg) therapy and incompatible (CD36+) apheresis platelets units, because presented a platelet count < 5000 × 109/L and haemorrhagic complications. No response was obtained and haemorrhagic complications were in progression. The risk of causing a secondary volume overload due to IVIG treatment was high. This forced to change the treatment introducing oral prednisone (maintenance dose of 2 mg/kg.) and high-dose methylprednisolone (2 g, IV bolus) before and after platelets transfusions. After two subsequent transfusions of apheresis platelets, any response was observed. Then the patient was treated with therapeutic apheresis (4) and Eltrombopag (50 mg/24 h). No initial improvement in the platelet count was observed. However, the detection of

platelets antibodies becomes negative several days later (MFI-188) and the bleeding symptoms improved until their complete resolution. The patient reached a normal platelet count been able to undergo the bone marrow transplant. In the last outpatient check-up, presented a trilinear graft. Nowadays she is in remission of her underlying disease (AA).

Summary/Conclusions: PR secondary to anti-GPIV in CD36 negative patients presents enormous morbidity and mortality. The probability to found compatible platelets donors is very low. Strategies should be focused on lowering the antibodies titre by apheresis techniques associating thrombopoyetin analogues. The pathophysiological similarity between PR and immune thrombocytopenia (ASFA 2019 guidelines) could support this indication. IC may have played a main role in the antibody and bleeding reduction/disappearance. The evidence describes that its effect is usually delayed and dose-dependent (5–7 sessions). A multidisciplinary approach and the combined use of all possible therapeutic strategies could have determined success in this patient.

Parallel Session 24: Blood services wider contribution to public health

PA24-L01 | Important role for donor and recipient biorepositories in global surveillance and epidemiological studies

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Since the 1970s, studies of archived samples from blood and plasma donors and transfusion recipients have contributed to advancing the safety of transfusions, as well as to surveillance for infectious diseases, development and performance assessments of donor screening and diagnostic assays, and epidemiological and pathogenesis research. Progressive approaches to establishing and executing studies utilising biorepositories have been critical to many of these contributions. Studies of blood and source plasma donor biorepository samples were critical to discoveries of several major pathogens, and to development of donor screening and diagnostic assays and infection staging systems for hepatitis viruses, retroviruses, arboviruses and parasitic infections. Donor repositories have provided the basis for immunological and molecular "observatories" for large-scale population studies to rapidly investigate the epidemiology of emerging infectious diseases, and have contributed to understanding of disease penetrance and correlates of protection of infection and symptomatic outcomes. Establishment of donor cohorts, with biobanking of longitudinal follow-up samples from acutely infected donors along with survey data, have led to significant contributions to disease pathogenesis and biomarker discovery research. Most recently, the global blood bank community has made major contributions to SARS-CoV-2/COVID-19 pandemic response through (1) donor-based serosurveillance studies using serial cross-sectional and longitudinal repositories and linked surveys, (2) studies of samples from donors reporting post donation illness (PDI) and (3) serial samples from infected donors enrolled into prospective cohorts to characterise virologic, immunologic and "omics" parameters that correlate with clinical outcomes including long-COVID. Beyond contributing to infectious disease surveillance, epidemiology, and pathogenesis research, donor data and biobanks have been linked to regional and national registries to investigate non-infectious health outcomes in donors and identify potential prognostic biomarkers of disease. Donor data and samples linked to component production, transfusion practices, and recipient characteristics and outcomes (vein-to-vein databases), enable powerful studies to identify donor, transfusion and recipient factors that impact transfusion efficacy and complications. Salient examples include the US REDS programs, ScanDat, the Danish Donor Study, the UK BioBank, French, Japanese, Netherlands national programs as well as more recent initiatives in Australia, Canada and many other countries. Application of "omics" technologies, including genomics, metabolomics, proteomics, glycomics, immune profiling and "exposome" analyses, to samples in these repositories has and will continue to be critical to contributing to surveillance and epidemiological research and advancing the field of Precision Transfusion Medicine.

PA24-L01.1 | Generating synthetic blood transfusion data for haemoglobin deferral prediction

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Background: Synthetic data generation is becoming an increasingly popular approach to make privacy-sensitive data available for analysis. Recently, we proposed an approach for synthetic data generation (Kroes, Journal of the American Medical Informatics Association, 2022) by means of a mixed sum-product network (MSPN), that demonstrated both high utility and privacy in simulations, but the method has not been applied to real world personal data.

Aims: To test the capability of the MSPN approach for generating an anonymised dataset from personal blood donor data which is capable to reproduce analysis results obtained from the original dataset.

Methods: Data from the Dutch national blood bank consisting of 250,729 donation records were used to predict donor haemoglobin levels by means of support vector machine (SVM) models. These analyses were replicated with synthetic data generated with the MSPN approach. Privacy was evaluated by quantifying to what extent sensitive information can be extracted by using background information (i.e., attribute disclosure), whereas the quality of the analyses was

evaluated by comparing precision and recall of the SVM models and the importance ranking of various predictor variables.

Results: Predictions from the SVM models trained on synthetic data were for 96% the same as the predictions made with the original SVM models. Precision was equal for both male and female donors, recall was 0.003 higher for males and 0.009 lower for female donors. The importance of the variables for Hb predictions, quantified and visualised with Shapley additive explanation values, were very similar. Opportunities for attribute disclosure were removed for all but two variables. Only the binary variables "Deferral Status" and "Sex" could still be inferred.

Summary/Conclusions: The similarities in predictions and predictive reasoning between the SVMs based on original and synthetic data indicate that the synthetic data generated by the MSPNs could be used instead of the original data without compromising predictive performance. This indicates the potential of this method for data sharing and explorative data exchange in practice. Future research should be targeted at further reducing the risk of attribute disclosure.

PA24-L02 | The effect of COVID-19 interventions on virus nasal carriage among Danish blood donors

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Background: The COVID-19 outbreak was unprecedented in scale and many questions regarding virus transmission and prevalence needed answering. In order to reduce the infection rate, health authorities introduced several interventions to restrict the pandemic. Two partial lock-downs of Denmark were initiated March 11 and December 09, 2020. COVID-19 interventions such as the closing of schools, public institutions and prohibition of group gatherings were implemented, only public employees with essential work were allowed to work and many private businesses were closed. The society was gradually reopened from April 15, 2020 and 08 February, 2021. The interventions against COVID-19 may play an important role in the transmission of not only severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) but also other common respiratory viruses and inadvertently lower the prevalence of these. More knowledge on this matter could impact future strategies to reduce the burden of respiratory viruses on morbidity, sick leave and health care expenses.

PA24-L02 Table 1. Overview of number of samples analysed and positive-percentage of human rhinovirus

	2018	2019	2020	2021
January	50 (8.0%)	32 (6.3%)	30 (6.7%)	100 (1.0%)
February	50 (6.0%)	67 (9.0%)	49 (2.0%)	100 (3.0%)
March	100 (5.0%)	97 (6.2%)	77 (2.6%)	100 (8.0%)
April	176 (6.3%)	155 (3.2%)	189 (0.5%)	65 (7.7%)
May	44 (2.3%)	50 (4.0%)	50 (4.0%)	50 (2.0%)
	420	401	395	415

Aims: We examined the prevalence of 24 virus sequences of common respiratory tract viruses in samples from the nostrils among healthy asymptomatic blood donors before and after the partial lock-downs in Denmark.

Methods: We analysed nasal swabs collected in 2018–2021 from 1631 participants (Table 1) in the Danish Blood Donor *Staphylococcus aureus* Study for 11 common respiratory tract viruses and subtypes using advanced PCR (Fluidigm Biomark HD, Integrated Fluidic Circuits). Further samples from 1369 participants are being analysed. We compared the prevalence of each virus among asymptomatic donors reporting for blood donation during the partial lock-down interventions and equivalent calendar months in the years before the lock-down using logistic regression adjusted for sex and age. Results are presented as odds ratio (OR) with 95% confidence interval (CI).

Results: The sex distribution in this study population was 56.7% male and the mean age was 41.4 years. In all periods, Human Rhinoviruses 1/2 (RV) were the viruses most frequently detected (4.4%) followed by Human Bocavirus (1.2%), Human Respiratory Syncytial Virus A/B (0.3%), Adenovirus 1/2 (0.2%), and Human Parainfluenza 1 (0.2%). We observed a lower odds of RV in April 2020 compared with April 2018, 2019 and 2021 (OR = 0.20, CI: 0.03–0.66). Similarly, the odds of RV in January 2021 was lower than in equivalent months in 2018–2020 (OR = 0.13, CI: 0.01–0.71). After partial or full re-opening, however, the odds of RV in May 2020 did not differ from equivalent months in other years (OR = 1.49, CI: 0.20–8.12). Adjustment did not change effect sizes. Of note, SARS-CoV-2 was not detected in any sample.

Summary/Conclusions: RV were the main common respiratory tract viruses detected in the nose among asymptomatic blood donors. Our results suggest that the effect of COVID-19 interventions after partial-lock-downs lowered the incidence of RV but the effect ceased quickly after reopening was initiated. No SARS-CoV-2 was detected which indicate sufficient deferral measures against COVID-19 in the blood centres.

PA24-L03 | Influence of sex, age, BMI, and smoking on 47 circulating inflammatory and vascular stress biomarkers in 9876 healthy individuals: Results from the Danish blood donor study

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Background: Large-scale blood donor cohorts and biobanks can be used to inform public health. Here we used plasma samples randomly picked from the biobank of The Danish Blood Donor Study to measure 47 inflammatory and vascular stress biomarkers. Information on expectable concentrations is scarce for plasma biomarkers in healthy individuals stratified by sex, age, BMI and common lifestyle factors such as smoking, that is, phenotypes that are not exclusive to the criteria defining a healthy individual. The emerging use of biomarkers in research and tailored care introduces a need for information about the association between inflammation biomarkers, basic demographics and lifestyle factors.

Aims: We examined the association between biomarkers of inflammation and vascular stress and sex, age, Body Mass Index (BMI), current smoking, and time of day of the sampling to present the influence of these lifestyle and demographic factors and to present expectable concentrations in healthy individuals.

Methods: A selection of 47 predefined biomarkers were measured in plasma samples from 9876 participants in the Danish Blood Donor Study. The participants were selected to ensure an equal sex and age distribution between 18 and 69 years. The measured biomarkers included several interleukins, cytokines, chaemokines and markers of vascular stress. Using adjusted linear regression models, we examined the association between biomarkers and sex, age, Body Mass Index (BMI), current smoking, and time of day of the sampling. Additionally, patterns were assessed and compared between strata using heatmaps.

Results: We observed a general increase in concentrations of measured biomarkers of inflammation and vascular stress with higher age, BMI and smoking. For multiple biomarkers, the concentration associated with age, BMI, and smoking. Additionally, concentrations but also associations differed significantly between male and female participants. Most biomarkers displayed time-of-day variation. For age, sex, and BMI we provide detailed information on these associations and on the observed concentrations.

Summary/Conclusions: This study provides solid and comprehensive information on concentrations of 47 circulating inflammatory and vascular stress biomarkers in healthy individuals. The results demonstrate the influence of sex, age, BMI, smoking, and time of day. The study emphasizes that in-depth knowledge about biomarker concentrations in healthy individuals is critical for improved understanding of disease pathology and for developing precision diagnostics and tailored care and decision support tools based on biomarkers. Furthermore, the established dataset allows us to investigate a plethora of new research questions when linked to a rich database of phenotypes.

PA24-L04 | Development of a nationwide repeat blood donor cohort to monitor SARS-CoV-2 serosurveillance and population immunity

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Background: Estimating population-level SARS-CoV-2 incidence, including vaccine breakthrough infections (VBTI) and reinfections (RI), is critical for public health surveillance. The prevalence of the population with a history of previous infection and the incidence of reinfections have both increased. Repeat, cross-sectional seroprevalence studies cannot estimate the incidence of reinfections.

Aims: We describe the objective and development of the first, nationwide repeat blood donor cohort (RDC); the RDC enables analyses of evolving SARS-CoV-2 infections and immunity in the U.S. population.

Methods: In this prospective cohort study, Vitalant and American Red Cross repeat blood donors were selected for inclusion in the RDC based on anti-spike (S) and anti-nucleocapsid (NC) serological testing results and reported COVID-19 vaccination history. During June 2020-July 2021, all blood donations were tested for SARS-CoV-2 antibodies and reactive samples frozen and archived. RDC donors were categorized according to SARS-CoV-2 infection and COVID-19 vaccination status at Q2 2021 and each quarter in 2022. Subsequent to July 1, 2022, serum/plasma samples were prospectively captured, frozen and stored from routine donations by RDC donors, and single samples were randomly selected each quarter and assayed for antibodies (Ab) to SARS-CoV-2 S (Ortho VITROS Anti-SARS-CoV-2 IgG Quantitative test) and NC (Ortho VITROS Anti-SARS-CoV-2 Total N Antibody test). Donors provided self-reported detailed vaccination history, infection history and clinical outcomes in response to quarterly online surveys. Participants were categorized into four groups, based on infection and vaccination history; previous infection was defined as any positive anti-NC test and vaccination status was reported on routine donation history questionnaires.

Results: The RDC, comprised of 142,612 repeat donors, was created to enable the following objectives and analyses: (1) establish methods to identify and discriminate primary infections, VBTI, and RI using longitudinal antibody results; (2) estimate quarterly (Q) seroprevalence and incidence from Q2 2021 to Q4 2022, weighted to better represent the US population; (3) characterise antibody kinetics following SARS-CoV-2 infection and vaccination; (4) determine the effectiveness of vaccinations for protection from infection and symptomatic disease; and (5) evaluate quantitative binding antibodies as a correlate of protection against infection and severe disease. The table shows initial results of population-weighted seroprevalence by history of infection and vaccination status from Q2 2021 to Q2 2022. Among all donors, the prevalence of those with serological evidence of previous infection increased from 20.3% to 58.4%, 2/3 of whom had also been vaccinated (hybrid immunity).

Table. Estimates of U.S. prevalence of previous SARS-CoV-2 infection and COVID-19 vaccination over time during Q2 2021–Q2 2022.

Q1: quarter 1 (Jan–Mar), Q2: quarter 2 (Apr–Jun)

Summary/Conclusions: This nationwide RDC is uniquely able to report U.S. estimates of evolving hybrid immunity which will include RI and VBTI rates. These data will be important to understanding the continued epidemic spread and evolving immunity to SARS-CoV-2 in the United States.

PA24-L04 – Table 1

Previously infected/ vaccinated	Number at baseline	Q2 2021	Q1 2022	Q2 2022
NO/NO	43,887	31.60%	7.60%	5.60%
NO/YES	65,992	48.10%	40.70%	35.90%
YES/NO	12,751	11.70%	19.90%	21.40%
YES/YES	19,969	8.60%	31.80%	37.00%
Total	142,599			

Parallel Session 25: The donor factor

PA25-L01 | Impact of donor factors on blood product quality and transfusion outcomes

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Background: Heterogeneity among blood donors reflects fascinating interactions between genetic, biologic, and lifestyle factors that contribute to inter-donor variability in the quality of blood products. These observations have led to the investigation of donor characteristics that may impact RBC transfusion effectiveness through the creation of donor-recipient linkage studies.

Aims: (1) To review the current understanding of donor-specific differences in RBC storage outcomes. (2) To discuss the molecular mechanisms, which contribute to donor differences in RBC biology. (3) To review donor-recipient linkage studies.

Methods: We reviewed data and outcomes from studies that linked donor factors with stored RBC characteristics including haemolysis, osmotic and oxidative injury, or with transfusion effectiveness/outcomes measured by haemoglobin increments or adverse events.

Results: Table 1 summarises key findings from cumulative studies.

Conclusions: Genetic, biologic, and lifestyle factors in blood donors demonstrate strong associations with RBC metabolism, biophysical characteristics, and susceptibility to haemolysis during cold storage. The clinical impact of these factors is under investigation. Identifying donor factors that predict RBC transfusion effectiveness is an essential step towards precision transfusion medicine.

PA25-L01 - Table 1: Selected donor factors that demonstrated significant associations with RBC storage or transfusion outcomes.

Donor factor	Stored RBCs	RBC transfusion effectiveness
Sex	Male donors: increased storage, osmotic and oxidative haemolysis	Males: higher Hb increments. Females: possible associations with adverse events
Sex hormone intake	TRT: increased oxidative haemolysis and decreased membrane deformability. Progesterone: antihaemolytic effects related to RBC calcium channel inhibition	Lower PTR of irradiated RBCs from TRT donors transfused into mice; Lower Hb increments in RBCs from TRT donors
Age	Increased oxidative haemolysis in RBCs from teenage donors. Age differences in MCV, RBC viscosity and hydration	Controversial- Possible younger donor RBC associations with risk of PTR morbidity or mortality
Obesity (BMI ≥ 30 kg/m^2)	Increased storage, osmotic and oxidative haemolysis; increased RBC metabolic indicators of inflammation and oxidative stress	Mouse model: Lower PTR in RBCs from donors with obesity
Ancestry	African/Asian: Lower osmotic fragility	Not determined
Genetic polymorphisms	SNPs in the *SEC14L4*, *G6PD*, *GPx4*, *PIEZO1*, *SPTA1*, *AQP1*, *MYO9B*, *HBA2* genes were associated with osmotic or oxidative haemolysis	*SEC14L4*, *G6PD*, *MYO9B*, *HBA2* SNP association with lower Hb increments
Sex-specific genetic polymorphism	Sex-specific SNPs in the *SPTA1*, *KCNA6*, *SLC4A1*,*SUMO1P1*, and *PAX8* genes modulated osmotic haemolysis	Not determined

Abbreviations: Hb, haemoglobin; PTR, posttransfusion recovery; MCV, mean corpuscular volume; TRT, testosterone replacement therapy; BMI, body mass index; SNP, single nucleotide polymorphism.

PA25-L02 | Removing the tattoo deferral for donors giving plasma for further manufacture gains much sufficiency, is safe, and can be safely extended to whole blood donors

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Background: Tattooing was among the most common blood donation deferral reasons in Australia (over 50,000 donors during 2010–2014), and until recently, resulted in a 4-month deferral from all donation types. Modelling estimated that the residual risk of hepatitis C virus (HCV) transmission would remain negligible at 1 in 34 million units transfused if the tattoo deferral was removed (Hoad, et al., Vox Sanguinis, 2019). Following regulatory approval, the guideline was changed on 27 September 2020 to allow donation of plasma for further manufacture only during the 4 months after receiving a tattoo in a licensed/regulated facility in Australia; whole blood donation remains subject to the 4-month deferral. Our review supports progressing this change to transfusible components.

Aims: To compare the cohorts of donors with a recent tattoo before and after the change to the guideline to determine whether there was any difference in rate of return to donation or incidence of blood-borne virus (BBV) infections that would impact on the residual risk. To assess whether these results supported a further change to allowing transfusible component donation.

Methods: All donors who answered yes to receiving a tattoo in the past 4 months on Lifeblood's questionnaire during the 2 years before and after the change were identified and followed up until 3 November 2022. Donor return was analysed by comparing the Kaplan-Meier curves for each cohort. The difference in the rates of returned donors and their donations over equivalent periods of time comprised the sufficiency gain; the change yield was the total number of plasma donations given during the 4-month restriction period. BBV incidence was analysed for donors with BBV results post-tattoo. A sensitivity analysis of the residual risk was performed to determine the risk change if additional HCV infections in donors occurred.

Results: Donors allowed to donate plasma for further manufacture 0–4 months after a tattoo in a licensed/regulated facility in Australia returned to donate significantly faster than donors subject to whole blood deferral (85 days compared to 278 days, $p < 0.001$). An extra 26 donors and 187 donations per 10,000 person-years of observation were gained from the change, while the extra plasma yield was 109 donations per 10,000 person-years of observation, or 41,458 donations in total. BBV incidence in both periods was zero, with upper 95% confidence limits of 0.31 and 0.69 per 1000 person-years of observation before and after the change, respectively. To breach a residual risk of 1 in 1 million, 195 HCV-infected tattoo donors would need to occur every year (incidence of 1.6%).

Summary/Conclusions: Allowing plasma for further manufacture donations immediately after a tattoo was associated with a substantial sufficiency gain from quicker return to donation by a higher proportion of donors. The zero incidence of BBV after the change, with the higher upper confidence limit reflecting the statistical uncertainty due to faster return, supports progressing this change to transfusible components with the residual risk remaining well below 1 in 1 million with even worst-case incidences that are not feasible. We have submitted this evidence to our regulator to consider extension to transfusible components.

PA25-L03 | Menstrual blood loss is an important determinant of Hb and ferritin in premenopausal blood donors

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Background: To prevent blood donors from developing iron deficiency (ID, ferritin <15 µg/L) and subsequent iron deficiency anaemia (IDA, haemoglobin (Hb) <120 g/L), blood services rely on information provided by the donor, including age and sex. Although a known risk factor for ID and IDA, the effect of menstruation on blood donors has been scarcely studied and to our knowledge is not considered in blood donation interval recommendations. Menstrual blood loss (MBL) can be measured using a pictorial blood assessment chart (PBAC), a semi-objective method that allows the individual to evaluate the number of used menstrual pads and tampons and the degree of staining. While disadvantages include underestimation of blood loss, the method is easy to include into clinical practice and has a lower cost compared to methods involving laboratory analysis.

Aims: We aim to investigate whether the effect of MBL explains variation in ferritin and Hb levels, to quantify its effect size and explore if asking donors questions related to menstruation could detect risk of ID or IDA.

Methods: Donor InSight III (2015–2016) is a cohort study of Dutch whole blood donors. The cohort includes data on ferritin and Hb levels and questionnaire data on health and lifestyle. Female donors were asked to supply a PBAC. To deduce the effect of MBL as explained in aims, we stratified female donors by menopausal status and used three separate analysis methods. First, we studied the association of variables related to log(ferritin) and Hb using Bayesian linear regression models. Secondly, we quantified the average variance explained of log(ferritin) and Hb by each of the variables used in the linear analysis. Thirdly, we measured association of variables to risk of ID and IDA in the participants who reported menstruation using Bayesian logistic regression models. Exclusion criteria was pregnancy, BMI ≥50, ferritin ≥200, PBAC score ≥400 and age <18 or ≥70 years.

Results: 482 premenopausal and 499 postmenopausal women were included in analyses. Premenopausal women had lower mean ferritin (28.81 µg/L) and Hb (133.80 g/L) levels compared to postmenopausal women (40.86 µg/L and 136.72 g/L) and there was a negative

association between PBAC score and ferritin/Hb. Based on variable importance analysis, PBAC accounted for the majority of variance explained for Hb and second only to the number of days since last blood donation for ferritin. Heavy menstrual bleeding (transformation of PBAC score ≥150, a validated proxy for MBL of ≥80 mL) and duration of menstruation (number of days of flow) were associated with increased risk of IDA but not ID, while use of a levonorgestrel-releasing intrauterine device (LNG-IUD) was associated with decreased risk of ID. Age was not found to be associated with iron status, but number of years since menarche was associated with decreased risk of IDA.

Summary/Conclusions: Along with blood donation, MBL is likely the most important determinant of iron status in premenopausal blood donors. As the effect of age on ferritin and Hb levels disappears when accounting for MBL, this suggests blood donors could benefit from donation interval guidelines based at least in part on information of MBL, heavy menstrual bleeding or use of hormonal contraception, specifically LNG-IUDs. Our research shows that it would be crucial to find methods to easily account for menstruation in blood donor management. Further study on ways to implement questions regarding menstruation into the donor selection process is needed.

PA25-L04 | Effectiveness of ferritin-guided donation intervals in blood donors: Results of the stepped-wedge cluster randomised find'em trial

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Background: Blood donors are at increased risk for iron deficiency and anaemia. The current standard of Hb monitoring is insufficient to ensure the maintenance of proper iron reserves and donor health, as Hb levels do not fully reflect iron stores. Novel iron management strategies such as iron supplementation and extended donation intervals are warranted, including ferritin measurements to monitor iron deficiency.

Aims: To determine the effects of ferritin-guided donation intervals for whole blood donors on Hb and ferritin levels, Hb deferral, iron deficiency (ferritin < 15 ng/mL), donor return, and iron deficiency-related symptoms.

Methods: Between November 2017 and November 2019, a ferritin-guided donation interval policy was gradually implemented nationwide and evaluated through a stepped-wedge cluster-randomised controlled trial by Sanquin, the national blood service in the Netherlands. All blood collection centres implemented the policy at one of six randomly allocated time points. The new policy entails ferritin measurements in all new donors and at every fifth whole blood donation in addition to Hb monitoring. Subsequent donation intervals are extended to 6 months if ferritin is 15 ≤ 30 ng/mL and to 12 months if ferritin is <15 ng/mL. The primary outcomes are ferritin and Hb levels, iron deficiency, Hb deferral (females <12.5 g/dL, males <13.5 g/dL), and donor return. These were assessed in all donations during four pre-defined measurement weeks before, during and after the implementation period. Secondary outcomes are iron deficiency-related symptoms, specifically restless legs syndrome (RLS), fatigue, pica, cognitive functioning and warm glow, and were assessed during the second and third measurement week using questionnaires.

Results: A total of 1,634,700 donations were made by 412,888 whole blood donors during the study and implementation period. We performed additional measurements in samples of 37,621 donations by 36,099 donors, of which 52% was female and the median age was 43 years. Ferritin-guided donation intervals were associated with higher log-transformed ferritin levels at all time points in the trial, up to 0.56 log-transformed ng/mL as compared to control (95% CI 0.49–0.63). Hb increased as well, up to 0.39 g/dL (95% CI 0.32–0.45). Decreased odds of 13% (95% CI OR 0.72–0.92) for iron deficiency and 20% (95% CI OR 0.53–0.94) for Hb deferral were found compared to control. Odds of donor return decreased with time since policy implementation, up to 56% (95% CI OR 0.34–0.52). We found no evidence for improved self-reported iron deficiency-related symptoms after implementation of the new policy, but odds of reporting restless legs syndrome (RLS) increased with time since policy implementation. RLS was up to 185% more likely to be reported after implementing ferritin-guided donation intervals as compared to control (95% CI OR 1.82–4.48).

Summary/Conclusions: Ferritin-guided donation intervals are associated with significantly higher overall Hb and ferritin levels, as well as lower prevalence of iron deficiency and Hb deferrals in whole blood donors. No improvements in iron deficiency-related symptoms were found, warranting further research into health effects of iron deficiency in donors. However, reporting of RLS increased, which may be due to increased awareness. Our observations suggest ferritin-guided donation intervals are beneficial for Hb levels and iron stores, but additional efforts are required to retain donors.

PA25-L05 | Impact of donor sex, age, BMI, Hb level, ferritin level, outdoor temperature, time of day and time between donations on perceived donor haemoglobin recovery after donation

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Background: Blood banks are required to monitor blood donor iron status and defer donors with haemoglobin levels below internationally prescribed threshold levels (135 and 125 g/L or 8.4 and 7.8 mmol/L for males and females respectively). It was shown previously (Janssen, Transfusion, 2022) that the majority of donor deferrals are in fact caused by measurement and biological variability, which -given the

PA25-L05 – Table 1

Male donors		Female donors	
Variable	Scaled regression parameter	Variable	Scaled regression parameter
(Intercept)	−0.044	(Intercept)	−0.006
dRefHb	−0.363	dRefHb	−0.323
Hb	−0.260	Hb	−0.291
dDontime	−0.095	dDontime	−0.078
dTemp	−0.090	dTemp	−0.075
lTime	0.088	lTime	0.067
lTime:lFer	−0.068	numdon	0.034
numdon	0.031	Age>50	−0.031
lFer	0.028	lTime:Hb	−0.028
BMI	0.027	BMI	0.024
lTime:age	−0.021	lTime:Age>50	0.018
lTime: dRefHb	−0.021	lFer	−0.011
lTime:BMI	0.015		
lTime: numdon	0.012		
Age	−0.010		

substantial proportion of deferrals in practice- warrants further research to improve our understanding of Hb change and variability in order to improve donor deferral strategies.

Aims: To quantify the main drivers of donor haemoglobin recovery after donation and variation in subsequent Hb measurement outcomes.

Methods: We analysed routinely collected Hb and ferritin measurements in Dutch whole blood donors between 2012 and 2022 (5.277.940 donations attempts by 772.641 donors). As Hb levels recover exponentially, the change in Hb after donation was estimated by linear regression of the change in Hb level between donation and subsequent donor visit as a function of the logarithm of the time between these measurements. Donor age, sex, BMI, ferritin and Hb level at donation, number of previous donations, reference Hb level (measured at the start of the donor's career), as well as difference in outdoor temperature and difference in time of day at donation between donations were used as predictor variables.

Results: The analyses show that the most important parameters for recovery, so those that determine the rate of change in Hb after donation, are (1) the Hb level relative to the donor's reference Hb level, (2) the actual Hb level at donation, (3) the difference in time of day of donation, (4) the difference in (daily average) outdoor temperature at donation, and (5) the (logarithm of the) time between donations. The scaled linear regression coefficients, which indicate the relative importance of the predictor variables are shown in the table below. The models predict that a 30 year old male donor with an Hb level of 9 mmol/L which is 0.25 mmol/L under his reference Hb level will on average recover in 62 days, whereas a similar male with an Hb level of 10 mmol/L will require 230 days to recover. Would these Hb levels be 0.50 mmol/L under the donor's reference level, recovery would take 41 and 144 days respectively. A donation eight hours later in the day on average decreases Hb levels by 0.16 and 0.12 mmol/L and an increase in daily average outdoor temperature decreases Hb levels by 0.15 and 0.09 mmol/L for male and female donors respectively.

Summary/Conclusions: Blood banks are required to monitor donor Hb levels and defer donors when fixed threshold levels for Hb levels are not met. However, measurement variability, biological variability and differences in outdoor temperature and timing of donations impact Hb levels of donors. These factors should therefore be accounted for when judging donor eligibility. Our analyses also show that donor recovery is strongly affected by donor Hb and reference Hb levels. This implies that pre-set donation intervals may be sufficient for some donors, but not for others. The results from this paper can be used to further guide individualized donation intervals.

Parallel Session 26: Fetal maternal immunology

PA26-L01 | Rh disease prevention for women with partial D: Is routine antenatal anti-D prophylaxis useful ?

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Background: Variant *RHD* alleles are often responsible for a weakened expression of the D antigen. Partial D phenotypes are expressed by some of these variants and characterised by an altered RhD protein. Pregnant women with partial D may develop allo-anti-D if their foetuses' red blood cells (RBC) express the conventional D antigen. Routine antenatal prophylaxis (RAP) consists of the administration of 1000 to 1500 UI anti-D immunoglobulin (RhIg) to D-negative women at the beginning of the third trimester to prevent maternal anti-D immunization caused by small foetal-maternal haemorrhages. It is often proposed to women with partial D, or a weakened D antigen expression in the absence of *RHD* genotyping results, with little evidence to support this practice. Indeed, some RhIg may bind to the patient's RBC and not be available to clear foetal D-positive RBC from the maternal blood.

Aims: We aimed to determine the proportion of women with *RHD* variants giving birth in the Ile de France Region (Paris area) and the usefulness of RAP for women with partial D.

Methods: We collected data from samples received between January 1, 2020 and December 31, 2021 at the National Reference Center in Perinatal Haemobiology, that performs Rh disease prevention tests to determine the need for RhIg injection for patients in 27 maternity wards in the Paris area.

Maternal *RHD* genotyping results (when available) were collected for patients with a weakened D antigen expression. For patients who received RAP, the D phenotype of the newborns and the results of the antibody screening test were collected to determine if RhIg was still present at delivery.

Pharmacokinetic curves of anti-D concentrations were determined through logarithmic regression for each D variant, considering the date of the last RhIg injection for women who received RAP and/or targeted antenatal prophylaxis due to an intercurrent event in the third trimester. Results were compared to a control group of D-negative pregnant women who delivered during the same period.

Results: Among 11,609 samples received, 217 patients (1.9%) had a weakened expression of the D antigen : 19 weak D type 1 (9%), 6 weak D type 2 (3%), 14 weak D type 3 (6%), 83 weak D type 4.0/4.1 (38%), 34 DAR (16%), 15 DAU (7%), and 8 rare variants (4%). Maternal *RHD* genotyping was not performed or the variant not identified in 38 (18%) patients.

Among patients who had a D-positive newborn and received only RAP : 5% (2/38) of patients with weak D type 4.0/4.1 had detectable anti-D at delivery, in contrast with 58% patients with DAR (7/12), 60% patients with DAU (3/5) and 83% in the D-negative control group (20/24). Pharmacokinetic curves show a progressive decrease of anti-D concentration over time for DAR and DAU variants, similar to D-negative controls, but a rapid decline in the days following RhIg injection for weak D type 1 and weak D type 4.0/4.1.

Summary/Conclusions: The usefulness of RAP seems to differ between D variants, suggesting it may be useless for weak D type 4.0/4.1 (supporting the recent evolution of the national recommendations for these variants) but could be useful for patients with DAR or DAU variants. Future studies will need to evaluate the appropriate RhIg dose (probably higher than 1500 UI/mL) or the benefit of multiple RhIg injections at the third trimester, to be more efficient in preventing anti-D immunization in women with partial D variants.

PA26-L02 | Natural history of human platelet antigen (HPA)-1a alloimmunised pregnancies: Prospective observational cohort study

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Background: Foetal and neonatal alloimmune thrombocytopenia (FNAIT), the platelet equivalent of haemolytic disease of the foetus and neonate (HDFN), can cause major intracranial haemorrhage (ICH) and organ bleeding in children during pregnancy and shortly after delivery. FNAIT results from maternal platelet-directed alloantibodies recognizing human platelet antigen-1a (HPA-1a), which is also expressed on endothelium and the placenta. If a pregnant woman has produced anti-HPA-1a, timely antenatal intervention with intravenous immunoglobulins (IVIG) can prevent the occurrence of severe ICH.

Aims: To assess the incidence of clinically detectable severe foetal and neonatal alloimmune thrombocytopenia (FNAIT) among human platelet antigen-1a (HPA-1a) immunised pregnancies and control pregnancies in a pregnant population in which both the pregnant woman and the obstetric care giver or neonatologist were blinded for the potential presence of HPA alloimmunisation.

Methods: Study design: Observational study

Participants Between 1-3-2017 and 1-5-2020, 153,106 women that were offered routine red cell antibody screening at the 27th week of pregnancy were eligible and were typed for HPA-1a.

Study outline Clinical data were collected of the HPA-1a negative women and a group of HPA-1a positive women (ratio 1:3), HPA-1a status was not reported to caregivers and researchers. HPA-1a antibody screening was performed in HPA-1a negative women. In HPA-1a immunised women antibody quantitation, HLA-DRB3*0101 typing and foetal HPA-1a typing were done.

Main outcome measures Proportion of neonates with severe FNAIT, defined as major bleeding and/or death related to bleeding, within HPA-1a immunised pregnancies without intervention. Secondary outcomes included mild FNAIT (minor bleeding and/or thrombocytopenia for which treatment was given), pregnancy- and neonatal outcome

Trial registration Clinicaltrails.gov NTC04067375

Results: We observed that 2.43% (3722/153,106) of the pregnant women were HPA-1a negative. Antibody screening was performed in samples from 913 pregnancies of 881 HPA-1a negative women (32 were included twice). Anti-HPA-1a was detected in 85 pregnancies of which 82 concerned HPA-1a positive foetuses. One pregnancy was excluded: this woman was treated with intravenous immune globulin (IVIg) because her previous child was diagnosed with FNAIT. In total, 81 HPA-1a immunised and incompatible pregnancies, 820 HPA-1a negative non-immunised pregnancies and 2704 pregnancies from HPA-1a positive women were included. One child (1.2%, 1/81) was diagnosed with severe HPA-1a mediated FNAIT (severe ICH) and three children (3.7%, 3/81) with mild FNAIT (two with haematomas and one with mucosal bleeding). Major bleeding was observed in 0.1% (3/2749) of the children of HPA-1a positive women. The incidence of clinically detectable severe anti-HPA-1a mediated FNAIT was 2.6 per 100,000 pregnancies. 15% (12/81) of the children from HPA-1a-immunised pregnancies were born prematurely (<37 weeks of gestation) compared to 5% (132/2749) of children of HPA-1a positive women ($P < 0.001$). Median birthweight percentile of neonates from immunised pregnancies was 0.46 [IQR 0.21 to 0.70] compared to 0.52 [IQR 0.26 to 0.77] in neonates from HPA-1a positive women. Hypertensive disorder during pregnancy was reported in 11% (9/81) of the immunised cases compared to 4% (120/2704) in HPA-1a positive pregnant women.

Summary/Conclusions: The incidence of major bleeding in FNAIT is 11 in 10,000 HPA-1a negative pregnancies. Premature delivery, lower birthweight and hypertensive disorders occurs more frequently in HPA-1a immunised pregnancies. The results of this study can be used to consider an HPA-1a screening program in pregnancy.

PA26-L03 | Natural course of haemolytic disease of the foetus and newborn after pregnancy with intrauterine transfusions

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Background: Haemolytic disease of the foetus and newborn (HDFN) is generally considered to worsen with each subsequent pregnancy with an antigen positive foetus. We found no adequately sized studies quantifying the risk of severe HDFN in pregnancies following a foregoing pregnancy in which treatment with intrauterine transfusions (IUT) to the foetus was necessary.

Aims: The aim of this study was to assess the likelihood of recurrence of the necessity of IUTs in a subsequent pregnancy with an antigen-positive foetus, if in an earlier pregnancy at least one IUT was given because of HDFN. As secondary outcome we assessed the interval between the gestational age (GA) at first IUT in the first immunised pregnancy and first IUT in the subsequent pregnancy with need for IUTs.

Methods: We constructed a database of all pregnancies complicated with RhD and K alloimmunization and at risk for HDFN from 1999 until 2017 and registered if IUT treatment was given. Of all women that received one or more IUTs, we assessed the need for IUT in a next pregnancy with an antigen-positive fetus.

Results: During the study period 331 women were treated with IUTs for RhD or K mediated HDFN, of which 18% had a subsequent pregnancy with an antigen positive foetus. If in a foregoing pregnancy an IUT was given for HDFN, there was a risk for recurrence of the need of IUT(s) treatment in a subsequent pregnancy with an antigen positive foetus in 91% (53/58) of cases. This concerned all pregnancies women ($n = 14$) treated with IUT before 25 weeks of gestation. Characteristics of the group of 53 women with recurrent IUTs versus the five women without an IUT in the subsequent pregnancy were: a lower gestational age at the first IUT in the first immunised pregnancy (28 vs. 32 weeks), a higher percentage of foetuses with hydrops (13% vs. 0%) and more IUTs per pregnancy (3 vs. 1.5).

If in a subsequent pregnancy an IUT was needed, the gestational age was a median of 5 weeks earlier at first IUT than in the foregoing pregnancy (23 weeks, range 16–34 vs. 28 weeks, range 16–35 respectively). However, the range in this interval was quite large (−13–10).

Summary/Conclusions: This study is the first to report on the natural course of HDFN after a pregnancy complicated by IUT. A high risk of recurrent need for IUT at a lower gestational age was observed for the majority of women. For women with a history of IUT and their caregivers, this information is essential to enable adequate preconceptional counselling and identification of high risk patients.

PA26-L04 | HLA class I antigen knock out endothelial cells, a tool for the detection of endothelial-specific anti-HPA-1a alloantibodies

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Background: The scariest consequence of anti-HPA-1a mediated FNAIT is intracranial haemorrhage (ICH). Studies have shown that endothelial-specific anti-HPA-1a alloantibodies play a major role in FNAIT-mediated ICH. It is believed that these subtypes of alloantibodies target αvβ3 on endothelial cells, which affects endothelial function. These endothelial-specific alloantibodies are detected using standard methods such as monoclonal antibody immobilization of endothelial antigens (MAIEA) and flow cytometry. During pregnancy, anti-HLA immunization has been documented in 54.4% of women which increases with the number of pregnancies up to 74%. However, both detection and functional analysis of endothelial-specific anti-HPA-1a alloantibodies are highly affected by the presence of anti-HLA class I alloantibodies in serum.

Aims: The current study aims to overcome the effects of anti-HLA class I antibodies on the evaluation of endothelial-specific anti-HPA-1a alloantibodies.

Methods: A CRISPR-Cas9-PITCh system to delete exons 2 and 3 of the beta-2-microglobulin gene (B2M) and thereby eliminate HLA class I surface expression was designed. An endothelial cell line (EA.hy 926) was transfected with this CRISPR-Cas9-PITCh system. Analysis of transfected EA.hy 926 cells detected the absence of HLA class I in about 2%–3% of total cells. Using mAb W6/32, HLA class I negative cells were single-cell-sorted and further cultivated. To confirm the HLA class I elimination, exons 2 and 3 of the B2M gene were amplified from the genomic material of sorted cells (HLA class I deficient) and compared with wild-type cells in PCR. The type of HLA class I on wildtype EA.hy cells was determined. Finally, binding of the matched anti-HLA class I antibodies in serum ($n = 10$) to wild-type and transfected cells were evaluated in a MAIEA assay and compared to a negative control.

Results: In contrast to wild-type cells, specific primers for the B2M gene amplified a PCR product of 535 bp, indicating the absence of exons 2 and 3 in transfected cells. Further evaluation of HLA class I expression on the cell surface in flow cytometry using mAb W6/32 showed no binding on transfected EA.hy cells. The HLA class I genotyping of wildtype EA.hy cells revealed expression of HLA class I types A*24, 25, B*15 and C*03. Analysis of sera containing HLA-matched anti-HLA class I antibodies with wild-type cells and HLA class I KO cells in the MAIEA assay showed no reactivity of these sera with HLA-class I KO cells (mean OD 0.2–0.5), while exhibiting a strong reactivities with wild-type cells (mean OD 3.4–3.9).

Summary/Conclusions: These results showed that the B2M gene deletion does not affect the proliferation and growth profile of HLA class I KO EA.hy cells. However, the absence of HLA class I makes these cells suitable tools to evaluate the binding of endothelial-specific anti-HPA-1a alloantibodies in both HLA class I positive and negative sera. Furthermore, HLA-Class I KO EA.hy 926 cells can serve as an effective tool to analyse the sole functional relevance of endothelial-specific anti-HPA-1a alloantibodies implicated in the mechanism of ICH without the effect of HLA class I antibodies.

PA26-L05 | Pre-administration of baicalin inhibits red blood cell immunization in a mouse model of red blood cell transfusion

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Background: Baicalins, a flavonoid compound originally isolated from the root of the Chinese herb Huangqin (Scutellaria baicalensis Georgi), is an anti-inflammatory agent and has a good safety record in the clinic. YIN-CHEN-TANG is widely used in preventing haemolytic disease of the newborn(HDN) caused by Rh and ABO maternal-foetal incompatibility since 1970s in China. Baicalin is a major component of YIN-CHEN-TANG. It could reduce the severity of experimental autoimmune encephalomyelitis (EAE), asthma, colitis, systemic lupus erythematosus (SLE) and other immune diseases. However, its potential in inducing transfusion tolerance remains to be explore.

Aims: The aims of our study are to evaluate if pre-administration of baicalin could inhibit red blood cell (RBC) immunization and to elucidate the underlying mechanism of baicalin as a major component of YIN-CHEN-TANG in preventing HDN.

Methods: We used human red blood cells with adjuvant Lipopolysaccharide (LPS) and transfused mice to induce antibodies, as an experimental system to study the effect of baicalin in inducing transfusion tolerance. Mice were divided into a human RBC transfused positive control group administered with human RBC and LPS intravenously three times every two days, a control group preadministered dexamethasone (DEX; 10 mg/kg/day) intraperitoneally daily for 1 weeks before human RBC transfused, a treatment group preadministered baicalin (250 mg/kg/day) intraperitoneally daily for 1 weeks, and a normal control group. Assessment of human RBC immunization was performed by measuring serum immunoglobulin G (IgG) and immunoglobulin M (IgM) against human RBC weekly. And the lymphocyte changes in spleen were also monitored by flow cytometry.

Results: We found that baicalin treatment decreased serum IgG but not IgM production significantly after human RBC being transfused, with a concomitant reduction in Th17 cells and increase in CD4 regulatory T cells in both spleen and mesenteric lymph nodes. And there were no significant differences in the percentage of Th1, Th2, Tfh and Tfr CD4 subpopulation among all groups.

Summary/Conclusions: Our results indicate that pre-administration of baicalin could inhibit RBC immunization especially IgG production. Considering its good safety records in clinic, it may be exploited for suppressing transfusion immunization events especially as preventive drugs in necessary. In addition, our results elucidate the inhibitory effect in antibody production of baicalin may be a possible mechanism for YIN-CHEN-TANG as a widely used Chinese herbal medicines in preventing HDN.

PA26-L06 | Fetal *RHD* genotyping by droplet digital PCR for D variant mothers or children

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Background: For foetal RHD genotyping, we perform multiplex PCR for RHD exon 5 (negative for RHD*pseudogene(Dψ)) and exon 7 with cell-free DNA isolated from plasma of serologically D negative pregnant women. The test result is used to guide antenatal prophylaxis or in case of RhD alloimmunisation to identify foetuses at risk for haemolytic disease of the foetus and newborn.

The majority of D negative mothers have a complete deletion of the RHD gene and therefore only foetal RHD PCR signals can be detected. However, in case of a maternal variant RHD gene, the foetal RhD prediction may be hampered by the presence of maternal RHD sequences. The most frequently occurring variants are Dψ in mothers from African origin and DVI type 2 in mothers from European origin. In these cases only exon 5 is available for foetal RHD prediction.

Aims: To improve applicability and accuracy of foetal RHD typing in women carrying the most frequently occurring RH variants.

Methods: We developed four different ddPCRs: exon 4 specific for c.577G/c.602C, exon 4 specific for c.577G/c.609G, exon 5 specific for c.654G/c.1048G and exon 7 specific for c.960G/1048G. With these assays we can discriminate DVI mothers (only maternal signal exon 7) from Dψ mothers (maternal signal exon 4:c.602 and exon 7). More importantly, in these mothers foetal RHD typing will be based on two foetal signals (exon 4:c.609 and exon 5). Additionally Dψ or DVI foetuses will be recognised.

The ddPCRs were validated with foetal DNA isolated from maternal plasma gestation week 27–32 with known neonatal RhD typing.

Results: We analysed samples from 21 Dψ pregnant women with five D positive and 16 D negative foetuses; four DVI pregnant women with two D positive and two D negative foetuses and five D negative pregnant women with two Dψ and three DVI foetuses. All results were conform the expected results.

Summary/Conclusions: We developed two new prenatal ddPCR genotyping assays on RHD exon 4. In combination with the exon 5/7 ddPCR we are able to reliably perform foetal RHD typing in mothers and foetuses carrying the most frequently occurring RHD variants RHD*pseudogene and RHD*DVI. The assays can be easily implemented in diagnostics.

Parallel Session 27: Harmonization of apheresis starting material for cell therapies manufacture–Is it needed and how? (AABB / ISBT joint session)

PA27-L01 | Managing donors during increasing demand for cell therapeutics

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The past decade has seen dramatic changes in oncology and, to a lesser extent, regenerative medicine. So far, the most relevant development in cellular immunotherapies was the successful insertion of chimeric antigen receptors (CAR) into T cells. These CAR-T cell therapies have shown remarkable clinical effects in various hematologic malignancies such as B cell lymphoma or B cell acute lymphoblastic leukaemia. Typically, such innovative cellular immunotherapies are manufactured from lymphocytes/mononuclear cells collected from the peripheral blood by apheresis technology. To date, this starting material is mainly obtained in the autologous setting from patients in need for the CAR-T cell product. To address these laborious and costly procedures, requiring the manufacture of unique products from and for individual patients, precise genome editing may allow to develop concepts for the production of cellular immunotherapies from allogeneic donors.

This talk will present the current concepts of autologous and allogeneic cellular therapies and discuss the potential relevance of patient/donor traits/profiles on their quality as well as practical issues of donor management (e.g., intervals between donations, donor identification, eligibility and deferral policy, timing and need for cryopreservation). In addition, potential donor profiles and donor blood management, as well as aspects of collection techniques for cellular therapies manufactured from bone marrow will be discussed.

PA27-L02 | Growing variations in cell collections requirements

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With the expansion of the cell & gene therapy space, increased variations in the requirements for cell collections are a cause for concern for the apheresis community. This presentation will discuss why there are variations in collections' requirements and provide solutions trying to mitigate these variations.

Parallel Session 28: IT in blood management

PA28-L01 | Uptake of electronic blood management systems in transfusion–A UK survey

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Background: Serious Hazards of Transfusion (SHOT), the UK haemovigilance scheme, data demonstrate that ABO incompatible transfusions often result from errors at component collection and/or administration. Electronic blood management systems (EBMS) provide controls that can prevent these errors. SHOT data demonstrate effectiveness of EBMS in prevention of error and recommended in 2017 that use should be standard practice; however, reports continue to highlight lack of system implementation. The SHOT SCRIPT group was formed in 2019 with an aim to review and improve the use of electronic systems in the transfusion process. A survey was sent to all SHOT reporters to ascertain the status of electronic systems in use, including EBMS.

Aims: To review the status of EBMS in UK hospitals.

Methods: The survey was sent to all registered SHOT reporters in the UK. Data collection was open 30 November 2020–14 February 2021.

102 responses were received

A completion rate of 88% was recorded on SurveyMonkey™

The survey contained 48 mandatory questions

7 questions related directly, and 2 questions indirectly, to EBMS

Not all questions were mandatory and so responses to questions were variable

SHOT requested a single response was submitted per transfusion laboratory to ensure the impacts of transfusion IT throughout the hospital, and facilities supplied, were represented.

Results: EBMS was used by 57 of 97(58.8%) respondents. Haemonetics BloodTrack was most commonly used 38/57(66.7%), 16/57 (28.1%) used MSOFT Blood360 and 3/57(5.3%) used Fordman BARS. Nearly all hospitals with EBMS (52/57, 91.2%) had blood refrigerator control for collection of components. EBMS at administration was used by 21/57 (36.8%). Full vein to vein functionality was used in 17/57(29.8%) of hospitals. Where collection and administration modules were used these had been been implemented at the same time 37/57(64.9%), where modules had been implemented at different times this ranged from 1 to 7 years for full implementation. Of the 56 who responded about upgrades, 26 stated none were offered for EBMS, with 30 offered 1–2 upgrades per year. Where upgrades were offered these were only all taken by 50% of respondents. Release notes were stated as available in advance of upgrades by 42 of 54 respondents. 42 of 89 respondents stated finances and resources were the greatest barrier to implementing electronic systems to support transfusion safety. Technical issues (11/89), IT support (10/89) and engagement from senior management (9/89) were also cited as barriers.

Summary/Conclusions: Survey results demonstrate deficiencies in implementation of EBMS at collection and administration in UK hospitals. For health services in the UK to undergo digital innovation encouraged by UK governments, appropriate resources must be allocated to enable these advancements, whilst continuing to supporting excellent care. It is impossible to instigate new initiatives whilst managing systems that are not currently functional. Organisations must also recognise the importance of safer transfusion IT systems and the risks implicated by not introducing these available technologies. SHOT has been recommending the use of electronic systems since 2017, yet just under half of survey respondents did not have access to these systems. Where EBMS are in use appropriate resources must be available to support upgrades to ensure that system deficiencies are resolved and improved functionality implemented. SCRIPT continues to work with suppliers, hospitals and health care bodies to improve uptake of EBMS in transfusion practice.

PA28-L02 | The practice and application effect analysis of platelet electronic matching

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Background: For patients requiring long-term platelet transfusion, the HLA and HPA genotype matching technique between donors and recipients is the most reliable method to resolve the ineffectiveness of immune platelet transfusion. Due to the high polymorphism of HLA genes and antigens and the rarity of HPA low-frequency antigens, the establishment of a large database of HLA and HPA genotypes of apheresis platelet donors is an effective means to realize the compatibility of HLA and HPA among donors and recipients. At present, platelet genotyping data in Zhejiang Province has not been fully shared, the platelet gene pool of blood banks is small and patients often cannot match suitable platelets. In addition, the operation of donor search, recruitment, blood locking and other links also needs manual operation or marking, and the informatization level of the whole genotype coordination process is low. Therefore, we carried out platelet electronic matching related work.

Aims: To construct a provincial platelet genotyping database, and to realize closed-loop management of platelet matching application, patient genotyping, platelet coordination, locking, dispensing and infusion efficacy feedback.

Methods: Firstly, a safe, stable and efficient private network connection mode was adopted to support blood stations and hospitals to access the Zhejiang platelet electronic matching information system. Secondly, the centralised sharing mode was adopted to realize the sharing of platelet genotyping data of blood donors in Zhejiang Province through unified data standards and interface specifications. Then, the coordination process of platelet donor and recipient genotypes was sorted out to realize the effective connection of relevant functional modules such as application for matching test, donor recruitment, platelet collection, blood testing, and blood inventory management.

Results: The platelet electronic matching information system of Zhejiang Province has been established. At present, there are 76,600 genotyping data of blood donors in platelet gene bank of Zhejiang Province. Hospital-side applications include gene matching application, access to matching reports, access to matching platelets, and feedback of matching platelet infusion efficacy. Blood-bank side applications include patient genotyping results and antibody information input, platelet donor search, platelet donor recruitment and collection, and matching platelet locking and distribution.

Summary/Conclusions: The platelet electronic matching information system in Zhejiang Province realizes the closed-loop management of platelet matching. At present, this system has been widely used in blood stations and hospitals in Zhejiang Province, which indeed improves the application value of platelet donor genotype data and increases the probability of successful matching for patients requiring long-term repeated platelet transfusion. In 2022, 421 cases were successfully matched through the provincial platelet electronic matching information system, and the waiting time for electronic matching (the time interval between the generation of search report and blood distribution) was significantly shortened, with an average of 3 days.

PA28-L03 | Evaluating the inventory impact of utilising low titre platelets in regional hospitals

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Background: Due to short shelf life and natural scarcity, it is sometimes necessary to provide patients with ABO mismatched platelets. Such practices may increase the risk of acute hemolytic transfusion reaction (AHTR). Thus, providing patients with platelets suspended in O plasma having low-titer Anti-A and Anti-B antibodies (LtABO) could reduce the incidence of AHTR. In a previous study it was shown that fewer outdates and shortages are expected at regional hospitals currently stocking one unit of A platelets and one unit of O platelets (1A, 1O) with two units of LtABO platelets (2LtABO). However, stocks of LtABO units are expected to be naturally limited and must, therefore, be distributed in the most effective manner possible. This study considers the use of an inventory policy consisting of one unit of A platelets and one unit of LtABO platelets (1A, 1LtABO) as a strategy to conserve the supply of low titre units within a blood supply chain network.

Aims: Regional hospitals often experience demand for platelets on an irregular basis. They are, however, required to stock some number of platelets (typically one A-unit and one O-unit) for emergencies; outdates are common, with discard rates sometimes >> 50%. A study was completed to determine the impact of replacing a (1A, 1O) inventory with 2 or 3 units of LtABO at regional hospitals.

Methods: Five regional hospitals using a (1A, 1O) inventory policy provided case examples for this study. A simulation model representing ordering and demand was created for each case site. It is known that a 2LtABO policy dominates a (1A, 1O) policy at these sites. In this study, a low titre conserving policy (1A, 1LtABO) was tested at each

of the five sites and results were compared against the current inventory practice (1A, 1O), as well as against the 2LtABO policy.

Results: A (1A, 1LtABO) policy results in fewer shortages than a (1A, 1O) policy at each of the case sites. At sites with demand exceeding 177 units per year, a (1A, 1LtABO) policy also results in fewer outdates. At sites with demand less than 177 units per year, wastage under a (1A, 1LtABO) policy was not different from a (1A, 1O) policy. A (1A, 1LtABO) inventory policy was not different to a 2LtABO inventory, in terms of wastage and shortage for sites with an annual demand less than 177 units per annum, but at sites with demand greater than 177 units an increased wastage rate was observed.

Summary/Conclusions: A 2LtABO platelet ordering policy provides improved inventory metrics for all sites currently holding 1 unit of A platelets and 1 unit of O platelets. However, for sites with highly episodic demand of less than 177 units per annum, a (1A, 1 LtABO) policy provides less wastage, no increase in shortages, and conserves the low titre platelet supply for the larger regional blood supply chain network.

PA28-L04 | Improve platelet supply management with 7-day platelet in Singapore

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Background: Blood Services Group (BSG) in Singapore secures the nation's blood supply and ensures all patients have access to adequate and safe blood products for transfusion. With the increasing demand of high-quality blood products every year, there is a need for constant improvement on the blood component inventory.

Platelet concentrate is one of the most important blood components used in transfusion medicine. It is mainly used in treatment for patients with severe thrombocytopenia or mass bleeding. Its short shelf-life and high variations in daily demand make management of platelet inventory a challenge. In July 2021, BSG adopted the bacterial testing strategy of Large Volume Delayed Sampling at 48 hours (LVDS-48) for platelet products to improve blood safety where we increased the ability to detect low level of bacterial contamination and therefore extending platelet shelf-life from 5 to 7 days. The change of platelet shelf life could impact platelet supply management and outdate.

Aims: This study aims to compare Singapore's platelet inventory and frequency of outdates of BSG and hospitals after conversion of platelet shelf-life from 5 to 7 days.

Methods: A retrospective study was performed by comparing BSG's platelets inventory and outdate rates before implementing LVDS-48 in year 2019 (5-day platelet) and after implementing LVDS-48 in year 2022 (7-day platelet). Data from year 2020 to 2021 were excluded from this study due to the irregular demand and supply of blood products impacted by COVID-19 pandemic.

Results: The *t*-test showed that 7-day platelet inventory (M = 149, SD = 37.36 $n = 365$) was hypothesised to be greater than 5-day platelet inventory (M-127.12, SD = 33.61, $n = 365$). This difference was significant, t (728) = 8.47, $p = 0.00$. On average, 7-day platelet stockpile was 17.3% higher than 5-day platelet stockpile. The Box and Whisker plot showed that the 7-day inventory distribution was relatively higher for most of the days with a wider range and it has improved weekday's platelet inventory especially. Platelet shortage occurrence was also fewer with the implementation of 7-day platelet. In addition, the BSG's platelet outdate rate reduced from 0.8% to 0.5%, while hospitals outdate rate reduced from 4.2% to 3.1%.

Summary/Conclusions: Overall, 7-day platelet has significantly improved platelet supply management with increased daily platelet stockpile and decreased outdate rates. The change has improved low platelet stockpile on weekday due the blood collection pattern (low on weekday and high on weekend) and during long holiday. However, demand remains highly unpredictable; platelet production planning could be studied to further improve platelet supply management.

PA28-L05 | Factors relevant for successful implementation of electronic identity control before blood transfusion

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Background: Bedside check of identity is a vital step in preventing transfusion error. The manual procedure requires two members of staff. Electronic identity control can replace one of the staff members. A pilot study in our hospital has shown that electronic identity control will save time, improve workflow and fully implemented, estimated to reduce working hours by 3000 h/year (27,000 transfusions/year). Electronic control has not shown to be inferior to manual control regarding transfusion errors. For a health care system with limited resources, it seems obvious to implement electronic identity control and expecting the staff to be keen to use it. In our hospital, the dept. of haematology had access to electronic control since 2017, but a follow up in 2021 shows that it is only used in 15% of all transfusions. In 2017 the initial training was performed by transfusion practitioners, subsequently training is performed by experienced nurses.

Aims: We wanted to examine, why electronic identity control is only used in a minority of transfusions in department of haematology.

Methods: A questionnaire was used to identify which factors influence the decision of nurses to use electronic identity control. The questionnaire focused on the following themes: Training, facilitation, preference, technology and change. The questionnaire was sent by e-mail to all nurses, printed copies were put into the office together with a basket of candy and it was introduced at staff meetings.

Results: Of 30 nurses, 19 replied (63 %). All nurses are trained in giving blood transfusion and do it on a daily basis.

The answers showed that the positive attitude to electronic procedure was decreased from 100% in 2017 to 48% in 2022. The answers revealed that issues with technology was a major issue but also training. Six were not trained, 10 were trained by colleagues and 3 by transfusion practitioners. Four replied that the training was not sufficient, compared to six who found it sufficient. The electronic procedure itself is not a problem, as 11 replied it was easy to perform and

only three that it was not easy, and then insufficient technology (slow Wi-Fi, old laptops) was stated as cause. Fourteen replied that they would choose electronic procedure because they did not need to ask a colleague to help. Opposite, 10 replied, that the manual procedure is preferred because they had more experience in this procedure. When asking a colleague for help in the manual procedure, this is seen as a necessary disturbance. The majority (98%) did not see change as challenge for successful implementation. They were used to changes and saw changes in general as a necessity.

Summary/Conclusions: Several factors have an impact on the use of electronic identity control. The major factors are technology, training and facilitation. Given that technology is out of control of the transfusion practitioners, efforts should be put on facilitation and training. It is vital that the initial positive attitude is maintained by facilitation, for example, retraining, visits, positive narratives involving data on use of electronic control. The follow-up can be performed by either transfusion practitioners or dedicated nurses.

PA28-L06 | Cost-utility analysis of screening of pregnant women for human platelet type 1a-alloantibodies mediated fetal neonatal alloimmune thrombocytopenia

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Background: Foetal and neonatal alloimmune thrombocytopenia (FNAIT) results from maternal alloantibodies which can cause severe intracranial haemorrhage (ICH) in foetuses and newborns. Screening for human platelet antigen-1a (HPA-1a) antibodies during pregnancy could allow for timely intervention with antenatal treatment with intravenous immunoglobulins (IVIG), to prevent the occurrence of ICH.

Aims: As the incidence of severe ICH due to FNAIT is low, we aimed to assess the cost-effectiveness of screening for anti-HPA-1a as part of a prenatal screening program.

Methods: A decision analysis model was developed to assess lifetime costs and effects of antenatal anti-HPA-1a screening with subsequent diagnostic and treatment interventions compared to the current situation without screening in the Netherlands. Model parameters were based on measurements from a recently completed nation-wide HPA-1a screening study in the Netherlands (manuscript in preparation), literature or expert opinions.

In the model the following interventions were used in different decision trees: HPA-1a- and HLA-DRB3*0101 typing (costs taken in every pregnancy), HPA-1a antibody testing week 20 and week 27; fetal HPA-1a typing; HPA-1a antibody quantitation; antenatal treatment with IVIg; hospitalisation, laboratory and clinical (ultra sound/MRI) investigation and treatment costs (platelet transfusions), life time costs.

One-way-sensitivity analysis and probabilistic sensitivity analysis were performed to address the uncertainty of the model parameters and to quantify the impact on the costs and QALYs. Three additional scenario's were tested; one to determine costs for inclusion of quality control of the program including platelet counts at birth of every newborn, a second model using improved risk stratification making it possible to reduce the number of pregnancies to be treated and a third model with failure of IVIG therapy in a given number of pregnancies.

Results: Adding of screening for HPA-1a to the current antenatal screening program of the Netherlands will lead to an additional cost of 4.7 million euro per year, and a gain of 226 Quality-Adjusted Life Years (QALY) per year, indicating an incremental cost-effectiveness ratio (ICER) of €20,782 per QALY gained. One-way-sensitivity showed that the uncertainty around the incidence of ICH, lifetime costs of disabled children and the probability of having antibody quantitation > 3.0 IU/mL at 20 weeks had the highest effect on the ICER. Performing platelet count in all HPA-1a negative mothers as a quality control (scenario analysis 1) would lead to yearly additional costs of €26,387 and no additional effects. If diagnostic assays become available improving the selection of high-risk pregnancies (scenario analysis 2), costs increment would be €2.9 million euro / year instead of €4.7. It is however currently uncertain to what extent this might lead to missing cases at risk for ICH. If yearly one case with ICH would be missed (scenario analysis 3), a gain of 192 QALYs was expected resulting in a cost-utility ratio of €26,559 per QALY gained.

Summary/Conclusions: An antenatal HPA-1a screening program including HPA-1a typing of pregnant women, HLA-DRB3*0101 typing, antibody testing and quantitation, foetal HPA-1a typing and treatment of pregnancies at risk with IVIG can be cost-effective. To obtain more knowledge on the usefulness of risk stratification based on HPA-1a antibody quantitation or antibody characteristics in a screening setting and to determine the efficacy of IVIG treatment in HPA-1a alloimmunised pregnancies identified by screening, a pilot HPA-1a screening program is warranted.

Parallel Session 29: Finding rare–well done!

PA29-L01 | Managing rare blood: A sustained challenge around the globe

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Finding rare blood for patients in need is challenge that requires the efforts of many, including the attending clinician, the transfusion

service, the blood supplier and the donor community, all around the world.

The first challenge is in recognizing the need for rare phenotype red blood cell units. This requires immunohematology skills and resources to recognise the presence of antibodies to high prevalence antigens, as well as the ability to phenotype and or genotype red cell antigens and their genes. These resources include laboratorians with the training, skills and experience to identify antibodies as well as the laboratory tools and techniques to facilitate these investigations. Many transfusion services initiate investigations for antibody identification and refer to national or international reference laboratories once the need for specialised tools or techniques is recognised.

The tools and expertise needed for identifying patient antibodies are mirrored on the donor testing side. The laboratory services of the blood operator must be able to screen donors effectively to identify those with rare phenotypes. These donors may be recognised through mass screening for antigens or, increasingly, blood group antigen genes; or they may be recognised due to antibodies seen in the donor. Once identified it is imperative that the blood supplier have a process to find these specific donors when a patient in need presents for transfusion. Both the internal search capabilities and the strategies for recruitment and retention of rare donors are critical to the effective supply of rare red cell units.

For some blood suppliers maintenance of a frozen inventory of rare red cell units is part of the strategy utilised to ensure timely availability of rare blood. The ongoing management of a rare frozen bank is an additional challenging task. Over the decades that rare units may remain in inventory both donor selection criteria and donor testing methods may change leading to a requirement for periodic review of frozen red cells for transfusion safety and suitability.

Once donors and patients are identified as individuals with rare phenotypes, notification and education related to the specific rare type are important. The specific phenotype may have bearing on patient health and the availability of blood for transfusion. In addition, awareness of the nature and need for rare phenotype red blood cells will often encourage donors and their family members to regularly donate and to respond to recruitment initiatives.

The need for rare phenotype red cells may arise anywhere in the world. Some phenotypes are more readily available in some regions than others, necessitating a process for international collaboration and exchange in order to ensure that those with rare phenotypes may have access to red cells that may not be available in their own region. This type of international collaboration is bolstered by institutions such as the ISBT Rare Blood Working Party and the International Rare Donor Panel which support the supply of rare blood everywhere.

PA29-L02 | Rare blood donor programs: ISBT working party survey

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Background: The ISBT Working Party on Rare Donors (WPRD) promotes international collaboration, education and exchange of information related to finding and providing rare blood. Key activities aim to increase rare donor numbers, by promoting growth of rare donor panels and to identify and assist with overcoming challenges with supply.

Aims: Provide an overview of rare donor programs and collate information to assist overcoming the challenges experienced with importing or exporting rare blood.

Methods: A survey was sent to all WPRD members focussing on the following elements:

- Rare Donor Program
- Red Cell Product Specifications
- Frozen Inventory
- Ordering & Shipping

Information about the following rare phenotypes was requested - Ge: −2, −3, Jk(a−b−), K_o, Kp(b−), M^kM^k, RH:-34−, U−, p, Sc:-1, En(a−), At(a−), Di(b−), Jr(a−), Rh_{null}, Vel−, D− − and O_h.

Data analysis was based on individual programs to allow for more than one program or database in a country and separate survey responses were provided.

Results: Members from 20 countries out of the 27 (74%) represented on the WPRD provided responses. In 19 countries there is some form of rare donor program. Two programs are facility based, one is regionally based, two are working towards national programs and 15 are national programs.

Fifteen programs used a similar definition for a person with a rare blood group. *Negative for a high prevalence antigen where the frequency of the antigen negative phenotype is < 1 in 1000 or where a combination of antigen negative phenotypes has a prevalence of <1 in 1000*. The remaining 6 programs generally varied in the frequency for example, ≤1:500, <4/1000 or <1% of the negative phenotype.

Sixteen respondents maintained a frozen inventory, 14 used the Haemonetics® ACP® 215 Automated Cell Processor for glycerolisation and deglycerolisation. Frozen expiry ranged from 10 to 40 years with 11 assigning a 10-year expiry, seven could retain exceptionally rare phenotypes beyond this expiry. SAG-M was the most common additive for thawed cells with an expiry of 24–72 h, two respondents assigned a 7-day expiry when using a closed system. A longer thawed

expiry, 7–14 days was assigned when AS-3 or saline with low concentration dextrose was used in a closed system.

There were 11,539 donors identified with the rare phenotypes of interest, 41% (4679) were group O and 3.1% (356) were group O negative. The most common rare phenotype was Di(b-), 39% (4460) found in eight programs with most donors identified in Japan (4324). The Kp(b–) phenotype was found in the greatest number of programs, whilst M^kM^k and Sc:-1 phenotypes were reported by the least number of programs, and M^kM^k also had the lowest number of donors (3). Most programs had small numbers or no donors with some of the rare phenotypes.

The number of rare units supplied each year varied significantly with six programs supplying less than 10 units, seven supplied 10–100, three supplied 100–1000 and three supplied >1000 units per year.

Eighteen programs indicated that they add rare donor phenotypes to the ISBT International Rare Donor Panel and 11 utilised the ISBT WPRD Blood Shipment form. Where incompatible blood is transfused, 10 indicated that they would utilise the ISBT Outcome of Incompatible Transfusion form.

Summary/Conclusions: The definition of a person with a rare blood type varied slightly between respondents, reflecting demand and local population variation. The low numbers or lack of donors with some of the very rare phenotypes highlights the need to increase the number of rare donors and establish new programs. The continued cooperation between member countries of the WPRD is essential to support patients with rare blood needs.

PA29-L03 | Development of RBC and platelet rare blood screening panels based on MALDI-TOF mass spectra

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Background: Rare blood screening might have been constrained due to shortages of appropriate serological reagent. To some extent, comprehensive genetic testing makes up for this deficiency. Several medium to high-throughput technologies have been applied in rare blood screening.

Aims: Here, we aimed to develop red blood cell (RBC) and platelet genotyping systems based on matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectra.

Methods: Sequences which contain polymorphisms of interest were obtained from GenBank. Specific amplification and extension primers were designed using online software Assay Design Suite (Agena Bioscience, USA). DNA samples were extracted from anticoagulant peripheral whole blood. The multiplex PCR, SAP and iPLEX reactions were performed using Veriti™ 96-Well Fast Thermal Cycler (Thermo Fisher Scientific, USA). The products were desalted, dispensed onto a 96-well SpectroChip and then detected using the MassARRAY Analyser system (Agena Bioscience, USA). The results were analysed by Typer 4.0 software (Agena Bioscience, USA). 42 rare and 36 random samples, as well as 34 random samples were serotyped and/or genotyped by routine serological methods and Sanger sequencing for verification of all loci of the developed RBC and platelet panels respectively.

Results: The RBC and platelet panels have been developed as follows. In the RBC panel, a total of 61 RBC antigen specific loci from 21 blood group systems are assigned to two reaction wells. In the platelet panel, 35 platelet antigen polymorphisms, 10 CD36 polymorphisms and 8 neutrophil antigen polymorphisms are detected simultaneously in two reaction wells. The reporting systems for the two panels have also been developed to output predicted phenotypes. The serotyping and genotyping results of the selected samples were completely identical with the results of mass spectrometric.

Summary/Conclusions: We have successfully developed the RBC and platelet panels for rare blood screening based on MALDI-TOF mass spectra.

PA29-L04 | Demographic study and transfusion challenges related to the rare D+C+E+c−e− (R_ZR_Z) phenotype: The French experience

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Background: D+C+E+c−e−, also known as R_ZR_Z, is a rare phenotype worldwide. The prevalence of the R_ZR_Z blood type is often considered $<1/10^4$. Due to the scarcity of the r^y haplotype (*dCE*), almost all D+C+E+c−e− individuals display a R^Z (*DCE*) haplotype in homozygous state. R_ZR_Z patients are at risk to form anti-c and/or anti-e, leading to a difficulty to find compatible donors.

Aims: The aim of this study consisted in the description in France of the ethnic background of the R_ZR_Z population (donors and patients), immunohematological features of donors and patients, and rare blood needs.

Methods: Data were extracted and processed from the French National Registry of People with a Rare Blood Type.

Results: 277 R_ZR_Z people are enlisted in the national registry. Out of people of European ancestry, the ethnic backgrounds are as follows: 32 (12%) Amerindians living in French Guiana, 30 (11%) Hispanic sounding name people, 30 (11%) Asians, 27 (10%) Middle Easterners, 24 (9%) North Africans, and 15 (5%) Turkish. Among people of European ancestry, we notably found 14 individuals from Italy and 6 from Portugal.

ABO distribution is as follows: 47% O, 35% A, 13% B, 5% AB. Interestingly, two individuals are Fy(a–b–), 1 originating from the Comoro Islands and 1 from Tunisia.

13 patients are alloimmunised, among which 6 show a Rh-related antibody: (i) an elderly female patient with anti-c, anti-Jk^a, anti-Lu^a; (ii) a pregnant woman with a low-titre anti-c, anti-P1; (iii) a 40-year-old male patient with anti-e, anti-C^w; (iv) an elderly female patient with

anti-c; (v) a multiply-transfused female patient with anti-e, anti-f; (vi) an elderly male patient with anti-e.

43 individuals (19 O, 15 A, 7 B, 2 AB) have at least 1 red cell unit cryopreserved at the French National Rare Blood Bank, for a total of 199 units. Among O donors, 2 are Fy(b–), Jk(a–), S–; 1 is Fy(a–), Jk(b–), S–; 1 is Fy(b–), Jk(b–), S–. There are no O, Fy(a–), Jk(a–), S– donors. Only 14 donors are considered active (> 1 donation over the past 2 years). Of note, 6 of them (43%) are over 50 years old.

During the past 15 years, 17 R_ZR_Z patients were transfused: 15 with no anti-c or anti-e (prevention of Rh alloimmunization), 1 with anti-e, 1 with anti-e and anti-f. 158 cryopreserved RBC units were transfused. 3 patients accounted for 33% of the transfused units, with a maximum of 17 units in 2 patients: 1 with myelodysplasia, 1 with acute leukaemia (transferred from French Guiana to Paris).

Summary/Conclusions: According to previously published data (Mourant AE et al. Oxford University Press 1976), a R^Z haplotype frequency > 2% was especially reported in populations from Israel, Italy, Kuwait, India, Vietnam, Taiwan, Japan, and South Korea. Frequencies > 10% were mainly found in North, Central and South American Indians, which is consistent with our high rate of R_ZR_Z individuals found in French Guiana. Our data also demonstrate for the first time the significant prevalence of R^Z in North Africa and Turkey. As shown through this study, there is a real need to renew our population of R_ZR_Z donors. There are currently no blood drives in French Guiana, while our study shows that people from this region account for 12% of our R_ZR_Z individuals. As a result, this may open the debate of the implementation of a targeted rare donor program in this territory, to better ensure transfusion safety of patients living both in French Guiana and mainland France.

PA29-L05 | The American rare donor program's support of patients with sickle cell disease

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Background: The American Rare Donor Program (ARDP) has a mission of supplying rare blood products to alloimmunised patients. ARDP is made up of 90 member facilities, of which 76 are blood collectors. Most requests for rare blood products are for red blood cell (RBC) units. Many of these are for patients with sickle cell disease (SCD).

Aims: This study aimed to analyse the requests received in calendar year 2022 (CY2022) for rare RBCs for patients with SCD by clinical condition, phenotype, and fill rate.

Methods: The ARDP database was queried for all requests received by patient diagnosis. Those requests for patients with SCD were further analysed by clinical condition, if provided, phenotype, and fulfilment information. Fulfilment data were evaluated to determine for which requests all, some or none of the requested units were identified and if they were shipped. Cancellations of requests were also enumerated.

Results: In CY2022, ARDP received 1273 requests for rare blood products, of which 1251 (98.3%) were for RBC units. Of these, 558 requests (43.8%) were for 233 patients with SCD. A single request was submitted for each of 130 patients with 19 being the maximum number of requests for a single patient. Based on the clinical information provided with the requests, 265 (47.5%) were for treatment of anaemia, 134 (24%) were for patients experiencing sickle crisis, 75 (13.4%) were for exchange transfusions and 61 (10.9%) were in support of pregnancy/delivery. Of all requests for patients with SCD, 52% required RBCs lacking multiple common antigens, 27% were for RH allele-matched RBC units and 21% required RBCs lacking a high-prevalence antigen. Nine percent of requests were cancelled within one day of order. Overall, 90% of uncancelled requests were filled or partially filled, 86% of requests were filled, while 3% were unable to be filled. RH allele matched requests had the lowest fill rate by rare type at 78.8%. No cases involved importation of blood from outside of the US; one request that was filled was for a patient outside of the US.

Summary/Conclusions: The ARDP receives more than 1000 requests for rare blood products per year. Most of these are for RBC units. By analysing data from requests received in CY2022, we show that nearly 44% of requests for RBC units were for patients with SCD. Overall, 90% of these requests were able to be fulfilled or partially fulfilled. Based on the estimated number of patients with SCD in the US (~100,000), the requests coming from member centres to the ARDP are in support of 0.2% of this patient population. Based on a recent survey of ARDP members (Nance S, Transfusion 2021), it is very likely that many more rare RBC units are being provided to hospitals for transfusion to patients with SCD directly by ARDP member facilities from their inventories of liquid and/or frozen RBC units or by direct recruitment of their rare donors. With the rate of alloimmunization in patients with SCD being the highest in any patient population, the ARDP, its member centres and rare blood programs around the globe are a critical resource for transfusion support of patients with sickle cell disease.

Parallel Session 30: DEHP bags in transition… are we ready?

PA30-L01 | Update on non-DEHP blood containers

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In order to be soft, flexible and withstand the forces applied during processing and everyday handling, PVC blood bags require a plasticizer. The plasticizer di(2-ethylhexyl) phthalate (DEHP) has been used in blood bags from their development during the mid-20th century, as it has proven to add a unique, stabilising effect to the red blood cell (RBC) membrane. Throughout the years, it has become an established

fact that DEHP aids in preserving the RBC membrane integrity. Storage of RBCs in a non-DEHP environment has been linked to unacceptable haemolysis levels, increased RBC microvesicle formation and reduced RBC post-transfusion survival in the circulation. For these purposes, DEHP has remained the almost exclusive plasticizer of choice for RBC storage up until now.

However, toxicity concerns have encouraged a revision of the EU regulation on the use of phthalate plasticizers in medical devices such as blood bags. The result of the revision enforces a termination of the previous exemption of usage of DEHP in blood bags. Consequently, there is an urgency to develop blood bags based on alternative plasticizers.

Finding a plasticizer that can replace DEHP, yet not compromise the RBC quality, is a very high priority. Yet, it has proven to be a challenge. Studies have been conducted using 1,2-Cyclohexanedicarboxylic acid, diisononyl ester (DINCH), di(2-ethylhexyl) terephthalate (DEHT) or n-butyryl tri-n-hexyl citrate (BTHC) as substitute plasticizers, with varying results. So far, most have pointed towards a necessary exchange not only of plasticizer, but also RBC additive solution, to ensure sufficient RBC quality. Such an exchange will require a tremendous validation effort since, in short, all RBC components downstreams of the unseparated whole blood will be affected. If the blood bag manufacturers choose different plasticizers and/or additive solutions, the required efforts will be even larger.

This presentation explores the different plasticizer options studied so far, the current availability of commercial blood bags and the joint European efforts in establishing validation guidance. The aim is to try to determine how far Europe has come on the road to non-DEHP blood bags.

PA30-L02 | In vitro evaluation of red blood cells, platelets, and plasma componentized with an automated blood processing system and stored in a non-DEHP plasticized bag system

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Background: Di(2-ethylhexyl)phthalate (DEHP) plasticized polyvinyl chloride (PVC) has been used in blood bag systems for the collection of whole blood (WB) and storage of its components for >70 years. Revisions to European regulations have called for DEHP removal from medical devices including blood bag systems. The quality of red blood cell (RBC), platelet (PLT), and plasma products stored in blood bags manufactured without DEHP (non-DEHP) requires evaluation prior to industry use.

Aims: This study evaluated non-DEHP material performance through *in vitro* metrics for the resulting RBC, PLT, and plasma products generated by the Reveos® Automated Blood Processing System (Terumo BCT, Inc., Lakewood, CO, USA) when using the non-DEHP configuration of the Reveos LR EXT disposable set. This is a hybrid plasticizer system in which the primary collection bag, plasma bag, PLT bag, and residual leukocyte bag vinyl are comprised of a Di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH) plasticized PVC, and the RBCs are stored in a proprietary non-DEHP ErythroMate vinyl.

PA30-L02 – Table 1

Parameter (unit)	*N*	Mean ± SD (Range)
Volume (mL)	77	269.1 ± 19.7 (229.4–329.3)
Haematocrit (%)	77	62.4 ± 1.8 (58.8–66.6)
Total Haemoglobin (g/unit)	77	56.8 ± 5.8 (46.1–73.7)
Day 35 LR-RBC Haemolysis (%)	76	0.19 ± 0.12 (0.06–0.80)
Day 42 LR-RBC Haemolysis (%)	77	0.24 ± 0.16 (0.07–1.13)
rWBC (WBC $\times 10^6$/unit)	77	0.07 ± 0.16 (0.02–1.22)
RBC leukoreduction duration (h:mm)	75	0:32 ± 0:08 (0:18–1:07)
RBC recovery across filter (%)	77	93.2 ± 1.2 (90.7–100)

Methods: Non-DEHP materials were maintained throughout collection, componentization, storage and sampling. WB from healthy donors was collected into non-DEHP sets containing CPD and held at 22°C ±2 for 2–24 h prior to componentization. WB was separated on the Reveos System in the Reveos LR EXT disposable using either a 2-component (2C) or 3-component (3C) protocol ($N = 77$). RBC products were combined with PAGGSM additive solution, leukoreduced, and stored at 4°C ±2 for 42 days with interim sampling on Day 35. Single-donor PLT units were stored at 22°C ±2 for 7 days with interim sampling on Day 5. Plasma units were frozen within 6 h and stored at −30°C ±5 for ≥30 days prior to end-of-storage thawing and sampling. Cell quality was assessed based on EDQM-defined criteria.

Results: RBC units had acceptable quality for all EDQM endpoints (Table 1). Single-donor PLT products ($N = 45$) also had acceptable volume (63.2 ± 3.0 mL) and yields (97.1 ± 23.5 PLT $\times$ 10^9/unit). PLT quality parameters were acceptable through 7 days of storage; Day 5 $pH_{22°C}$ (7.3 ± 0.4), Day 7 $pH_{22°C}$ (7.2 ± 0.4) and retained positive swirl. At end of storage, plasma units had acceptable volume (245.0 ± 37.1 mL, $N = 53$), Factor VIIIc content (99.6 ± 26.7 IU/dL, $N = 51$), rWBC (0.06 ± 0.07 WBC $\times$ 10^6/unit, $N = 53$), rPLT (14.2 ± 9.6 PLT $\times$ 10^9/L, $N = 53$), and rRBC (0.09 ± 0.12 RBC $\times$ 10^9/L, $N = 52$) content.

Summary/Conclusions: All products passed the EDQM requirements for each parameter measured. The non-DEHP disposable system achieved 42 days of LR-RBC storage in PAGGSM while maintaining acceptable cell quality. A 42-day LR-RBC storage duration would make the transition from DEHP to non-DEHP possible for most European countries without negatively impacting the available blood supply. Additionally, all evaluated PLT parameters achieved passing results on 5 and 7 days of storage in the non-DEHP system supporting a 7-day single-donor PLT product storage duration, compared to the standard storage duration of 5 days.

PA30-L03 | In vitro evaluation of red cell concentrates and plasma prepared and stored in non-DEHP bags from whole blood collected in full non-DEHP in-line system

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Background: Di-ethyl-hexyl-phthalate (DEHP), main plasticizer used for whole blood collection systems, must no longer be used in medical disposables after May 2025 in Europe. Preparation of regulatory transition of whole blood disposables and storage containers to non-DEHP plasticizers requires extensive in-vitro evaluation of red blood cell concentrates (RCCs) and plasma quality during storage period.

Aims: This study assesses the impact of alternative plasticizers and additive solution (respectively PVC-Citrate/PAGGSM & PVC-DINCH) material change on quality of RCCs and plasma until the end of storage period to determine whether the selected non-DEHP materials can maintain acceptable haemocomponents quality, especially for RCCs having DEHP a well-known stabilizing effect on the red blood cells membrane.

Methods: Whole blood was drawn from usual donor population following the common repartition–% male and female donors and % ABO group–using non-DEHP FQ422FR blood bag disposable (Fresenius Kabi) with PAGGSM as additive solution. In-vitro quality of RCCs ($N = 32$) has been assessed according to regulatory major change evaluation file at day 1 before and after processing (centrifugation and separation as routinely used) then at D21, D28, D35, D42 and D49 of storage at 4 ± 2°C. Separated plasma units ($N = 32$) were connected to a filtration disposable (non-DEHP FS013FR, Fresenius Kabi) to perform leukoreduction to meet French residual leucocytes limit ($\leq 1 \times 10^4$/L). Plasma was evaluated before filtration, before freezing and after 14 days, 6 and 12 months of storage at −25°C.

Results: RCCs and plasma units met the French regulatory requirements with 95% confidence interval. The following parameters were analysed and compared with the historical data:

PA30-L03 Table 1: RCCs quality parameters during storage

Parameters	Haemolysis (%)	ATP (µmol/L)	Glucose (mmol/L)	Lactate (mmol/L)
D1	0.05 ± 0.10	880 ± 91	34.9 ± 2.0	5.6 ± 0.9
D21	0.15 ± 0.12	913 ± 115	25.3 ± 2.3	21.9 ± 2.8
D28	0.19 ± 0.12	858 ± 108	22.2 ± 2.5	26.1 ± 3.3
D42	0.36 ± 0.17	669 ± 100	17.7 ± 2.6	33.1 ± 3.9
D49	0.48 ± 0.24	554 ± 93	15.8 ± 2.8	35.9 ± 4.3

- RCCs Haemoglobin, Haematocrit, ATP, Glucose, Lactate, Haemolysis, K+, pH, pO_2 and pCO_2 showed comparable results to the control group (LR- RCC processed in PVC-DEHP blood bags). The absence of DEHP in the collection and storage bags did not show any negative effect on the quality of RCCs stored until D49, especially in their main membrane stability indicators shown in Table 1.
- Plasma total protein, Albumin, IgG, IgM and IgA, complement activation factors (C3a and C5a), prothrombin time, APTT, coagulation factors (fibrinogen, FVIII, FII, FV, FVII, FX, FIX, FXI, VWF and ADAMTS13), coagulation inhibitors (Antithrombin, Proteins S and C), fibrinolytic factors (Plasminogen, α2Anti-plasmin, TAT) as well as thromboelastometric parameters were similar to control group (plasma derived from PVC-DEHP blood bags).

Summary/Conclusions: RCCs prepared and stored in PVC-Citrate/PAGGSM Top and Bottom In-Line system (Fresenius Kabi) met French quality requirements after processing and 42 days storage for every parameter tested and more particularly for haemolysis. These conclusions are reassured by the results obtained after 49 days of storage. The quality parameters for Plasma prepared and stored in PVC-DINCH are likewise compliant to French requirements.

PA30-L04 | Evaluation of containers with innovative PVC plasticizer used for 42-day storage of BCR-AS

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Background: Polyvinyl chloride (PVC) plasticized with di-ethylhexyl phthalate (DEHP) is used for the manufacture of containers for blood preparation and storage. DEHP improves the properties of PVC and upgrades its durability as well as stabilizes the RBC membrane and prevents haemolysis. Since there appeared evidence of DEHP toxicity, the EU Regulation 2017/745 was implemented which banned the use of DEHP in medical devices if the maximum concentration exceeded 0.1% (w/w). So DEHP had to be replaced with another, non-toxic plasticizer at no loss to the quality of stored red cells.

Aims: Qualitative assessment of a set of containers for blood collection, preparation and storage made of modified PVC with no DEHP (Prototype Renolit Bloodprotect 42Plus). The qualitative tests were performed using Red Cells, Buffy Coat Removed in Additive Solution (BCR-AS).

Methods: 16 trials were conducted with 32 units of whole blood (WB). Blood was collected in the Regional Blood Transfusion Center in Warsaw, according to Polish guidelines. The WB units were collected into quadruple sets of DEHP-free plastic containers. Each study included at least 2 ABO- and Rh-compatible WB units. WB units were subjected to preparation 1 to 2 h of collection. The 2 units of compatible BCRs obtained during preparation were then pooled, and the pool was divided into two equal-weight BCR units. A BCR unit in a plastic with DEHP container was the control while the study group was a unit poured into a container made of the material under investigation. Additive solution (SAGM) was added to both containers which were then stored under standard conditions for up to 42 days at 2–6°C. The quality control samples were collected on the first and forty-second day of storage. The tests were performed using the following analysers: haematology Sysmex XP-300 (RBC, Hb, Ht), biochemistry Cobas Integra 400 plus (glucose, sodium, potassium, LDH), Cobas b 221 analyser (pH, pCO_2, pO_2). Haemolysis was tested using spectrophotometry.

Results:

Summary/Conclusions: The quality of RBCs/in additive solution with no buffy coat stored for up to 42 days in containers made of modified PVC Prototype RENOLIT BLOODPROTECT 42Plus (without DEHP) is comparable to the quality of RBCs/in additive solution with no buffy coat stored up to 42 days in conventional containers (with DEHP).

PA30-L04 Table 1 Quality control tests of BRC-AS on days 1 and 42 of storage

	Control group		Study group	
Parameters	**1 day**	**42 day**	**1 day**	**42 day**
V (ml)	288 ± 14	277 ± 13	287 ± 14	276 ± 13
WBC ($\times 10^9$/unit)	0.81 ± 0.03	0.13 ± 0.05	0.82 ± 0.03	0.11 ± 0.03
RBC ($\times 10^{12}$/unit)	1.89 ± 0.13	1.81 ± 0.13	1.91 ± 0.13	1.82 ± 0.12
Hb (g/unit)	55.61 ± 3.7	53.83 ± 3.8	56.23 ± 3.8	54.08 ± 3.8
Ht (%)	56.58 ± 2	60.64 ± 2	56.55 ± 2	61.29 ± 1.5
Haemolysis (%)	0.00	0.16 ± 0.2	0.00	0.17 ± 0.1

PA30-L04 Table 2 Biochemical tests of BCR-AS on days 1 and 452 of storage

	Control group		Study group	
Parameters	**1 day**	**42 day**	**1 day**	**42 day**
Glucose (mmol/l)	33.72 ± 0.7	19.81 ± 1.2	33.66 ± 0.8	18.99 ± 1.3
Sodium (mmol/l)	151.63 ± 2.0	118.00 ± 2.0	150.75 ± 3.0	116.25 ± 3.0
Potassium (mmol/l)	4.25 ± 0.6	>30	4.32 ± 0.6	>30
LDH (IU/l)	54.73 ± 22	554.09 ± 174	49.18 ± 18	656.55 ± 166
pH	6.93 ± 0.03	6.43 ± 0.02	6.93 ± 0.03	6.42 ± 0.02
pCO_2 (mmHg)	71.93 ± 6.8	142.54 ± 9.8	74.79 ± 6.9	119.08 ± 8.6
pO_2 (mmHg)	33.18 ± 4.2	49.16 ± 7.3	32.92 ± 4.2	62.07 ± 8.1

PA30-L05 | Comparison of the in vitro quality of red cell concentrates, prepared from whole blood collected in DINCH-PVC and stored in BTHC-, DEHT- and DINCH-PVC storage bags

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Background: Di(2-ethylhexyl)-phthalate (DEHP) is the main plasticizer used for whole blood collection and storage bag. Because of its potential toxicity, the European Commission is sun setting the use of DEHP in Medical Devices in European per the REACH Annex XIV process. Therefore, alternative plasticizers are investigated for use in blood collection systems. A complicating factor here is that the alternative plasticizers lack the ability to stabilize red cell membranes as observed with DEHP, resulting in higher levels of haemolysis. Newer storage solutions, such as PAGGS-M, could help reduce haemolysis during storage in non-DEHP bags. The present study was designed to evaluate red cell quality during storage in three different non-DEHP storage bags.

Aims: To compare the *in vitro* quality of red cell concentrates (RCC) in PAGGS-M prepared from whole blood (WB) and collected in non-DEHP collection systems, during storage in BTHC-, DEHT- and DINCH-PVC storage bags.

Methods: Eighty WB units (500 ± 50 mL), collected in DINCH-PVC (Fresenius Kabi, GQ422NL) were processed into plasma, buffy coat and RBC after overnight hold at ambient temperature. After adding the PAGGS-M additive solution, RCCs were leukodepleted over the inline filter into the BTHC storage bag. In two separate studies, a total of forty pairs of ABO-compatible RCCs were mixed and equally divided over one of the original BTHC bags ($2\times$ $n = 20$) and either a DEHT- ($n = 20$) or a DINCH-PVC bag ($n = 20$). RCCs were statically stored at 2–6°C and analysed for haematological and metabolic properties at day 35 and 42 of storage. Differences between the storage bags were analysed using paired *t*-test. A *p*-value of less than 0.05 was considered significant.

Results: During storage of RCCs, the red cell concentration remained stable in all three storage bags. A slight increase of MCV was observed during storage. The cell swelling was more pronounced during storage in DEHT as compared to BTHC and DINCH. Haemolysis increased during storage, but at day 42 all units complied with the European requirement (<0.8%). Haemolysis was significantly higher (paired *t*-test) in DEHT and DINCH as compared to BTHC. In the BTHC vs DEHT comparison, haemolysis at day 42 amounted to 0.35% ± 0.12% and 0.53% ± 0.20% ($p < 0.01$), respectively, while in the BTHC versus DINCH comparison these values amounted to 0.34% ± 0.12% and 0.43% ± 0.13% ($p = 0.016$) respectively. Glycolytic activity, as measured by glucose consumption and lactate production, was significantly ($p < 0.01$) higher during storage in BTHC as compared to DEHT and DINCH ATP levels were better maintained during storage in BTHC and DINCH as compared to DEHT ($p < 0.01$). At day 42, all units had ATP levels >2.7 µmol/g Hb (Sanquin internal guideline), a level associated with acceptable 24 h *in vivo* recovery.

Summary/Conclusions: RCCs prepared from WB collected in a DINCH collection bag, stored in PAGGS-M in BTHC, DEHT- or DINCH-PVC storage bag for 42 days, comply with the requirements for haemolysis and ATP content. Red cell stability (as measured by haemolysis) and glycolytic activity were significantly better maintained during storage in BTHC- as compared to DINCH- and DEHT-PVC. Currently, at Sanquin, a second haemovigilance surveillance is ongoing to evaluate the adverse event frequency after transfusion of RCCs stored in BTHC/PAGGS-M.

Parallel Session 31: A, B, maybe?

PA31-L01 | Identification of three novel ABO blood group alleles

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Background: Today we know more than 300 *ABO* variant alleles. Most ABO subtypes are caused by single nucleotide substitution in the exons 6 or 7, which encodes for the catalytic domain of ABO glycosyltransferases. The characterization of the molecular background of these variants helps to understand the underlying mechanisms causing the different phenotypes.

Aims: Identifying the underlying genetic background causing discrepant serological results in three different patient and donor samples.

Methods: The ABO antigen determination was done by standard gel column agglutination testing (Grifols; BioRad) and tube technique. Reverse typing was performed using commercial A, B, and O test cells (Grifols). Molecular investigation was initially performed by sequence specific primer (SSP)-PCR detecting common *ABO* variants (ABO-Type variant, BAG Diagnostics). All seven *ABO* exons containing the adjacent flanking intron regions were amplified and sequenced using published and in-house primers. The effect of the amino acid substitution on the glycosyltransferase A was predicted using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/)

Results: The erythrocytes of the first proband showed 3+ mixed field resp. 1-2+ agglutination with monoclonal anti-A and anti-AB, and no agglutination with anti-B. In reverse typing, the serum showed no agglutination with A and O cells, and 4+ agglutination with B cells. The erythrocytes of the second proband showed no

agglutination with monoclonal anti-A, -B and -AB, but a weak agglutination with polyclonal anti-AB. The antigen determination in tube showed no agglutination with anti-A, -B and -AB. In reverse typing, the serum showed 3+ agglutination with A cells, 4+ agglutination with B cells and no agglutination with O cells. The erythrocytes of the third proband showed a very weak agglutination with monoclonal anti-A and anti-AB, and no agglutination with anti-B. The antigen determination in tube showed no agglutination with anti-A, -B and -AB. In reverse typing, the serum showed no agglutination with A and O cells and 3+ agglutination with B cells. By SSP-PCR the characteristic single nucleotide variations known for alleles *ABO*A1.01* and *ABO*O.01.01* (proband 1 and 2) resp. *ABO*O.01.02* (proband 3) could be identified. In two samples, sequencing of *ABO* revealed heterozygous substitutions c.952G>A (proband 1) resp. c.973T>C (proband 2) in exon 7. These mutations induce the amino acid substitutions p.Val318Met resp. p.Trp325Arg, both located within the catalytic domain of the glycosyltransferase. In proband 3, a heterozygous splice site mutation c.28+5G>A (IVS1+5G>A) in intron 1 was identified.

Summary/Conclusions: Here we report three novel A variant alleles, *ABO*A1.952A* (accession number ON931623), *ABO*A1.973C* (accession number OQ236607) and *ABO*A1.28+5A* (accession number OQ236608), all associated with the *ABO*A1* allele. Substitution c.952G>A has been associated with a B_{el} phenotype (*ABO*BEL.05*), however on the *ABO*A1* background the weakening of the A expression is less reduced. Based on serological data, the substitution p.Trp325Arg most likely reduces the glycosyltransferase activity. According to PolyPhen-2 this substitution is predicted to be probably damaging (PolyPhen-2 score 1.00). The serological data implies that the splice site mutation c.28+5G>A causes an A_{el} phenotype, which is in agreement with the published results for the allele *ABO*B.28+5A* (Hong *et al*., Journal of Transl. Med. 2021).

PA31-L02 | Truncated glycosyltransferase coding regions in novel ABO alleles give rise to weak A or B blood group expression and discrepant typing results

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Background: Correct ABO blood-group matching between donor and patient is crucial for safe transfusions. However, both acquired and inherited factors complicating interpretation of typing results occur. Among them numerous genetic variants have been described to result in weak A or B expression, most of them amino acid substitutions altering the activity and or specificity of the ABO transferase.

Aims: The focus of this study was to investigate the underlying reason causing inconclusive ABO serology in samples referred to our laboratory.

Methods: *ABO* genotyping and sequencing were used to characterise ABO-discrepant blood samples ($n = 13$). Detected genetic variants were inserted in a GFP-containing bicistronic vector to assess A/B antigen levels following overexpression in HeLa cells. Flow cytometric analysis was performed to evaluate ABO expression profiles on both red blood cells and the transfected cell line.

Results: Seven novel alleles with nonsense mutations predicted to truncate the encoded ABO glycosyltransferases were identified. Whilst these variants spontaneously could be mistaken to represent *O* alleles, routine serology showed signs of ABO glycosyltransferase activity. Alleles based on *ABO*A1.01* backbone displayed the following variants: c.42C>A; p.Cys14* ($n = 1$), c.102C>A; p.Tyr34 ($n = 2$), c.106dup; p.Val36Glyfs*21 ($n = 3$) or c.181_182ins; p.Leu61Argfs*21 ($n = 3$). Serological red cell typing with anti-A showed weak A expression in all but two samples (both of the c.181_182ins variant). Plasma typing was negative or only weakly reactive with A_1 test cells. Although the main erythrocyte population stained negative with monoclonal anti-A by flow cytometry, 10%–31% of cells showed variable levels of A antigen for all samples tested among the four A variants. Transfection studies confirmed significantly decreased A expression in all four variants compared to wildtype, the c.42C>A variant least impacted, that is, showed the highest percentage of positive cells. The remaining variants were found on *ABO*B.01* background: c.1_5dup; pGly3Trpfs*20 ($n = 2$), c.15dup; p.Arg6Alafs*51 ($n = 1$) or c.496del; p.Thr166Profs*26 ($n = 1$). Although absence of plasma anti-B was noted in all, B antigen expression was barely detected on erythrocytes in traditional serology and flow cytometry alike. Overexpression showed varying degrees of decreased B expression levels in all three variants, most pronounced for c.496del, for which B expression was virtually completely abolished whilst the decrease observed with c.15dup was marked but left some remaining activity. However, for the c.1_5dup variant, results did not reach significance but a showed a clear downwards trend.

Summary/Conclusions: Samples displaying aberrant ABO serology revealed seven principally interesting alleles. Despite the presence of truncating mutations, normally expected to result in null alleles in any blood group system, low levels of A or B antigens were detectable in cases where the alterations affected *ABO* exons 1-4 but not exon 7. This is compatible with the previously proposed concept that alternative start codons in exons 2–5 can be used to initiate translation of functional ABO glycosyltransferase. However, more research is required to prove if N-truncated fragments of ABO glycosyltransferases exist *in vivo*.

PA31-L03 | Beyond the hemagglutination assay for ABO-histocompatibility: ABH-glycan-functionalized beads allow precise characterization of ABO antibodies

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Background: Immune risk assessment in solid organ transplantation has been revolutionized by use of single antigen Luminex bead technology to measure HLA antibodies with specificities to donor antigens. ABO-incompatible (ABOi) organ transplantation, undertaken due to severe donor shortages, relies on quantification of ABO antibodies (ABO-Abs) using the hemagglutination assay (HA), which is known to be plagued by poor reproducibility and variable sensitivity, is cumbersome for determination of IgG vs IgM isotypes, and cannot detect A/B-glycan-subtype specificities of ABO-Abs.

Aims: Our aim was to quantify IgG and IgM anti-A and anti-B antibodies by Luminex mean fluorescence intensity (MFI) values using glycan-functionalized beads, and compare MFIs to HA titres.

Methods: ABO single antigen beads (ABO-A and -B subtype I-VI glycans synthesised and coupled to individual Luminex beads) created by our team were used to measure serum ABO-Abs. Sera from healthy adults of ABO-A, ABO-B, and ABO-O groups ($n = 119$; 60% female) were tested for anti-A and/or anti-B; Abs bound to A/B-glycan-coupled beads were detected using fluorescent-labelled anti-human IgG and IgM antibodies. The same samples were tested by 'standard' HA (no enhancement with anti-human globulin; no DTT). Serially diluted sera (50 µL) were incubated with 25 µL of 1% A1 and B reagent erythrocytes at room temperature; HA titre was reported as the last dilution showing visual agglutination. For comparison to anti-A HA, Luminex beads MFI values for A-II, A-III and A-IV glycan subtype target beads were averaged as all three glycans are present on group A1 erythrocytes.

Results: Although a pattern of increasing median MFIs versus HA titres was evident for IgM anti-A and anti-B, this was not the case for IgG, where no correlation of MFI with HA titre was observed; indeed, a wide range of MFIs was detected at every HA titre step for both IgM and IgG anti-A and anti-B Abs as shown in the table below. Levels of one isotype antibody did not predict levels of other isotype antibodies. No significant sex-based differences in titres or MFI were detected (data not shown).

PA31-L03 – Table 1: Examples of ABO titre ranges of antibody as detected by luminex assay.

Examples of titre results	Approximate IgM MFI range	Approximate IgG MFI range
Anti-A1: 1/64	0–15,000	5000–30,000
Anti-A1: 1/128	0–18,000	100–22,000
Anti-B: 1/64	0–15,000	0–28,000
Anti-B: 1/128	0–14,000	0–24,000

Summary/Conclusions: These results demonstrate that HA titre alone is insufficient for accurate assessment of ABOi transplant risk related to ABO-Abs. Each HA titre includes an unpredictable range of ABO-Abs as detected by Luminex. IgG antibodies can contribute to non-AHG HA titres but are notably under-estimated by HA. Using only titre thresholds, patients may unnecessarily be denied access to ABOi transplants. In contrast, the Luminex bead-based ABO-Ab assay will allow more accurate ABOi transplant risk assessment and inform the relative contributions of specific IgM and IgG isotype anti-A and anti-B antibodies. This method can also be used for precise determination of ABO antibodies in haemolytic disease of the newborn as well as assessment of ABO antibodies in platelet units.

PA31-L04 | Four cases of recipient blood group serum typing accommodated to donor organs after ABO-incompatible kidney transplantation

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Background: ABO-incompatible kidney transplantation (KT) has become a high-profile strategy in the field of organ transplantation due to limited options for donor organs. This procedure has been made possible through intensive desensitization of donor-specific ABO antibodies before and after transplantation, including apheresis and B cell-depletion. However, not all cases come down to clinical rejection after the desensitizing treatments have been completed. Several studies suggest that donor-specific down regulation of antibody production plays a key role in preventing rejection, implying the importance of monitoring ABO antibodies in KT recipients.

Aims: This study is aimed at analysing the pattern of blood group serum typing after ABO-incompatible KT.

Methods: We retrospectively analysed patients who underwent ABO-incompatible KT from May 2009 to December 2015. ABO blood types of each patient before and after KT were investigated. The results of typing were cross-checked by using manual method with typing plates and column agglutination method.

Results: Among 101 recipients, 4 recipients showed changes in serum typing after KT, resulting in ABO discrepancies. All of their original blood types were type O, which were preserved for at least 2 months

after KT. At follow-up several years later, their serum typing was no longer type O; rather showed the same results as the donor's serum typing. 3 cases went through changes in serum typing from O to B, and their anti-B isohemagglutinin antibody titters before desensitization ranged from 1:8 to 1:128, which were turned to negative within 6 years after KT. The other case was about a change from O to A, showing decreasing anti-A antibody titter from 1:256 to 1:1 for 9 years after KT. 2 recipients, including the case of O to A, were diagnosed as antibody-mediated rejection (AMR) of kidney during follow-up periods, while the other 2 recipients have not suffered clinical rejections.

Summary/Conclusions: This analysis demonstrated that immune tolerance can occur in some KT recipients, beyond the extent of down regulation of antibody production. Because the immune tolerance results in ABO discrepancies, separate transfusion guidelines and monitoring are necessary for the recipients. The study also suggests that regular check-ups of blood types for all KT recipients be recommended.

PA31-L05 | Novel evidence that the ABO blood group shapes erythropoiesis and results in higher hematocrit for blood group B carriers

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Background: The haematopoiesis is orchestrated by gene regulatory networks that progressively induce lineage-specific transcriptional programs. To guarantee the appropriate level of complexity, flexibility and robustness, these networks rely on transcriptional and post-transcriptional circuits involving both transcription factors (TFs) and microRNAs (miRNAs). Albeit the expression of glycosyltransferases encoding for ABO blood group (BG) antigens share the same post-transcriptional circuits, little is known about the interference of the ABO BG transcriptional network with the erythropoiesis. Recently, we reported that miRNAs play a critical role in the regulation of the ABO BG system by simultaneous targeting TFs and the glycosyltransferase (GT). In addition, numerous miRNAs present in RBCs show a partially BG dependent expression pattern and comprise potential binding sites in the 3'UTR of the mRNA of TFs involved in the regulation of erythropoiesis.

Aims: We therefore aimed to analyse if the ABO BG type has an impact on the erythropoiesis.

Methods: We conducted a large-scale ($N = 245.000$) analysis of the haemoglobin (Hb) content in RBCs and haematocrit values in first time healthy blood donors with different ABO blood group. MiRNAs and TF profiling were performed in HSC-erythroid differentiation cultures representing different ABO BG (predominantly heterozygous) by quantitative PCR. Candidate miRNA, pre-miR-215, was stably overexpressed in CD34+ HSPCs of BG A donors using lentiviral gene transfer and erythroid differentiation assessed by flow cytometric analysis of CD235a positive cells.

Results: Over the large population of first time blood donors, the Hb content is slightly but significantly increased in individuals with BG B compared to BG A (13.92 ± 0.94 vs. 13.82 ± 0.92 mg/dL). In addition, in vitro cultures revealed that HSCs from BG B individuals ($N = 14$) showed a significant accelerated erythropoiesis compared to BG A HSCs (A: 22.5% ± 3.0%, B; 31.3% ± 2.2% proerythroblast at day 5 of differentiation). Markers of terminal eryhthroid differentiation, such as CD235a appeared 2 days earlier in BG B, as compared to BG A HSCs. Individuals with BG B showed decreased mRNA and protein expression levels of TFs (RUNX1, HES-1) and increased expression levels of corresponding miRNAs (miR-215-5p, -182-5p) in erythroid precursors as compared to BG A. Consistent with these data, overexpression of miR-215 in HSPCs of BG A donors lead to downregulated mRNA levels of *RUNX1* and *GT* as well as decreased A-antigen expression in conjunction with an accelerated erythropoiesis.

Summary/Conclusions: Our study for the first time reveals an interference of the ABO BG and the velocity and capacity of RBC differentiation, which also translates into clinically meaningful differences in the haematocrit depending on the ABO BG. We show that ABO BG dependent differences in miRNA repertoires/compositions of HSCs affect erythroid lineage specific TFs and thus the velocity and yield of hematopoietic differentiation. A deeper understanding of the gene regulatory networks of erythropoiesis and the interference with a BG specific gene regulation may help to shed light on the many clinical manifestations, where differences in susceptibility and disease progression have been observed in dependence of the ABO BG, such as infarction, thrombosis and infection.

PA31-L06 | Correlation of ABO antibody titres with physiological/biochemical parameters in Japanese blood donors, and comparison between donors of 2010 and 2021

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Background: ABO antibody titter testing is important, not only because low-titter blood components are needed in ABO incompatible HLA-matched platelets and emergency pre-hospital blood group O whole blood, but also it is an important part of ABO blood grouping. A report published in 2007 has shown that, compared to 20 years before, ABO antibody titters in Japanese blood donors had decreased significantly, but the reason is not known. Previous studies have shown that ABO antibody titters are correlated with age and gender, but their correlation with other physiological/biochemical parameters such as BMI, γ-GTP and total cholesterol are not yet known.

Aims: To elucidate the correlation of donors' factors with ABO antibody titters.

Methods: Serum/plasma from 5371 (collected in 2010) and 6768 (collected in 2021) random blood donors were tested for ABO antibody titters by the ABO reverse typing by an automated microplate system, and classified into low, middle and high titters according to the agglutination results obtained with diluted samples. (1) Multivariate regression analysis to analyse the association between ABO antibody titters and age, gender, biochemical parameters (ALT, γ-GTP, globulin, total cholesterol, and glycosylated albumin), and BMI, using samples collected in 2021. (2) Fisher's exact test to compare the ratio of blood donors with low/high antibody titters in 2021 and 2010 according to age and gender.

Results: (1) ABO titters were significantly correlated with age and gender ($p < 0.001$), except for gender in anti-A of blood group B. BMI showed significant but negative correlations with anti-A and anti-B (standardised partial regression coefficient $\beta = -0.062, -0.085$, $p < 0.01$) in blood group O donors. In addition, significant but negative correlations between γ-GTP and total cholesterol with anti-B of blood group A ($\beta = -0.055, -0.047$, $p < 0.05$) were observed. Also, a significant positive correlation between globulin ($\beta = 0.084–0.143$, $p < 0.01$) and ABO antibody titters in all blood groups was observed. (2) Ratio of donors with high anti-B titter in middle to high (30–49, 50–69) age male and high (50–69) age female in 2021 were significantly lower compared to the same cohort in 2010 (Table 1). Similarly, the ratio of blood group O donors with high anti-A titter in high (50–69) age male in 2021 was significantly lower ($p < 0.001$) compared to 2010. There were no significant differences in the ratio of donors with low antibody titters according to age, gender, and blood group type between donors of 2021 compared to 2010.

PA31-L06 - Table 1.

(a) High anti-B titres (Blood Group A)

Gender	Age	2010	2021	*p*-value
Male	16–29	41 / 157 (26.1%)	44 / 197 (23.3%)	
	30–49	**62 / 529 (11.7%)**	**55 / 783 (7.0%)**	**0.004**
	50–69	**42 / 289 (14.5%)**	**28 / 724 (3.9%)**	**<0.001**
Female	16–29	55 / 118 (46.6%)	75 / 209 (35.9%)	
	30–49	61 / 214 (28.5%)	69 / 287 (24.0%)	
	50–69	**21 / 103 (20.4%)**	**27 / 246 (11.0%)**	**0.026**
All		**282 / 1410 (20.0%)**	**298 / 2446 (12.2%)**	**<0.001**

PA31-L06 – Table 2.

(b) High anti-B titters (Blood Group O)

Gender	Age	2010	2021	*p*-value
Male	16–29	46 / 131 (35.1%)	65 / 193 (33.7%)	
	30–49	**74 / 418 (17.7%)**	**72 / 561 (12.8%)**	**0.037**
	50–69	**46 / 220 (20.9%)**	**50 / 535 (9.3%)**	**<0.001**
Female	16–29	60 / 99 (60.6%)	93 / 155 (60.0%)	
	30–49	66 / 175 (37.7%)	98 / 261 (37.5%)	
	50–69	**36 / 108 (33.3%)**	**48 / 221 (21.7%)**	**0.031**
All		**328 / 1151 (28.5%)**	**426 / 1926 (22.1%)**	**<0.001**

Summary/Conclusions: (1) ABO antibody titers seem to be associated with physiological and biochemical parameters of healthy individuals. (2) In 2021, a significantly decreased ratio of individuals with high antibody titers, compared to 2010, was observed, especially the anti-B antibody titer among donors in the high age group.

Parallel Session 32: Joint ISBT / EHA session–CAR-T cell therapeutics and stem cells

PA32-L01 | Update on CAR-T therapeutics

C Chabannon

Abstract not available.

PA32-L02 | New perspectives on the role of blood services in CAR-T

N Worel

Abstract not available.

PA32-L03 | Pressure ulcer treatment with autologous bone-marrow mononuclear stem cells. EudraCT 2008-003015-12 results

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Background: The use of Advanced Therapy Medicinal Products (ATMPs) is a promising therapeutic alternative, especially appropriate in those cases in which conventional treatment has not given good results, mainly in the case of bone marrow mononuclear cells (BM-MNCs) due to their properties promoting vascularization and recruiting paracrine factors that could induce angiogenesis and reduce cell necrosis resulting in wound healing. In this context, pressure ulcers (PUs) are defined as Localised damage to the skin and underlying tissue that generally appears over a bony prominence due to intense and prolonged pressure and/or friction. PUs appear mainly in immobilized people as patients with spinal cord injury. These wounds are a high source of morbidity and mortality due to the risk of infection and constitute a significant economic and social burden. Traditionally, treatment consists of an initial debridement followed for the removal of bone projections and the contribution of covering tissues (flaps). However, surgical treatment still has a high risk of recurrence and limits the possibility of carrying out a similar treatment for other ulcers.

Aims: Evaluate, in terms of safety and efficacy, the results obtained after cell therapy treatment in paraplegic patients with grade III/IV pressure ulcers included in the Clinical Trial EudraCT 2008-003015-12.

Methods: Autologous Bone marrow was extracted by iliac crest function and diluted 1:1 in saline with heparin 100 UI/ml. BM-MNCs isolation was performed at a Clean Room under GMP conditions stablished by Directive 2003/94/CE (Manufacture Certificate ES/020HV/19). MNCs were isolated by a Ficoll density gradient at 400 g for 25 min, mononuclear layer was transferred to a 50 mL conical tube and washed twice with saline at 550 g for 10 min. A minimum of 50·106 BM-MNCs were obtained. Cells were resuspended in a final volume of 14 mL heparinized saline and then filtered. A small volume was used for cell counts, characterization and sterility tests. About 10–12 mL were filled in a syringe and was taken to operating room where surgeon debrided the PU to eliminate necrotic tissue. When the wound was completely cleaned, surgeon closed the ulcer with non-absorbable suture and applied the cell solution by infusion or infiltration. Afterwards, patients should maintain prone position for 3 weeks to avoid premature opening of surgical intervention.

Results: Ninety-two paraplegic patients with grade III/IV PUs were treated with autologous BM-MNCs being the most common locations ischial injuries (69.8%) followed by sacral (17.4%) and trochanteric (12.8%). The main number of cells applied for treating the wounds was 104.42·106. No significant differences between control (conventional) and treatment group were observed in terms of immediate postoperative complications. Comparing follow-up results in two groups, significant differences ($p < 0.001$) appear in percentage of dehiscence at 6 months and 1-year after surgery (41% and 39.53% respectively in cell therapy group). Mean length of hospitalization was significant shorter in the ATMP group ($p = 0.037$).

Summary/Conclusions: After finishing the Phase I/II clinical trial it could be concluded that although BM-MNC treatment is an adequate and less aggressive alternative that minimises length of hospitalization resulting in a decrease of costs, significant differences were identified in long-term follow-up results in favour of conventional treatment of PUs.

PA32-L04 | Criteria of storage for cord blood units at Japanese red cross Kanto-Koshinetsu cord blood bank

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Background: The number of cord blood transplantations (CBTs) exceeds the number of adult unrelated stem cell transplantations in Japan. It is the responsibility of the cord blood banks (CBBs) to improve the quality of the processing and storage by analysing the processing data.

Aims: The purpose of this study is to analyse the criteria for storage of cord blood (CB) units in the Japanese Red Cross Kanto-Koshinetsu CBB, and to provide data that will be the basis for creating appropriate CB processing and storage criteria to efficiently store CB units with higher quality.

Methods: The Kanto-Koshinetsu CBB received 29,795 units from CB collection centres and 5486 (18.4%) were stored as transplantable units from 2014 to 2021. The collection centres were instructed to send the CB unit only when they collect >60 mL. The factors investigated for this study were gender, blood type, gestational period, volume of CB, total number of nucleated cells (TNC) at pre-processing and post-processing, number of CD34+ cells, colony forming units (CFU), time from collection to cryopreservation, time from arrival at the CBB to cryopreservation and cell viability (CD45+ cells, CD34+ cells). The criteria for storage were; over 12 × 10E8 TNC, start freezing process within 36 h from collection, no blood clot (size not specified), and adequately completed documents (consent form, questionnaire, family history, and delivery record). From 2021, if the TNC count was from 12–14 × 10E8, and the CD34+ cell count was over 2.5 × 10E6, then the CB was processed and stored. Units with over 14 × 10E8 TNC were processed and stored without a pre-processing CD34+ cell count. All processed units were tested for TNC, CD34+ and CFU.

Results: The Kanto-Koshinetsu CBB has received about 4000 CB units annually. From 2014 to 2021 the average time from collection to reception of 29,795 units was 18.0 h. All CB was obtained following gestational periods of 32 to 42 weeks. Collection at 37 weeks or less was performed at the discretion of the obstetrician. The most common reason for not storing a CB unit was insufficient cell numbers (pre-processing TNC <12 × 10E8), accounting for 47.9% of the total number of units received, followed by insufficient CB volume (<60 mL) for 19.4% and the presence of a blood clot for 10.3%. The average volume of 29,772 units received was 76.8 mL, with the maximum 234.2 mL and the minimum 20.9 mL. There was no correlation between the CB volume and pre-processing TNC (R2 = 0.4618). The storage rate for all CB units received was 18.4% (5486/29,795 units). For the 5486 stored units, the post-processing CD34+ cell number was not correlated with the collected CB volume (R2 = 0.0579). The shorter the gestational period, the higher the post-processing CD34+ cell count in the CB (35–37 weeks vs. 38–41 weeks, $p < 0.05$). The longer the gestational period, the more pre-processing TNC (37–38 weeks vs. 39–41 weeks, $p < 0.05$). The collected volume of CB did not correlate with the gestational period. Of 2335 units with 12–14 × 10E8 pre-processing TNC, 1123 units (48%) exceeded the CD34+ minimum criteria of 2.5 × 10E6. According to these results, we should expand the criteria for units with TNC <12 × 10E8 and the CB volume <60 mL from a gestational period of 38 weeks or less, by measuring the CD34+ cell count. In this way we can secure additional CD34+ rich units.

Summary/Conclusions: According to the transplant centres' changing preference for choosing CB based on the CD34+ count rather than the TNC, our CBB introduced pre-processing CD34+ cell counting for units with TNC of 12–14 × 10E8. There are units with a high CD34+ count when the gestational period is less than 38 weeks. The CB volume limit could also be lowered.

Parallel Session 33: Adverse events

PA33-L01 | ABO incompatible transfusions still a significant risk: 15 years of data from the serious transfusion incident report program, Australia

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Background: The STIR program has been running for 15 years in four jurisdictions in Australia and incorrect blood component transfused (IBCT) has been a reportable category, including ABO incompatible (ABOi) transfusions. Reporting to STIR is voluntary; and through personal communications it is known not all events are reported. Reporting involves registered health services completing an initial notification form, with an investigation form sent for completion. On return, a member of the STIR expert group reviews the information and validates type of adverse event, imputability and severity.

Aims: To review the incidence of ABOi reports and identify any common contributing factors.

Methods: Data from the STIR program from July 2007 until December 2022 was collected in relation to IBCT and more detailed information was assessed for the ABOi transfusions including location, contributing factors and outcomes.

Results: Since 2007, there has been 166 IBCT reports received, with 161 validated; 32 related to ABO compatible transfusions, where although wrong patient or blood group transfused, they were fortuitously compatible. Most of these (30) related to red cell (RC) transfusions, the remainder were FFP (1) and platelets (1).

There were 14 ABOi transfusions validated, 9 RC and 5 FFP. There were 6 emergency and 7 routine transfusions, 1 unknown.

Almost all locations were represented with theatre (2), ambulatory services (2), emergency department (ED) (4), wards (5) and 1 unknown having incidents.

The outcomes for these reports have been varied. Two patients died, thought related to the underlying condition although ABOi transfusion, could not be ruled out as a contributing factor, 3 resulted in ICU admission, 4 required an increased length of stay and 3 reported no increase in care.

There are some common factors leading up to these incidents. In 6, errors in collection or delivery of blood products from satellite fridges or by pneumatic chutes occurred; bedside checking procedure did not occur or was incomplete in 6 cases, 4 started with an error in the laboratory, not picked up in bedside checks. Stem cell transplants with complex post-transplant product requirements were associated

with 2 cases. There was 1 event associated with a wrong blood in tube event reported.

Summary/Conclusions: ABOi transfusions continue to occur and often related to errors with the collection and or administration of the blood component. Nine percent of all IBCT reported to STIR are associated with an ABOi transfusion, 64% of these are associated with RC, which are also associated with the more serious outcomes of these incidents. Although education occurs in the majority of Australian health services around blood administration, often via an online platform, it is necessary to ensure this education is understood and put into everyday practice. Understanding of ABO component compatibility is important. Suitable blood groups for the use of FFP is an area where education of all staff is required. Errors have occurred due to both nursing and medical staff misunderstanding of compatibility for these products.

While larger haemovigilance programs, for example, SHOTUK, have had deaths reported, there have been no confirmed deaths associated with ABOi transfusions in our reports received, however there have been serious morbidity described with at least four events.

PA33-L02 | The impact of perioperative blood transfusions on major adverse cardiovascular and cerebrovascular events in elective-major abdominal surgery patients

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Background: Perioperative blood transfusions (PBT) can be beneficial for surgical patients. However, some studies indicate that they are associated with an increased risk of mortality and postoperative complications, such as infections, oxygen dysfunction and myocardial injury. In the elective major abdominal surgery (E-MAS) population, PBTs are common, yet transfusion outcomes are poorly defined.

Aims: To investigate the incidence and effect of PBTs on 30-day major cardiovascular and cerebrovascular events (MACCE), infectious complications and mortality in E-MAS patients.

Methods: A multicenter observational cohort study using data from the Myocardial Injury in Noncardiac Surgery in Sweden study is presented. Inclusion criteria were patients aged ≥ 50 years, who stayed at least one night in hospital. Data were collected between April 2017 and December 2020. PBT was defined as any red blood cell unit with or without fresh frozen plasma and/or platelets transfused intraoperatively and/or postoperatively on any of the three consecutive days following surgery. The primary outcome was 30-day MACCE, defined as non-fatal cardiac arrest, acute myocardial infarction, congestive heart failure, new cardiac arrhythmia, angina, stroke or any combination of these. The secondary outcomes were 30-day infectious complications and 30-day all-cause mortality. Univariate and binary logistic regressions were performed to assess the effect of PBT on MACCE, infectious complications and mortality.

Results: Among 762 patients, 155 (20.4%) received a PBT. Transfused patients were older, had a higher American Society of Anaesthesiology (ASA) class, and had lower preoperative haemoglobin compared to non-transfused patients (all $p < 0.001$). Transfused patients also had a higher incidence of coronary artery disease, longer duration of surgery, and experienced greater intraoperative blood loss (all $p < 0.001$). MACCE occurred in 70 patients (9.2%). Overall, 231 patients (30.4%) experienced an infectious complication and six (0.8%) died within 30 days. In the bivariate analysis (Table 1), PBTs were significantly associated with MACCE (OR 2.71, 95% CI 1.53–4.78, $P < 0.001$) but not infectious complications (OR 1.33, 95% CI 0.84–2.11, $P = 0.22$), nor 30-day all-cause mortality (OR 0.33, 95% CI 0.17–0.54, $P = 0.18$).

Summary/Conclusions: Patients undergoing E-MAS who receive a PBT are at a higher risk of MACCE. Careful consideration is required to assess the risk and benefits of PBTs in this cohort. Strategies to minimise the need for PBT such as preoperative anaemia management may also be considered.

PA33-L02 - Table 1. Univariable and binary logistic regression of perioperative blood transfusion and outcomes

Outcome	OR (95% CI)	*P* value	OR (95% CI)	*P* value
30-day MACCE	2.97 (1.77–4.97)	<0.001	2.71 (1.53–4.78) *	<0.001
30-day infectious complications	1.87 (1.29–2.69)	<0.001	1.33 (0.84–2.11) ø	0.22
30-day mortality	0.33 (0.17)–0.54)	0.18		

* Adjusted for age, sex, ASA class, number of comorbidities, estimated blood loss.
ø Adjusted for age, sex, ASA class, number of comorbidities, estimated blood loss, preoperative anaemia, length of surgery.
Abbreviations: CI, confidence interval; MACCE, major adverse cardiovascular and cerebrovascular events; OR, odds ratio.

PA33-L03 | Role of anti-HLA class I IgG subclasses in platelet activation in the context of platelet transfusion refractoriness

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Background: In patients with haematological and oncological disorders, a heavy platelet transfusion support is necessary to prevent life-threatening haemorrhagic complications. However, some patients experience a transfusion efficiency failure known as platelet transfusion refractoriness (PTR). One of the causes of PTR is anti-HLA class I (HLA-I) alloantibodies in the recipient's blood. Interestingly, not all patients with anti-HLA-I antibodies develop PTR upon platelet transfusions, raising the question of their mode of action on platelets and the antibodies' features that should be considered in a PTR context.
Aims: To study the effect of anti-HLA-I antibodies on platelets and the relevance of IgG subclasses.
Methods: Sera from alloimmunised patients presenting PTR or not were collected and analysed by Luminex regarding the IgG subclasses of anti-HLA-I antibodies. Either murine IgG2 or human chimeric IgG1 or IgG3 W6/32 mAbs (pan-HLA-I mAbs) were used to study the importance of IgG subclasses on platelet activation. Hirudinated human platelet rich plasma (PRP) or washed platelets were incubated with those different mAbs. Platelet activation status was assessed by flow cytometry through P-selectin (Psel) surface expression, phosphatidylserine exposure (AnnV), and complement recruitment (C3b). In some conditions, inhibitors of CD32a receptor (IV.3) or complement cascade activation (Eculizumab) were added.
Results: We found that the relative proportion of different anti-HLA-I IgG subclasses was different in patients suffering from a PTR as compared to patients with satisfactory transfusion yields. In the sera of 9 PTR patients, 5 contained IgG1, 4 IgG2, 2 IgG3, and 1 IgG4, while in the 7 sera of patients with adequate transfusion efficiency, all contained IgG1, 1 IgG2, 1 IgG4 but none of them had IgG3. Murine IgG2 W6/32 activated platelets as shown by Psel (10%) and AnnV (26%) exposure. IV.3 did not abrogate this activation but Eculizumab did, indicating an important role of complement in this process confirmed by the recruitment of C3 (40%) at the platelet surface induced by W6/32. Moreover, in the absence of plasma factors (washed platelets), W6/32 failed to activate platelets. To further characterise the impact of IgG subclasses, chimeric IgG1- or IgG3-W6/32 mAbs were incubated with platelets. The mAb-induced activation, assessed by Psel and AnnV exposure, was more important with IgG3-W6/32 (14% and 20%) than with IgG1-W6/32 (6% and 13%). Accordingly, C3b recruitment at platelet cell surface was significantly higher with IgG3-W6/32 (53%) than IgG1-W6/32 (17%).
Summary/Conclusions: These results highlight the importance of complement in anti-HLA-I mediated platelet activation, whereas CD32a mobilization had no effect. IgG subclasses of anti-HLA-I W6/32 influence complement recruitment at platelet surface and the associated activation. IgG subclasses should be considered in the context of PTR to provide a broader spectrum of compatible donors for refractory recipients.

PA33-L04 | Abstract withdrawn

PA33-L05 | SHOT about SOT- transfusion incidents in solid organ transplant recipients reported to SHOT

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Background: Patients undergoing solid organ transplant (SOT) may require intensive transfusion support. ABO-mismatches occur in SOT to meet demand with finite supply. Blood components for transfusion must be compatible with donor and recipient ABO/D type, and patients require specialised laboratory testing due to a potential complication of ABO mismatched transplant - passenger lymphocyte syndrome (PLS). In PLS antibodies produced by lymphocytes in transplanted organs cause alloimmune haemolysis of recipients red blood cells (RBC). These factors, combined with the involvement of many specialist teams may contribute to serious adverse events, such as incorrect blood component transfused (IBCT) and reactions in SOT patients.
Aims: To identify key themes and trends in IBCT errors in SOT recipients reported to SHOT and suggest mitigating actions to prevent future errors.
Methods: IBCT errors accepted by the Serious Hazards of Transfusion (SHOT) UK haemovigilance scheme from 2017 to 2021 involving SOT patients were reviewed.
Results: A total 59 reports were identified mostly in adults 54/59 (91.5%), with no fatalities, major morbidity or PLS. The majority were renal or hepatic transplants (50/59, 84.7%).
IBCT-specific requirements not met errors were 42/59 (71.2%); 15/42 (35.7%) not irradiated, 10/42 (23.8%) not hepatitis E negative (all components now negative in the UK), 8/42 (19.0%) inappropriate use of electronic issue, 3/42 (7.1%) incorrect phenotype, and others 5/42 (11.9%). Most were due to errors in communication by the clinical area to laboratory 26/42 (61.9%). Laboratory errors occurred in 16/42 (38.1%), alerts or information was not added to laboratory information management systems (LIMS) in 9/16 (56.3%), alerts were not heeded, and a lack of LIMS functionality was noted in 2 further cases.
IBCT-wrong component transfused (WCT) accounted for 17/59 (28.8%) cases; 13/17 (76.5%) wrong group (WG), 2/17 wrong patient (11.8%), 1/17 D mismatch (5.9%) and 1/17 wrong component (5.9%). WG cases involved RBC 10/13 (76.9%), plasma components 2/13 (15.4%) and platelets 1/13 (7.7%). All resulted in components compatible with the patient group, but not the donor, with one resulting in a delayed haemolytic transfusion reaction. Most were due to errors in the laboratory 12/17 (70.6%) with 8/12(66.7%) stating LIMS involvement; information on LIMS was not heeded or not updated/available in 4/8 (50.0%) each. Errors in communication by the clinical area caused 5/17 (29.4%).

Summary/Conclusions: Clear laboratory procedures with underpinning knowledge in staff are essential for safe transfusions. Laboratories must ensure that actionable flags are added to LIMS in a timely fashion. Algorithms to support safe component release for ABO/D mis-matched transplant patients should be integrated.

Clear communication between clinical transplant teams and the laboratory is vital. Patients receiving SOT are often under shared care of multiple hospitals, all of which must be kept up to date about the transplant, in particular the ABO/D group and specific requirements of blood components. Transplant centres and referring organisations must ensure that robust processes are in place for transfer of information across sites, and to transfusion laboratories. Transplant protocols must clearly state ABO/D requirements throughout the transplant process.

Transfusion errors in SOT patients may be underreported, it is important that these events are included in haemovigilance data to optimise learning in this complex patient group.

PA33-L06 | Delayed transfusions reported to the French haemovigilance database: 2011–2021

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Background: Timely provision and transfusion of blood components (BCs) is often vital, notably for acute bleeding and severe anaemia. Despite procedures to prevent such occurrences, transfusion delays putting the patients at risk do occur. In France, delayed transfusion (DT) are reported as serious adverse events (SAEs) on a mandatory basis to the national haemovigilance database (dedicated website), under the supervision of ANSM, the French competent authority.

Aims: To characterise and understand the causes/contributive factors of delayed transfusions in France, we analysed all the occurrences of DT reported to the French haemovigilance database. We subsequently focused on DT occurring in settings where the transfusion was deemed urgent by the clinical team (as notified on the medical prescription).

Methods: All reports pertaining to DT reported to the French haemovigilance database between 01/01/2011 and 31/12/2021 were collated and analysed with regard to occurrence circumstances, causes for delay and clinical impact. Data regarding DT were compared to overall transfusion practices (EFS data and Fillet et al, Vox Sanguinins, 2017). Despite mandatory reporting, underreporting of such adverse events is likely. Difficulties to identify DT may further contribute to underreporting.

Results: Over an 11 years period (and ≈ 2.9 million BCs transfused annually), 319 DT were reported in the French database, with increasing frequency (26 in 2011, 43 in 2021). Reported DT occurred in an emergency setting in 36.2% (113/312 evaluable cases vs. 6.1% for overall transfusion). Obstetrics and neonatology were 2 sectors where DT were overrepresented (11.4% of DT vs. 1% of total transfusion, and 6.7% vs. 0.6%, respectively). Implicated BCs included red cells concentrates (in 84.8% of reported cases), plasma (23.8%) and platelet concentrates (20.8%). Among the reports of TD, 18 reported a clinical outcome of the patient towards death (imputability to DT not assessed). Among DT in an emergency setting, 49.5% (48/97 evaluable cases) occurred during night shifts (7 PM to 7 AM). Such SAEs in an emergency setting occurred mainly in the operating rooms (61.5%, 24/39), obstetrics (75%, 12/16), intensive care (60%, 21/35), and the emergency department (48.8%, 13/29). Causes for DT in an emergency setting involved issues regarding patient identification (6.2%, 7/113 evaluable cases), patient blood typing / antibody screening (8.8%, 10/113), BCs issuing (14.2%, 16/113) or transportation (24%, 28/113), communication and IT (13.3%, 15/113), system failure in the clinical area such as staffing, workload or skill-mix (18.6%, 21/113). Multiple contributive factors were identified, involving for example BCs prescription, issuing and transportation (14.2%, 16/113). Human errors (incorrect decision or omission following the correct procedure to be applied in case of emergency) were identified in a large number of cases of DT.

Summary/Conclusions: Transfusion delays, with potentially catastrophic consequences, result most often from process failures across the multistep pathway from transfusion prescription to actual transfusion. Transfusion in an emergency setting, at night-time, in specific clinical sectors such as obstetrics and neonatology are at higher risk. Continuous training, including simulations, as well as regular process updating to prevent such occurrence are a priority in transfusion medicine.

Parallel Session 34: Case studies in transfusion ethics

PA34-L01 | Ethical considerations in transfusion medicine and biotherapies

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The practice of transfusion medicine can involve several ethical considerations. Blood is a precious medical resource with a limited shelf life, and its supply and demand can be uncertain.

The ISBT Code of Ethics was first developed in 1980 in response to the World Health Assembly resolution WHA 28.72 which called for the establishment of national blood services with voluntary non-remunerated blood donation (VNRBD) together with promoting the health of donors and recipients.

The Code has been endorsed by the World Health Organization, International Federation of Red Cross and Red Crescent Societies and by the International Federation of Donor Organizations. It has been updated and revised on three occasions, most recently in 2017 but its key objectives remain the same, that is, "to define the ethical principles and rules to be observed in the field of Transfusion Medicine". The Code can serve as a useful basis of national legislation and regulation and is a tool for advocacy. However, it reflects the views of a professional society and some of the content may therefore be perceived to be aspirational and it is reasonable to challenge and review this within the current developments within the field of our practice.

Biotherapies is a rapidly emerging field with the exponential application of cellular products. Medical professionals share duties with the recipient of the blood product, other patients and the community, including the blood and stem cell donors. While the four pillars of medical ethics (beneficence, non-maleficence, autonomy and justice) offer a general framework to approach any given ethical situation, decision-making can be challenging in some cases. Examples include resource allocation when demand is anticipated to exceed supply, blood refusal, informed consent, donor counselling and post-donation information, and introducing investigational biotherapy products. Institutional ethical committees and the Code can help make decisions in such situations.

This interactive session will use case studies to explore some ethical dilemmas in the field of transfusion medicine and biotherapies and application of the Code to these scenarios. We will also explore the next steps for update and revision of the ISBT Code of Ethics.

PA34-L02 | Ethical consideration in transfusion medicine and biotherapies

A Z Al-Riyami[1,2], S Allard[3], M Goldman[4]

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The practice of transfusion medicine can involve several ethical considerations. Blood is a precious medical resource with a limited shelf life, and its supply and demand can be uncertain.

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Parallel Session 35: Achieving efficiencies in blood component production

PA35-L01 | Automation in blood product manufacturing

S Krishnan[1]

[1]*School of Engineering, Swinburne University of Technology, Melbourne, Australia*

The processing of whole blood donations is carried out in a mostly manual manner involving several repetitive and ergonomically challenging operations. This is particularly true for the folding of blood packs, prior to separation into blood components. Swinburne University, Melbourne, Australia in collaboration with an Australian blood processing centre, through a Commonwealth-funded project, has designed and developed a proof of concept automated work cell for the prescriptive folding of blood packs with tubes and filters, in preparation for the centrifugation. An industry partner is currently integrating it into their production line.

This talk is aimed at providing an overview of the research approach, unique challenges, industry 4.0 value add inclusions, benefits and the robust processes followed in the implementation of a solution, not commercially available in the market.

PA35-L02 | Assessment of biological response modifiers in cold-stored group O whole blood product

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[1]*Research and Development, Australian Red Cross Lifeblood, Alexandria,* [2]*Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia*

Background: Early administration of blood products to trauma patients with severe bleeding improves their survival, with advantageous outcomes shown in both military and civilian settings. Low-titre group O whole blood (WB) stored at 2–6°C is increasingly being adopted for transfusion of trauma patients in pre-hospital and early hospital admission, and has demonstrated haemostatic capacity *in vitro* for at least 21 days of storage. Although robust data has been shown for platelet-, red cell- and plasma-specific storage parameters, there has been limited investigation of biological response modifiers (BRMs) in cold-stored WB, which may induce an inflammatory or anaphylactic reaction in the recipient.

Aims: The aim of this study was to evaluate BRMs in the supernatant of cold-stored group O WB product during 42-day storage.

Methods: WB ($n = 12$) was collected into CPD anticoagulant, held overnight, processed through a platelet-sparing filter, and stored at 2–6°C for 42 days. Samples were taken on day 1, 4, 7, 14, 21, 28, 35 and 42, and platelet-poor-plasma was prepared by centrifugation. BRMs were measured by ELISA, flow cytometry and cytometric bead array. Data were analysed using one-way ANOVA comparing each time-point to day 1, with a *post hoc* two-sided Dunnett's *t*-test.

Results: WB units were effectively leukoreduced, with 99.98% reduction in leukocyte count per unit and 85% platelet count recovery following filtration. There was a significant increase in soluble platelet-derived factors including PF4 ($p < 0.0001$) and sCD62P ($p < 0.0001$), and to a lesser extent sCD40L ($p = 0.037$). The concentration of inflammatory mediators HMGB1 ($p = 0.748$), S100A12 (EN-RAGE; $p = 0.274$) and C5a ($p = 0.988$) remained stable throughout storage, but was contrasted with a significant increase in C3a from day 14 of storage ($p < 0.0001$). There was a significant increase in the supernatant concentration of chaemokines RANTES ($p < 0.0001$) and MCP-1 ($p < 0.001$) throughout storage. The concentration of IL-6, IL-8, IL-13, MIP-1α and IFN-g was below the limit of detection. Platelet-derived microparticles numbers ($p < 0.0001$) and red cell-derived microparticles ($p < 0.0001$) increased during storage, whereas the number of white cell-derived microparticles was relatively low and did not change during storage ($p = 0.100$).

Summary/Conclusions: This study shows that biological response modifiers accumulate in cold-stored WB during storage. High BRM concentrations in WB may have clinical consequences for transfusion recipients in a trauma setting, although this is yet to be fully elucidated.

PA35-L03 | In vitro biochemical and functional comparison of amotosalen-UVA-treated buffy-coat platelet concentrates stored in pas-c or pas-e additive solution up to 7 days

B Hechler[1,2], N Brouard[1,2], F Rudwill[2], C Mouriaux[1,2], A Koll[2], D Haas[2], A Galvanin[2], D Kientz[2], P Mangin[1,2], H Isola[2]
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Background: Deterioration in the quality of platelet concentrates (PCs) during storage results from changes of various biochemical and metabolic parameters affecting platelet haemostatic properties and survival after transfusion. These lesions depend on the methods used for preparation and pathogen inactivation, the duration of storage and the type of platelet additive solutions (PAS) used. However, there exists no detailed comparison of PAS-C (InterSol/PAS-III, Fresenius) and PAS-E (SSP$^+$, Macopharma), a modification of PAS-C containing 5 mM KCl and 1.5 mM $MgCl_2$, for the conservation of buffy-coat (BC)-PCs treated with amotosalen-UVA (INTERCEPT Blood System, Cerus) with regard to the storage changes including *in vitro* platelet functional properties.

Aims: We evaluated the *in vitro* quality of BC-PCs treated with INTERCEPT and stored up to 7 days in either PAS-C or PAS-E.

Methods: A pool-and-split strategy was used to obtain two study groups ($n = 5$ per group): (i) double-dose BC-PCs collected into PAS-C/plasma (55/45) treated with amotosalen-UVA, (ii) double-dose BC-PCs collected into PAS-E/plasma (55/45) treated with amotosalen-UVA. The *in vitro* quality and function of the platelet components were tested over 7 days of storage post collection at 22–24°C.

Results: Platelet counts were conserved in both groups of PCs during storage, as was platelet swirling without the appearance of macroscopic aggregates. Storage in PAS-C resulted in a significant increase in mean platelet volume (MPV) as of day 3, as compared to storage in PAS-E, where it remained stable. Integrin αIIbβ3 and glycoprotein (GP) VI expression remained stable in both solutions, whereas GPIbα and GPV declined similarly in both groups. Storage in PAS-E resulted in a significant reduction in glucose consumption and lactate generation as compared to storage in PAS-C on day 5.5, with better maintenance of pH levels as of day 3. Notably, sufficient glucose was still available on day 7 in PCs stored in PAS-E compared to PCs stored in PAS-C. Spontaneous P-selectin exposure, a marker of α-granule secretion, was significantly reduced in PCs stored in PAS-E as compared to PAS-C as of day 1.5. The proportion of activated αIIbβ3 remained globally low and similar in both study groups. Spontaneous phosphatidylserine (PS) exposure at the surface of platelets, a marker for platelet activation and apoptosis evaluated by annexin V binding, significantly increased during late storage of PCs in PAS-C compared to PCs conserved in PAS-E where it remained stable. Mitochondrial transmembrane potential, evaluated using the tetramethylrhodamine methyl ester (TMRM) fluorescent dye retained in functional intact mitochondria, diminished significantly in PCs stored in PAS-C but not in PAS-E as of day 5. Lactate dehydrogenase (LDH) release, an indication of premature platelet lysis, was significantly reduced in PCs

stored in PAS-E as compared to PAS-C, as of day 5.5. During storage, both PAS-C and PAS-E platelets retained capacity to adhere to VWF and fibrinogen, and to form aggregates of similar thrombus volume on collagen in a microfluidic shear flow chamber.

Summary/Conclusions: Use of PAS-E/plasma improved platelet metabolism, reduced spontaneous activation, reduced apoptosis and reduced LDH release, as compared to PAS-C/plasma, especially during the late stages of storage, without differences in *in vitro* platelet adhesive properties. This study highlights the strong influence of the composition of the additive solution on the occurrence of storage lesions in pathogen-reduced PCs.

PA35-L04 | Quality of red cell concentrates from non-anaemic donors with signs of iron deficiency

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Background: Frequent blood donations can increase the risk of iron deficiency (ID; as measured by low ferritin levels) but not necessarily that of anaemia (as measured by low haemoglobin levels). At pre-donation screening, donors' haemoglobin (Hb) levels—but not ferritin levels—are measured, so that non-anaemic ID donors can qualify for blood donation. Besides posing concerns about donor safety, ID may also impact the quality of blood components during storage.

Aims: To monitor the physiological changes and quality indicators of red cell concentrates (RCCs) from donors with severe ID, donors with mild ID and donors with normal ferritin levels during product storage.

Methods: A specific donor population prone to ID was selected. Twenty-two female donors aged 19–64 years were randomly selected for inclusion over a 9-month period. A blood sample was collected to determine serum ferritin levels and classify donors in three groups: <11 µg/L ("severe ID"), 11–25 µg/L ("mild ID") and >25 µg/L ("controls"). Four whole blood (WB) samples were collected in tubes (V_{total} = 28 mL) and stored at T = 4°C overnight. RCCs were then prepared based on a method adapted for small volumes of WB. WB was leukoreduced by filtration and red blood cells (RBCs) were isolated by centrifugation and dispersed in AS-3 to obtain 50% haematocrit RCCs. RCCs were stored 42 days in small volume containers designed to reproduce the biochemical conditions of regular storage bags. The following parameters were assessed at day 0 and 42 of storage: complete blood count, RBC deformability (using a microfluidic device), ATP level (luminescence assay), reticulocyte count (flow cytometry) and haemolysis.

Results: Mean ± standard deviation (SD) ferritin levels were 9 ± 3 µg/L for donors with severe ID, 16 ± 3 µg/L for donors with mild ID, and 66 ± 19 µg/L for controls. The mean ± SD deformability of RBCs was similar among the three groups at day 0 (i.e., severe ID = 2.1 ± 0.1 a.u., mild ID = 2.0 ± 0.2 a.u., controls = 2.1 ± 0.1 a.u.) as it was for mean ± SD relative impairment of RBCs deformability over the storage period (i.e., severe ID = 33 ± 4%, mild ID = 29 ± 7%, controls = 31 ± 6%). Mean ± SD ATP levels were also similar at day 0 (severe ID = 2.5 ± 0.3 µmol/g Hb, mild ID = 2.7 ± 0.7 µmol/g Hb, controls = 2.8 ± 0.4 µmol/g Hb) and day 42 (severe ID = 1.5 ± 0.7 µmol/g Hb, mild ID = 1.5 ± 0.4 µmol/g Hb, controls = 1.4 ± 0.4 µmol/g Hb). At day 42, mean ± SD haemolysis levels were 0.5 ± 0.2% for donors with severe ID, 0.3 ± 0.1% for those with mild ID, and 0.4 ± 0.2% for controls.

Summary/Conclusions: The quality of RCCs from donors with mild or severe ID did not significantly differ from that of regular donors throughout storage. Their RBC deformability lowered throughout the storage period but at the same rate seen for the control group, an expected phenomenon associated to storage lesions. Isolation and storage of RCCs in this study differ from usual blood banks practice. However, haemolysis levels at the end of storage suggest the procedure was well tolerated by RBCs. Therefore, overall results suggest that ID, which might occur after frequent blood donation, may not impact the quality of blood products during storage, although ferritin levels should be monitored in frequent donors to prevent signs of ID.

PA35-L05 | Preparation of hypoxic red blood cells for transfusion of thalassemia study patients

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Background: Red blood cells (RBCs) are subject to metabolic and oxidative impairments accumulating during storage. RBC deformability progressively diminishes over time, affecting microcirculation perfusion. Hypoxic storage, where the oxygen content of RBC units is reduced prior to refrigeration and throughout storage, is a viable alternative to reduce oxidative stress. *In vitro*, hypoxic storage reduces oxidative impairments that occur during normal storage, providing more viable cells at transfusion (Yoshida T, et al. *Blood Transfus*. 2019;17:27–52). Metabolomic analyses of hypoxic RBCs have shown increased ATP synthesis and a decrease in oxidative stress biomarkers (D'Alessandro A, et al. *Transfus*. 2020;60:786–98). In animal models, hypoxic RBCs facilitated more effective resuscitation from haemorrhagic shock than conventionally stored RBCs (Williams AT, et al. *Shock*. 2020;53:352–62). Hemanext Inc. (Lexington, MA, United States) has developed a CE mark certified device to process and store RBCs hypoxically–CPD/PAGGSM Leukocytes-Reduced (LR), O_2/CO_2 Reduced–which may reduce transfusion burden in transfusion-dependent patients and attenuate the oxidative stress associated with acute major bleeding. Ahead of a post-market clinical investigation of patients with thalassemia in Italy, a validation study of the *in vitro* performance of hypoxic RBCs was conducted at the Regional Blood Bank in Genoa, Italy.

Aims: To evaluate RBCs stored hypoxically for 42 days after pre-storage O_2/CO_2 reduction.

Methods: The study was designed so that results would be applicable throughout the Italian regional blood centres. Informed consent was obtained from all donors. Each whole blood unit collected generated one unit of O_2/CO_2 reduced LR-RBCs. RBC filtration was performed either on the day of collection or after storage at ambient hold for up to 24 h. All units were processed using the system and stored at 1–6°C within 24 h of collection for 42 days. Control RBC units were conventionally processed and stored. Acceptance criteria (assessed after leukoreduction (post-LR) and on days 0, 21 and 42 of storage) were haematocrit (HCT) >50%, haemolysis at day 42 <0.8% and a negative blood culture. ATP and malondialdehyde (MDA) levels were measured as key energy and lipid peroxidation/oxidative stress biomarkers.

Results: Thirty RBC units stored hypoxically were evaluated and met all acceptance criteria at day 42 (**Table**). At day 42, we confirmed a high ATP content in hypoxic RBCs previously described in literature (D'Alessandro A, et al. *Transfus*. 2020;60:786–98) and found lower MDA levels compared with control RBCs (**Table**).

Summary/Conclusions: This is the first report validating hypoxic RBCs for transfusion, in preparation for a clinical study of chronically transfused patients with thalassemia. Compared with conventionally stored blood, hypoxically stored RBCs met acceptance criteria for transfusion, maintained high levels of ATP, and attenuated MDA accumulation, an indirect estimate of RBC membrane oxidation. It is expected that deoxygenation of RBCs will retain more physiological levels of key blood quality parameters vs conventionally stored RBCs.

PA35-L05 – Table 1

	Post-LR	Day 0	Day 21	Day 42
HCT, %, mean (SD)	62 (0.03)	61 (0.03)	66 (0.08)	63 (0.06)
Haemolysis, %, mean (SD)	0.10 (0.03)	0.10 (0.03)	0.20 (0.09)	0.25 (0.09)
Blood culture	–	Sterile	–	Sterile
ATP, µmol/g Hb, median	5.10	5.64	4.15	**Hypoxic RBCs** 3.55 **Control RBCs** 2.70
MDA, µmol/g Hb, median	115.82	102.40	40.87	**Hypoxic RBCs** 36.14 **Control RBCs** 76.74

Posters

Management and organisation

Organisational issues

P001 | Utilisation of LEAN start up methodology for the identification, development and pilot of novel services that can be provided to hospital transfusion laboratories

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Background: Service development is improved by customer input. LEAN Startup streamlines collective idea creation in extreme uncertainty, providing collaboration to match a service to customer wants. Uncertainty brought by pathology networks prompted Red Cell Immunohaematology (RCI) to use LEAN Start-up to develop services beyond its traditional testing role with the Bristol Royal Infirmary (BRI).

Aims:

1. Identify novel services that the RCI department could provide Hospital Transfusion Laboratories.
2. Develop novel services for RCI using LEAN Start-up principles and methodology
3. Pilot novel services and identify criteria relevant to expansion beyond pilot for example cost.
4. Identify the benefits of the novel services to NHSBT; RCI; User (NHS/Private) and the patient

Methods: The Value Proposition Canvas (VPC) Customer Profile, Kano model and the Business Model Canvas (BMC) identified themes and attributes for new service provision. The VPC Customer Profile identified the BRIs jobs, pains and gains. Process mapping gathered data relating to: (i) ISO15189 vertical audit training and completion; (ii) Sample verification, automation and manual crossmatch processes. Online Miro based events allowed redesign of processes.

Results: Three themes were identified (i) Quality assurance–Enables a self-sufficient laboratory, provides centralised document control, facilitates compliance; (ii) LEAN Laboratory–Part of daily practice, (iii) Training–Demonstrates effectiveness of a cross-organisational platform for competency. The VPC Value Map and BMC resulted with the Laboratory Solution Development Platform to support hospital partner service provision. The Build-Measure-Learn cycle resulted in three options: (1) The hospital partner bespoke service; (2) The hospital and RCI full partnership bespoke service (3) RCI led generic template service. Option 2 targeted two job themes: (i) Vertical audit training and completion to meet ISO15189 accreditation requirement. New training material was created. A new audit process allowed planning, process observation and report generation; (ii) Improve process flow. The laboratory layout was sub-optimal. A new layout reduced process waste, improved patient safety and blood readiness.

Summary/Conclusions: LEAN Start-up principles and related business tools allowed identification, development and pilot of a service beyond RCIs traditional testing role. Benefits were related to: A better understanding of the current market; An improved relationship between RCI and hospital; Empowerment of the HTL; improved patient safety and treatment times.

P002 | Knowledge economy: Impact on the developmental progress of blood systems in resource limited countries

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Background: Knowledge is offered to be received and stored, perceived and interpreted, and converted into signals for action. To standardise a basic amount of knowledge, teaching and learning systems, have been developed and are continuously further developed. The more exposure and repetition of knowledge, the better the perception, storage, interpretation and understanding. In principle there are three successive layers of education–primary, secondary, higher or academic; for each exists a system to improve on existing knowledge, optimizing the economy or value of knowledge. Personal perception, interpretation, storage and triggers to action are part of the individual knowledge economy (KE) with its levels of alertness and awareness, comfort and discomfort, and risks.

Aims: Using Knowledge Economy to make knowledge (information and education) and skills universally available and practiced to improve progress and development of transfusion medicine.

Methods: Observational (20 years); review of recent WHO, UNDP and World Bank (WB) literature and reports, and study and analysis of Blood Systems in Low- and Middle-Income Countries (LMIC); benchmarking against previous WHO and UNDP progress reports.

Results: WHO 2021 Global Database Report and UNDP 2018 Statistical Update show progress, but also a series of major challenges important for the still existing knowledge gap. Blood supply systems are manufacturing institutions; the demand (market) is created by the patient and ideally translates into a tailor-made supply of safe and clinically effective blood products manufactured from the crude source material human blood. This vein-to-vein blood transfusion chain needs specific knowledge to be acquired and used economically to develop into a patient-oriented blood transfusion organization of adequate economy-of-scale to secure consistency, quality and operational and managerial competency..

Analysing the needed vocational and professional education backgrounds of staff to be employed in any part of the vein-to-vein transfusion medicine chain, at primary, supportive or steering process level shows that most employees need a preliminary secondary education. However, UNDP 2018 Statistical update shows only 8–24% enrolment from secondary into tertiary professional

education, indicating a substantial knowledge gap and meagre to poor KE practice as a major investment in development, in sharp contrast to the advanced part of the world, home to only 16% of privileged people. Another weakness is the paucity of an educational unfrastructure and environment together with a lck of competent teaching cadre,

Summary/Conclusions: Knowledge economy is in the establishment of

- a well-constructed and sustainable education environment, climate and adaptive structure.
- a well-balanced and designed education scope and quality of knowledge.
- intellectual property, stewardship, and ownership.

These should be supported and accompanied by genuine curiosity, continued exploration and healthy appetite to science and evidence-based knowledge that drive development and determine the pace of acceleration towards advancement and improved effectiveness–safety, availability, accessibility, affordability, equity and equality.

P003 | Characterizing the transfusion continuum in a low-resource referral hospital in Kenya: A qualitative study of emergency transfusion measures

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Background: Blood is an essential medicine, but in many rural settings across the world, the closest stocked blood bank is hours away, leaving communities without reliable access to screened, banked blood for transfusion. The limited access to life-saving blood transfusion in such settings underscores the need for innovative solutions to bridge the gap between blood availability and need.

Aims: This study aimed to identify barriers and facilitators of timely transfusion and explore the feasibility of a just-in-time transfusion process when screened; banked blood is unavailable in a low-resource referral hospital in rural Kenya.

Methods: We conducted ten semi-structured interviews with surgeons, medical and clinical officers, nurses, blood bank staff and hospital administrators. Interview transcripts were analysed by four coding teams of dyads and triads, using a combined inductive-deductive approach as informed by a literature review. Emerging themes were validated through a member-checking session and supplemented by focus group discussions.

Results: Screened, tested blood was not always available when needed. Four key themes emerged around the course of action undertaken when no screened blood was available. (1) Just-in-time transfusion strategies, which employ point-of-care rapid diagnostic tests (RDT) rather than traditional laboratory testing despite concerns around the sensitivity of RDTs in detecting transfusion transmitted infections (HIV, Syphilis, HCV, HBV), may be clinically necessary in times of extreme emergency; (2) While the clinician must ultimately authorize emergency transfusion measures, the process must include shared decision-making amongst clinicians, patients & family, blood bank staff and hospital administrators; (3) While the ideal donor is a voluntary, previously tested donor from a low-risk population, all potential donors should be stratified based on compatibility, risk of transfusion-transmitted infection, and time constraint; (4) Aligned policies at at all levels are necessary to improve blood availability in standard and emergency circumstances.

From the information presented within interviews and focus group discussions, we developed a protocol outlining the overall transfusion process at the study hospital. This protocol reflected the standard transfusion process, as well as the steps taken in emergency settings when no screened, banked blood is available. Notably, the protocol highlighted the need for coordination and communication directives between all involved parties.

Summary/Conclusions: In this study, participants described scenarios where screened, tested blood was unavailable and emergency transfusion measures may be necessary to meet clinical needs. While our findings reflect the local context of one rural hospital, they emphasize the global need for rigorous research on best practices in emergency transfusion, and clear policy and regulation around blood availability and access.

P004 | Development of bone marrow donor registry in Kazakhstan

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Background: In Kazakhstan, more than 9000 patients are registered with oncological diseases of the hematopoietic and lymphatic systems and up to 1500 new cases are registered annually, of which about 15% of cases are paediatric patients with acute leukaemia.

For the development of bone marrow donation and transplantation treatment of oncohematological diseases, the National Registry of Hematopoietic Stem Cell Donors (HSC) was established in 2012 based on the Scientific and Production Center of Transfusiology, Astana.

One of the main tasks of the Register is the formation of a database of potential bone marrow donors in Kazakhstan.

Aims: To evaluate the dynamics of the Register's activities and determine the further algorithm of work.

Methods: We analysed of the Register's activity for 2022. Statistical data was processed in the Microsoft Excel.

Results: 17 regional blood centres of the country take part in recruiting new potential donors. In 2022, 1222 donors joined the Register, which is the highest figure compared to previous years (in 2021–1043 donors, 2020–489, 2019–473). Thus, the total number of the Register is approximately 9500 donors. All including donors were sampled at 5 loci at high resolution in accordance with the recommendations of the European Federation of Immunogenetics.

The majority of those recruited (63.5%) are males. The largest proportion among donors were the age groups from 25 to 35 years (54.9%) and from 35 to 45 years (26.1%).

The Register database includes donors of more than 30 nationalities. According to its ethnic content, the Register reflects the structure of the Kazakhstan population (Kazakhs–70.3%, Russians–19.2%, other nationalities–10.5%).

In the reporting year, the Register received 43 requests from transplant clinics, search centres in Kazakhstan and Russia.

From 2018 to February 2023, 9 unrelated allogeneic transplants were performed for patients, including 3 in 2022 for patients diagnosed with primary immunodeficiency, acute leukoblastic leukaemia and aplastic anaemia according to HLA-typing 9/10, 10/10 and 9/10, respectively. Thus, with the growth of the Register, the number of requests for which compatible donors are located increases.

Currently, the Register is included in the international Kazakh-Russian Register, which includes the Kazakh and 15 other Russian registers located in various regions of Russia. The total number of the base is about 140 thousand potential donors of HSC.

In August 2022, the Board of the World Bone Marrow Donors Association (WMDA) approved the Register's Temporary Membership in WMDA, which allows the Register to list data on potential donors in an international database and use it in a safe mode.

On September 17, 2022, the National Registry took part in the celebration of the World Bone Marrow Donor Day. An open Day, a press conference and interviews were held with the participation of donors, journalists, members of the public, active coverage of HSC's gratuitous donation in the media and social networks.

Summary/Conclusions: With a small number of donors, the National Register of the Kazakhstan shows signs of its viability. To improve the efficiency of the Register, it is planned to continue information and explanatory work with the population and potential donors, and establish international cooperation with foreign registers.

P005 | Management of blood services during emergencies

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Background: The United Nations defines a disaster as a situation or event which overwhelms the local available capacity, and thus requiring assistance at the national or international level from external sources; an unforeseen and often sudden event that causes great damage, destruction, and human, economic and environmental losses and impacts.

A disaster in blood supply is defined by:

- A sudden unusual increase in blood needs, **or**
- A temporary restriction of the blood transfusion centres (BTCs) ability to collect, test, process and distribute blood, **or**
- A temporary restriction of the local population ability to donate blood or to use the available blood stock in BTCs, **or**
- A sudden unusual influx of blood donors.

The possible scenarios of blood shortage during the emergencies include supply failure, reduced availability of blood, surge in demand and limited access.

Aims: To provide a benchmarking analysis of the actions that were taken for managing blood disasters in Lebanon by the known hospital-based blood banks, the government and the Lebanese Red cross (LRC) blood services, in order to strengthen or establish emergency preparedness plans.

Methods: Published articles related to blood management during disasters from PubMed, in addition to data collected from both the LRC blood services and the Lebanese Ministry of Public Health were analysed.

Results: Lebanon have some emergency measures in place and an overall acceptable, yet heterogeneous response in the management of blood supply during disasters. The wide diversity of encountered situations requires a clear identification of their impact on blood transfusion and the response plans. The LRC have played a substantial role in maintaining the national blood supply owing to the availability of mitigation and preparedness plans in addition to disaster activation procedures (Tables 1 and 2). Good examples includes the 2006 war with

P005 - Table 1. Mitigation plan

No	Item	Description
1	Premises	Availability of 13 blood centres fully operational
2	Assessment	Power supply and availability of generators (Check Generator Condition)
3	Blood testing	Capacity kept in each area despite centralization equipment maintained
4	Storage	Supplies in two different locations
5	LRC volunteers	List for walk-in blood donors
6	Connection	Assessment of networks paths, phone systems, and its redundancy
7	Back up	Critical equipment connected to a backup power supply

P005 Table 2. Preparedness plan

No	Item	Action
1	Disaster plan	Clear chain of command
2	Human Resources	Train staff and volunteers/drills
3	Supply chain	Emergency stock/different brands
4	IT	Hardware accessories, satellite phones, internet redundancy
5	Logistics	Fuel reserves, warehouse capacity, transportation by volunteers
6	Running Cost	60 days cash availability
7	Communication	Disaster awareness messages, update hospital contacts
8	Blood Stock	Walk-in blood donor list from LRC volunteers

Israel, the Syrian war and massive immigration to Lebanon, the Suicide attack in Southern Beirut, the October 2019 Revolution, the Covid-19 lockdown measures, the 2020 economic collapse and the Beirut port explosion.

Summary/Conclusions:

- The challenges faced by each country are different. The plan to secure the availability and sustainability of safe blood components during humanitarian emergencies should be custom-made for each blood system individually.
- Similarly to the European countries, the blood supply contingency and emergency plan should be established or strengthened (if available), implemented and regularly assessed and improved.
- This plan should define all the relevant risk scenarios, the key stakeholders and their anticipated actions in order to create effective risk mitigation strategies.

P006 | On-line inspections of polish blood establishments during the COVID-19 pandemic: 2021–2022

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Background: Pursuant to the Public Blood Transfusion Service Act of 1997, one of the tasks of the Institute of Hematology and Transfusion Medicine (IHTM), as the Competent Authority are regular inspections in 23 Polish Blood Establishments (BEs). Since 2009, IHTM inspections have also been performed according to EuBIS guidelines with great impact on the strengthening of blood component quality and safety. Each Polish BE is inspected at least once in 2 years with additional inspections performed when: (1) critical non-conformities are found during routine inspections, (2) major changes in work organization or key personnel have occurred. Every inspection team is composed of an immunohematology expert, a specialist in transfusion-transmitted infections (TTI); two specialists focused on the pathway from donor qualification to blood component issue, as well as implementation of the quality assurance system. Such approach to inspection strategy is dictated by the complexity of blood component preparation which is a process that requires in-depth knowledge of laboratory testing, preparation procedures, principles for implementation of the quality assurance system as well as conducting inspections and recommendation issue. Each inspected BE is required to present the timetable for implementation of the post-inspection recommendations and preventive actions. The routine strategy of on-site inspections was disrupted by the outbreak of the COVID-19 pandemic.

Aims: The study aim was to assess the IHTM recommendations issued following on-line BE inspections induced by the pandemic in the years 2021–2022.

Methods: 32 protocols from on-line BE inspections (2021–2022). All the inspections were based on a detailed analysis of documentation forwarded by the BEs, before and in the course of the on-line inspection as well as on the virtual discussion with BE personnel during on-line sessions.

The on-line inspections were primarily based on the submitted documentation, with no opportunity for observing the processes themselves, therefore the non-compliances found were not classified as critical, major, or other significant but rather referred to areas and scope of BE activity (documentation, work organization, qualification and validation, pathway from donor qualification to blood component issue, quality control of blood components, adverse events and reactions).

P006 – Table 1

Year		2021	2022
No. of inspected BE's		15	17
No. of recommendations issued		402	502
Classification of recommendations	Documentation	250 (62.2%)	405 (80.7%)
	Work organization	45 (11.2%)	25 (5.0%)
	Qualification and Validation	28 (7.0%)	23 (4.6%)
	Pathway from donor qualification to blood component issue	42 (10.4%)	32 (6.4%)
	Quality control of blood components	28 (7.0%)	10 (2.0%)
	Adverse events and reactions	9 (2.2%)	7 (1.3%)

Results: Recommendations issued during the on-line inspections as regards the inspected areas.

Summary/Conclusions: In the inspected BEs there was no integrated documentation system therefore it is necessary to 1. develop guidelines referring to uniform standards for the development, implementation and management of standard operating procedures and emphasize their significance for integration of the documentation system 2. Procedures for properly conducted self-inspections to include their educative role and proper quality control.

P007 | Workload indicators of staffing needs tool for calculating the manpower requirement of an Indian blood center

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Background: World Health Organisation (WHO) released the WISN tool which stands for "Workload Indicators of Staffing Need". It is a tool used to help healthcare organizations determine the appropriate staffing levels for their operations. This tool considers factors such as the workload required to provide quality care, the skills and expertise of staff members, and the availability of resources. When it comes to a blood centre, the staffing needs can vary depending on the size of the centre, the number of donations received, and the variable services offered. It is of utmost importance for staff adequacy at any blood centre to comply to Good Manufacturing Practice, Good Lab Practice and Good Clinical Practice guidelines. This endeavour aims at using this tool to determine the appropriate number of staff members needed to meet these goals at our blood centre.

Aims: To calculate the minimum manpower requirement for an Institution-based Indian blood centre using WISN tool.

Methods: For using the WISN tool at our centre, the staffing requirement was stratified into faculty, resident, nurse, junior technical staff (JTS), senior technical staff (STS), social worker, data entry operator (DEO), multi-tasking staff (MTS). After taking into account all the vacations, public holidays and other permissible leaves, the available workhours per year (AWY) was calculated for each stratum. Work activities carried out on a regular basis were categorized as "health service activities" carried out by all members of the professional category with reported statistics; "support activities" carried out by all professionals of that category; "additional activities" are performed by some members of the category, and both are not recorded in the service statistics. Subsequently, the pattern of activity was calculated to calculate the WISN elements viz. the service standard, standard workload, allowance standards and allowance factors were calculated. The data analysis using WISN elements to estimate the staff need, was done using MS-Excel.

P007 – Table 1

Staff stratification	AWY (hrs./year)	WISN predicted staff requirement and existing staff	Shortage and workload pressure
Faculty	1376	5 and 1	Staff shortage and 0.2
Resident	1944	3 and 2	Staff shortage and 0.67
Nurses	1338	8 and 4	Staff shortage and 0.5
JTS	2152	18 and 10	Staff shortage and 0.55
STS	1659	12 and 2	Staff shortage and 0.16
Social worker	1659	2 and 1	Staff shortage and 0.5
MTS	2152	7 and 5	Staff shortage and 0.85
DEO	2152	2 and 2	Staff adequate and 1

Results: The AWY was minimum for the nurses while it was maximum for the JTS, MTS, and DEO. Using the WISN tool, the broad service headings were Administration, Apheresis, Camps, Molecular IH Lab, Donor Screening, Donation, QC Lab, Component Lab, Automation Lab (for Immunohematology and Infections screening), Pre-transfusion testing Lab, Pre-donation Counselling, Education sessions, Post donation Counselling, Regn. for In-house Donation & Apheresis and at Camps.

Summary/Conclusions: We utilised the tool to establish the staff shortage at our centre and predict the requirement objectively based on the previous year workload and AWY for the staff. This can be extrapolated to other facilities with higher workload very easily to understand and plan the manpower requirement. This will facilitate decision makers and planners for effective and efficient management of workforce; thus, ensuring adequate manpower, with the right skill sets, are performing adequately and timely to achieve desired quality standards.

P008 | The design of an integral vigilance system for registered ATMPs based on human material in The Netherlands

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Background: Advanced Therapy Medicinal Products (ATMPs) form a group of medicinal products based on genetic material, cells or tissues. In the production of ATMPs based on human material (hmATMPs), human cells or tissues are used as starting material. The quality of this starting material can affect efficacy and side effects, and events such as loss of a product can have consequences for both patient and donor. To guarantee the safety and quality of hmATMPs, a comprehensive vigilance system is necessary covering the entire chain of donation, production and administration.
Aims: To develop a vigilance system for registered hmATMPs, covering the entire chain from donor to patient, to optimise safety and quality.
Methods: Based on international and national laws and regulations, an inventory was made of the requirements for reporting regarding the safety and quality of hmATMPs. Based on the effects on both patients and donors, the types of adverse occurrences that can arise in the various steps have been determined. A reporting route was designed based on the responsibilities and expertise of the authorities involved.
Results: In The Netherlands, the Medicines Evaluation Board (MEB) and the Healthcare and Youth Inspectorate (IGJ) are responsible for the oversight of the safety aspects of hmATMPs. The MEB receives input from the national pharmacovigilance centre Lareb, and IGJ receives registered and assessed reports through the national haemo- and biovigilance office Transfusion and Transplantation Reactions in Patients (TRIP). Incidents or donor complications that arise during the donation, procurement and testing of human bodily material in a tissue establishment, or are related to the process in the tissue establishment, are governed by the Dutch Act on safety and quality of substances of human origin. TRIP receives information about these adverse occurrences primarily through the tissue establishments. In case of adverse occurrences at the manufacturer or the applying facility, these organisations provide feedback to the tissue establishment, which subsequently notifies TRIP. Special attention is paid to donor vigilance, including donation complications during the donation of human body material and repeat procurement after loss of material due to an incident. Side effects during or after administration of an hmATMP are reported to Lareb, after which, in collaboration with TRIP, a relationship with incidents earlier in the chain and the consequences for the upstream donation can be investigated. Adequate traceability of the donor, the material and product is essential.
Summary/Conclusions: Framed by international and national legislation, a comprehensive vigilance framework is posited for registered hmATMPs, based on the concatenation of bio- and pharmacovigilance. Formalized cooperation between both vigilance systems is necessary to successfully implement the proposal and to increase knowledge of adverse occurrences and side effects in the hmATMP chain.

Management and organisation

Information technology

P009 | Abstract withdrawn

P010 | Leveraging on enhanced donor appointment and recruitment system to improve communication efficiency

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Background: The Blood Donors Programme (BDP) team from Singapore Red Cross previously would need to manually identify donors staying within close proximity of a mobile blood donation drive and/or donors who have donated in the previous drive (e.g., Using Excel or Access etc.) to send short message service (SMS) to inform them of the upcoming drive. In additional, the team would also need to extract donor list for blood appeals and to inform/update donors of upcoming events. The process is time-consuming and is heavily dependent on individuals who are assigned to the tasks.
Aims: The blood bank aim to leverage on system equipped with the necessary data to improve communication efficiency.
Methods: The project team worked closely with BDP and Blood Collection Team from Blood Service Group, Health Sciences Authority to review its work processes, assess the existing systems and gather requirements before developing the new modules in system. The program scripts are carefully written to run tasks for user which would replace the manual processes of identifying and communicating to donors.
Results: The project team successfully rolled out two new modules in system namely Mobile Drive Session and Mass SMS Notification module.
Mobile drive session module
This module allows user to create mobile drive records in the system. As the system have all the donation records, user would be able to extract donors staying within 1, 2 or 3 km radius of a drive by using the postal code and/or donors who have donated in the previously drive. SMS could be generated and send to these donors to notify them about the upcoming drive. This directed recruitment measure will likely reap better return of investment (ROI). In additional, previously it would take about 240 min to extract and inform donors. With the enhancement feature, BDP now only need about 9 min. This is equivalent to 57,750[1] min of saving in a year ([1]Based on 250 mobile drives a year that require to use this feature).
Mass SMS notification module
This module allows user to extract donor list by defined criteria (e.g., blood group, total donations, donation eligibility and age etc.) and schedule a date to send SMS or email. It would take about 180 min previously to extract and inform donors. BDP now only need

about 9 min after using the new feature. This is equivalent to 11,016[2] min of saving in a year ([2]Based on 136 of such requests in 2022).

Summary/Conclusions: The communication efficiency improves after using the two new modules. BDP could save about 81,006[3] min in a year by using the two new modules ([3]Total time saved for using the two new modules). Given the improved efficiency, BDP could also use the system to make last minute appeal to donors to step forward to donate or boost the appointment take up rate and channel their resources to step up engagement with blood mobile organiser.

P011 | Method for a biobank scale imputation of blood group antigens

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Background: A key element for successful transfusion is the compatibility of the patient and donor red blood cell antigens. Precise antigen matching reduces the risk for sensitization and other adverse transfusion outcomes. As large cohorts with genotyping array data are increasingly available, it would be useful to have methods to impute the red blood cell antigens from the genome data.

Aims: We developed an imputation method for determining blood cell antigens from genotyping array data.

Methods: Random forest models for 31 antigens in 11 blood group systems and for HPA1a were trained and tested using genotype and blood cell antigen data available for 1168 blood donors of the Blood Service Biobank. The algorithm and models were further evaluated using a validation cohort of 111,000 Danish blood donors genotyped with a different genotyping array.

Results: In the Finnish test cohort, the balanced accuracies determined by cross-validation were >98%, except 89% for hrS. We were able to replicate 26 out of 32 Finnish models in the Danish cohort. In the Danish cohort, the balanced accuracies were >91% in models trained with the Finnish cohort, except for Jkb, C, c, and D (ranging from 17% to 78%). When applying models trained with the Danish cohort, the balanced accuracies in the Danish test cohort were >95%, except for Coa, k, and Vel (ranging from 75% to 86%).

Summary/Conclusions: The blood antigen imputation models demonstrated high overall accuracies suitable for biobank scale determination of blood types. The method is applicable for screening of blood donors having rare blood types. Population-specific training cohort increased the accuracies of the models.

P012 | The information sharing between blood establishment and hospital is realized by establishing standard

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Background: Zhejiang Province took the lead in establishing a unified blood collection and supply management information system in China in 2005, realizing the real-time sharing of blood collection and supply information in the whole province. However, there are many development companies of clinical blood information system used by hospitals, and the function modules and coding rules are not uniform. As a result, the blood station cannot realize interconnection with many hospitals in the province, and it is difficult to realize the early warning of clinical blood inventory, the early warning of adverse transfusion reactions, and the scientific evaluation of clinical rational blood use within the province. There are great risks and difficulties in guaranteeing the safety of clinical blood use and responding to major public emergencies.

Aims: To provide a set of basic data standard with standardised terms, clear definition and unambiguous semantic context for information sharing of blood stations and hospitals. To realize the consistency and comparability of blood station information and hospital information in blood reservation, blood distribution, stock sharing, blood quality control index comparison and other applications, so as to ensure the effective exchange, statistics and sharing of information.

Methods: Investigate and analyse the current situation of the information system of blood stations and clinical blood use in the province, consult and study the relevant laws, regulations, policy documents and papers related to blood collection and supply of blood stations and hospital transfusion, sort out the needs of blood stations and hospitals for standardised management of clinical blood use, and determine the plan of information sharing; Through use case analysis and functional modelling, the content of the basic data set to be collected is clarified. Data elements were extracted, data element attributes were standardised and standardised according to the health industry information standards and data standards for information sharing between blood banks and hospitals were finally established.

Results: The standard content involves 6 basic data sets such as blood reservation, inventory and use, 66 data elements and 13 data element range codes, which have been applied to be the local standard of Zhejiang Province. Since its implementation, the standard has been applied to the whole process of information management from blood reservation to clinical infusion and the construction of provincial clinical blood quality control system. Examples of main effects: (1) The hospital sends blood reservation orders through the network, and the blood station adjusts the blood supply quantity according to the hospital's reservation quantity and inventory, and forms electronic orders, thus improving the accuracy of information and reducing labour workload. Up to now, 78 hospitals have been covered with 47,357 orders, with an average of 80 orders per day. (2) A clinical blood quality control index system including pre-transfusion evaluation and post-transfusion evaluation was established, and an electronic form of

archiving information of clinical blood use, auto transfusion and indoor quality control was automatically formed.

Summary/Conclusions: The establishment of standard is the key to the information system to play the application effect, is to avoid the "information island", realize the resource integration, information sharing and interconnection foundation. Through formulating the data standard of information sharing between blood stations and hospitals, the key problems of interconnection between blood stations and hospitals in the whole province are solved. It is of great significance to strengthen the management of clinical blood use in medical institutions, carry out adverse event monitoring of clinical blood use, carry out scientific evaluation of rational clinical blood use and establish a quality management system from blood vessel to blood vessel.

P013 | On boarding the whole of government (WOG) workflow management system for a robust occurrence reporting system

T - J[1]

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Background: The Blood Services Group (BSG) of the Health Sciences Authority (HSA), Singapore embraced the whole of government (WOG) initiative to digitally transform its work processes for a more productive work environment. The Quality unit embarked on a project to transform its paper-based occurrence reporting to a robust occurrence management system using the WOG Workflow Management System (WMS). The WMS sat on HSA's intranet environment and staff had easy access to the online tool.

The BSG was using a hardcopy form for its occurrence reporting. Reports were handwritten and difficult to decipher. Staff had to send an email notification of the occurrence to senior management within 24 h. Notification to Senior Management was not always timely. There were inevitable delays in report submission as the form had to be hand carried to the next reviewer. The Quality unit staff had to collate the hardcopy reports and submit to management for review. Keeping track of emails and reports was laborious and time consuming. Timely reporting and intervention were compromised.

Aims: The aims were as follows:

To leverage on technology to reduce human intervention

To develop an online occurrence reporting system using the WMS

To ensure Senior Management is notified within 24 h of an occurrence

To automate the reporting, review and approval of the occurrence report

To have an audit trail of the personnel involved with a date and time stamp

To auto alert staff of pending task to prevent delays in reporting

Methods: BSG's Quality unit and HSA's IT Specialist simplified BSG's occurrence reporting by reviewing the processes and removing unnecessary steps. User Acceptance Testing was performed to ensure specifications were met. The Occurrence Reporting workflow went live and staff were briefed on the new reporting tool.

Once the staff have completed a task in the workflow, the report would be automatically sent to the next reviewer with a click. The next reviewer could review and either "accept" or "reject" the report with comments. If the report is rejected, it would be re-directed automatically to the staff who had submitted the report. If the reviewer clicked on the "accept" tab, the occurrence would be directed to the next reviewer as pre-defined in the workflow.

Automatic alerts were sent as reminders for action. The system sent the consolidated reports to Senior Management for comments/acceptance/rejection and for Group Director's final approval.

Results: Reporting was seamless and timely as the system automatically sent the report to the relevant reviewer/approver with a click. An automatic email notification was sent concurrently to Senior Management upon the creation of a new occurrence report. 100% of occurrence reports had since been notified to senior management within the set target of 24 h. The description and immediate action taken for the occurrence was sent concurrently to Senior Management within 24 h. It took up to 7 days for immediate action submission previously.

The system generated an audit trail for a quick view of the staff involved at every stage in the occurrence report with a date and time stamp. As any delays were immediately evident, there was also self-surveillance to complete the task within the deadline. An automatic alert was sent to staff every day after the deadline until the task was completed.

A dashboard on the WMS gave a status update of the occurrence report. The WMS allowed BSG to move into a paperless work culture which was environmentally friendly and cost-effective.

Summary/Conclusions: The new workflow was fully automated, time saving, user-friendly and brought about greater productivity. The quality unit was able to track the reports online and ensure the necessary corrective/preventive actions were taken on a timely basis. The new online occurrence reporting system was a resounding success and was another milestone in ensuring quality and efficiency in BSG's work processes.

P014 | Mobile calculator application for human erythrocyte antigen frequency to assist blood banks in antigen typing and obtaining specific antigens-negative blood units

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Background: In transfusion medicine, compatible blood units are selected to be transfused to prevent transfusion reactions caused by antigen-antibody reactions. For patients with unexpected antibodies, corresponding antigens-negative RBCs are typically selected. When looking for specific antigen-negative units, blood banks perform antigen typing on several units of random RBC units. However, the

number of units to be typed varies depending on the person performing the antigen typing, because it is commonly estimated by mental arithmetic with reference to known frequencies sometimes outdated.

Aims: This study was conducted to investigate the most updated human erythrocyte antigen frequency in Korean population, create a mobile calculator application through which the frequency of specific antigen-negative unit and the number of units required to be typed can easily be calculated, and thereby improve routine process of blood banks.

Methods: To determine the true frequency, each human erythrocyte antigen frequencies were investigated through the most reliable sources in Korea. ABO and RhD data of blood donors accumulated on the Statistics Korea from 2012 to 2021 were analysed. RhCE phenotype data of RBC units accumulated on Blood Information Sharing System from June 2016 to January 2023 were analysed. Other human erythrocyte antigen frequencies were averaged considering the sample numbers of several references: 'Delaney, Transfusion, 2015', 'Hong, Ann Hematol, 2016', 'Kim, Ann Lab Med, 2018', 'Shin, Ann Lab Med, 2018', and 'Jekarl, Transfus Med, 2019'. Mobile calculator application was created using the Appsheet, a no-code development platform for mobile application based on spreadsheet data. The frequency of combinations of two or more independent antigens was encoded to be calculated by multiplying the respective frequencies. The number of units required to be typed was encoded to be calculated by taking the reciprocal of the frequency.

Results: Among 28,458,634 donor data on the Statistics Korea, frequencies of A, B, O and AB were 34.2%, 26.8%, 27.5% and 11.5%, respectively. Frequencies of D+ and D- were 99.6% and 0.4%, respectively. A total of 220,171 RhCE phenotype data were recorded on the Blood Information Sharing System. The frequencies of RhCE phenotypes between D+ and D- individuals showed significant difference. Among RhCE phenotypes of D+ individuals, Ce was the most common (41.7%), followed by CEce (37.9%), Ec (9.1%), Cce (7.3%) and Ece (3.5%). Among RhCE phenotypes of D- individuals, ce was the most common (63.1%), followed by Cce (18.7%), Ece (13.9%), CEce (2.0%), Ce (1.6%) and Ec (0.4%). In MNS blood group, frequencies of M-, N-, S- and s- were 24.1%, 25.6%, 89.7%, and 0.9%, respectively. Frequencies of Fy(a-), Fy(b-), Jk(a-), Jk(b-), Di(a-) and Di(b-) were 0.8%, 84.0%, 26.1%, 22.0%, 89.2% and 0.8%, respectively. Based on the frequencies investigated, the mobile calculator application was created for calculating selected antigens frequencies and units to be typed to obtain selected antigen combinations.

Summary/Conclusions: To the best of our knowledge, the most reliable human erythrocyte antigen frequency in Koreans was investigated with reference to the most extensive sources at the present time. Based on that frequency, the mobile calculator application was created to assist blood banks in estimating necessary units to be typed. The results of this study are expected to streamline the blood preparation and release process.

P015 | Abstract withdrawn

P016 | Evaluation of a new-generation nucleic acid testing middleware to improve process efficiency through full real-time monitoring of the NAT instrumentation

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Background: Blood and plasma donations are routinely screened by using nucleic acid testing (NAT) technology. Data from automated NAT instruments (i.e., Procleix Panther and Procleix Xpress) are collected by a middleware, a software that tracks individual donor blood samples, collects results, and connect them to the laboratory information system (LIS). The Bloodstream software (BSSW) (Grifols) was developed to connect bidirectionally with the Procleix Panther and Procleix Xpress systems and with LIS, automatically managing the entire NAT process.

P016 Table 1. Results obtained with BSSW.

Case study	Results
1	- Easy to configure auto-approve and export automatically. User-friendly and intuitive configuration and navigation. - Flexible to configure instrument thresholds. Laboratory dashboard allowed to monitor the entire NAT process automatically, optimizing process time. - TAT: Flexible to change between dynamic and rigid times. The workflow was improved because there was data about sample processing time.
2	- BSSW allowed to configure and control assay reagents stability times - BSSW managed reagent stability information which was not managed by NATM. Reagent stability expiration was configured to interpret the reagent validity based on reagent tracking information
3	- Easy to operate bidirectionally between LIS and NAT instruments to order tests and send results. - It was suggested to introduce multiple sample requests with different types of tests at the same time if the information is not available in the LIS.
4	- Test results were searched and exported faster with BSSW compared with NATM, although they were not easily modified. - Little training was required to handle assay results.
5	- There were no major issues when managing pool results. - Information about donation and the use of different filters was appreciated and better compared with NATM.
6	- The time to create a report with BSSW was similar to NATM.

Aims: The aim of the study was to collect data and user feedback of the new generation middleware, BSSW, to manage NAT results in routine laboratory environment.

Methods: The BSSW was evaluated at the Blood Transfusion Centre of Granada (Spain) between May and June 2022, using two Procleix Xpress and three Procleix Panthers instruments. A questionnaire was conducted with six case studies evaluating the most important BSSW implementations: (1) configurations: approval and export, instrument thresholds, laboratory dashboard and turnaround times (TAT); (2) assay reagent stability: configuration, entry of reagent preparation information and review of conclusions; (3) managing test orders; (4) modification, approval, release, and review of assay results; (5) managing pooling results; and (6) reports and Key Performance Indicators (KPIs). For each case, user's satisfaction was compared with the previous software (NAT Manager, NATM) in terms of usability, intuitiveness, time, performance and suitability of training.

Results: Data reported by the investigators are included in Table 1.

Summary/Conclusions: The BSSW middleware represented a user-friendly software that simplified, standardised, and automatized procedures during NAT blood screening. Likewise, it provided a unique interface point for centralised data routing between NAT instruments and LIS. Multiple sample requests were not simultaneously created when information was not available in the LIS. Overall, BSSW improved the whole process efficiency, required less user interaction and reduced workload.

P017 | Computerisation of the transfusion chain

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Background: Paper medical records are on the way out and electronic solutions are becoming indispensable in the healthcare system, for instance hospital wristbands contain barcodes. Also transfusion management is changing. Known irregular antibodies of patients in The Netherlands are registered in an electronic database (TRIX), blood products have barcodes and transfusion reactions (TR) can be reported in a transfusion module in the hospital information system (HIS) and laboratory information system (LIS).

Aims: Our aim was to investigate the extent of computerisation of the hospital transfusion chain in The Netherlands.

Methods: TRIP, the Dutch national haemovigilance office, invited the haemovigilance contact persons of the Dutch hospitals to respond to an online questionnaire about the state of digitisation of the transfusion chain, including the way of issuing components, reporting TR and about short term plans in this area.

Results: Thirty-six of the 81 hospitals responded to the questionnaire (44%), including three university hospitals. In 2021 these 36 hospitals were responsible for 43% of the red blood cell transfusions in The Netherlands (167,682/388,386 units).

Thirteen responding hospitals have a transfusion module in both LIS and HIS (36%; group 1). Twenty-two hospitals (61%) make use of a transfusion module in either LIS or HIS (group 2). One hospital does not use a transfusion module in LIS or HIS (group 3).

In 9/13 group 1 hospitals the units and patient identification sticker are scanned at issue (69%), and in 4 they are both scanned and checked by two staff members. In group 2 thirteen hospitals employ checking by two staff members at issue (59%) while 9 others also use scanning (41%).

The pre-transfusion check is by scanning the unit and patient wristband in 10 group 1 hospitals (77%), among which in 7 hospitals confirmation of administration of the unit is automatically registered. In group 2 this is the case in 7 hospitals (23%), with 4 automatically confirming administration. Nineteen hospitals employ the check by two staff members, with four also scanning at the bedside.

Reporting TR to the transfusion laboratory is done using the module in 8 group 1 hospitals (62%), two also phoning. In five hospitals TR are discussed by telephone only (38%). In group 2 hospitals, 19 report TR by phone (86%), and six also do this in HIS. Three respondents indicated that the TR is reported using the transfusion form.

Six hospitals in group 1 intend to further optimise their own system and are experiencing issues in which one hospital indicated a desire to meet professionals from other hospitals to discuss problems and experiences. Hospitals in group 2 report plans to progressively roll out digitisation.

Summary/Conclusions: Of the hospitals which responded to the questionnaire, checks at issue and before starting transfusion are more often performed using computer systems in hospitals with linked LIS and HIS which employ a transfusion module. There are major differences between Dutch hospitals in the extent of digitisation, and most of them are progressively increasing their level of digitisation. Inter-hospital exchanges could contribute to solving implementation difficulties and speed up the process. In the end progressive digitisation has the potential to increase safety in the transfusion chain, reduction of staff workload and conceivably lead to increased reporting of TR.

Management and organisation

Cost effectiveness

P018 | Fresh frozen plasma waste reduction

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Background: Fresh frozen plasma (FFP) is a blood product that has been available since 1941. Initially, it was used as a volume expander, but currently is indicated for the management and prevention of bleeding in coagulopathy patients. The evidence on FFP transfusion is scant and of limited quality. An audit on transfusion practices suggested that 25% of all thawed FFP were inappropriately ordered and/or transfused.

Maternity & Children Hospital-Dammam is enrolled in value-based projects as part of the national health strategic plan. At the baseline audit, the FFP wastage was found to be variable, and a Quality improvement project was launched to decrease the wastage below international upper limit (10%).

Aims:

- Reduce FFP wastage
- comply with national and international KPI recommendations
- Maintain Patients' safety
- save FFP units for potential patients and other possible future projects

Methods: The wastage is calculated on a monthly basis and plotted on a chart. At the end of each year, the monthly surpass will be counted as a percentage of the whole year. In order to accomplish this project, the problem was approached on so many levels simultaneously. Proper definition of the wastage, personnel training, staff communication improvement, patient blood management and proper utilisation of thawing devices among other steps have made this project of improvement possible.

Results: In 2020, when the problem was identified, 58% of the months had on overshoot of wastage calculated. After the first year of the launch of the project, the percentage decreased to 33%. In 2022, we had a wastage of only 8% which to only one month surpass in 12 months. This means that in 2 years, there was a significant improvement in the wastage from 58% to 8%. This has saved the hospital more than 200,000 US dollars worth of fresh frozen plasma.

Summary/Conclusions: After the launch of the improvement project, there was a remarkable positive change in the wastage and utilisation of FFP units without risking the safety and well being of the patients.

P019 | Abstract withdrawn

Management and organisation

Training and education

P020 | Abstract withdrawn

P021 | Impact of contextualized blood banking training in improving the safety and quality of blood transfusion services: Case of the Cameroon Baptist Convention health services

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Background: Blood transfusion is lifesaving though associated with varying complications from infectious to more fatal ones. Poor knowledge, unskilled personnel and limited financial resources have been highlighted as some of the causes for decreased blood safety in Sub-Saharan Africa (SSA). Despite the presence of diverse training programs, there is paucity data on the impact of the trainings in improving the services and quality of blood transfusion.

Aims: To do a service improvement project for laboratory technicians and scientists through needs assessment training, and evaluation in selected health facilities within the Cameroon Baptist Convention Health Services (CBCHS)

Methods: A contextualized curriculum was designed following a gap analysis survey carried out within 9 of the principal health facilities located in 6 regions of the country. The hospital blood banks are attached to the laboratory department and this training was delivered to 13 of these heads with a background in medical laboratory science. It was done from April 2022 to September 2022 involving 2 days of in-person training, totalling 78 h. The six training modules included an introduction to quality blood products, the development of a quality team, strategies for donor recruitment, donor retention, quality assurance and haemovigilance. Practical assignments were given to be implemented in their various hospital's post each module. Moreover, a WhatsApp group was created to facilitate interaction post the onsite training and for follow-up. Educational materials were gotten from institutions like the National Blood Transfusion guidelines, the World Health Organization (WHO), the African Society for Blood Transfusion (AfSBT), the International Society of Blood Transfusion (ISBT), the Association for Advancement of Blood and Biotherapeutics (AABB), the European Directorate for the Quality of Medicines and Healthcare (EDQM), evidenced-based publications and presentations at scientific conferences. Powerpoint presentations and case presentations were the main means of teaching whereas brainstorming, group work, presentations and team-building exercises were used to facilitate interaction. The impact of the training was graded 3 months

after the end of the training via a feedback form. This was entered and analysed on Microsoft excel 2013. The grades were ranked as followed: 1–4 were considered 'poor'; 5–6 as 'fair'; 7–8 were considered 'good' and 9–10 as 'excellent'. The impact was assessed on 7 processes in blood banking.

Results: Out of the 13 participants, 10 underwent the complete training among which were 40% females. All of the participants followed the national blood transfusion service standards while none had done any formal training within the last 24 months. The mean ± SD scores of the impact post the training were as follows for the processes included; donor recruitment/retention (7.6 ± 1.6), blood donors management (7.4 ± 2.1), screening for TTIs (7.1 ± 2.1), blood distribution and inventory management (7.2 ± 2.2), quality assurance (7.3 ± 1.9), quality control (6.3 ± 2.1) and teamwork (7.3 ± 1.9).

Summary/Conclusions: Overall the training had a good impact on most of the blood bank processes except for quality control where there was a fair impact. This thus shows the effectiveness of contextualized blood banking training to improve safety standards in blood transfusion services, especially for an organization like the CBCHS which is a major provider of health services in the country.

P022 | E-learning in transfusion medicine: A scoping review

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Background: E-learning/online education has been increasingly used in the health care sector and health sciences. While well-established and long-running, regional and national transfusion medicine e-learning programs are present in some countries, there is limited published data on the use of transfusion e-learning programs and their effectiveness.

Aims: This scoping review aims to summarise the published literature on existing transfusion e-learning programs, their characteristics, covered topics and target audiences and how (knowledge, skills and attitudes) learning outcomes and effectiveness are assessed compared to other means of education. We also aimed to identify knowledge gaps in the published literature.

Methods: Using pre-identified search terms, we performed a literature search of MEDLINE, PubMed, Embase, CINAHL, APA PsycInfo, Education Collection, Web of Science Conference Proceedings citation Index, Transfusion Evidence Library databases until March 28 2022. We also searched ClinicalTrials.gov & WHO International Clinical Trials Registry for unpublished clinical trials. Eight reviewers reviewed the literature in pairs, and extracted the data. We excluded unpublished and non-English literature, abstracts, conference proceedings, review articles, editorials and commentaries.

Results: From 2946 references and 266 ongoing trials, we screened 48 full-text articles. A total of 26 studies were included in the scoping review. These included observational ($n = 18$), randomised trials ($n = 4$) and case-control studies ($n = 2$). Two studies were a qualitative evaluation of implemented e-learning programs.

Most programs were developed by academic/university affiliated institutions and were developed in-house (Table 1). The format of the

P022 - Table1: Characteristics of e-learning programs in the included studies ($n = 26$)

Characteristics		N (%)
Type of institutions	Academic/University affiliated	18 (70)
	Non-academic/non-university affiliated	8 (30)
Source	In-house developed	16 (62)
	External	8 (31)
	No details	2 (7)
Format	Module	10 (38.5)
	Virtual/video simulation	10 (38.5)
	Other	6 (23)

P022 - Table 2: Learners and assessments in the included studies ($n = 26$)

Characteristics		N (%)
Type of learners (multi-choice)	Medical students	7 (27)
	Physicians (interns, residents)	5 (19)
	Nursing/Midwives	6 (23)
	Others	9 (35)
Assessment levels of knowledge based on Kirkpatrick model (multi-choice)	Level 1 (reaction)	16 (62)
	Level 2 (learning)	12 (46)
	Level 3 (behaviour)	1 (4)
	Level 4 (results)	4 (15)
	Not specified	2 (8)

e-learning varied between the institutions. The programs covered different audiences (Table 2). The number of subjects in these studies ranged from 7 to 538, while number of topics ranged from 1 to 7. The topics covered a wide range of transfusion practice from donation to patient blood management, with the commonest being on blood administration ($n = 17$), and transfusion reaction ($n = 12$). Eighteen programs incorporated some form of learner's assessment, and 17 obtained learner's feedback. Most programs assessed level 1 & 2 learning outcomes (Table 2). Eight studies compared e-learning to other forms of education, while two described the resources &/or costs required for developing these programs. Three programs were accredited.

Summary/Conclusions: This scoping review documents the use of e-learning in transfusion education. Most programs were developed by academic/university affiliated institutions and included some sort of learner's assessment. However, few programs were accredited or assessed the effectiveness of the e-learning programs in comparison with other forms of education. Further studies are required to assess the effectiveness of e-learning, and the cost-effectiveness of its implementation in transfusion education.

P023 | Introducing 'transfusion evidence round-ups': An ISBT and systematic review initiative collaboration with a goal to share knowledge to improve transfusion practice worldwide

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Background: In 2020, ISBT began a collaboration with the Systematic Review Initiative [SRI] to improve the dissemination of the evidence base for transfusion practice to new audiences. The SRI, based within the UK blood services is a producer of high-quality systematic reviews and creator of two open access evidence libraries (Transfusion Evidence Library and Stem Cell Evidence), all used worldwide by clinical, research and guideline developer audiences.

Aims: The aim of this collaboration is for ISBT and the SRI to work together to develop a quarterly "Transfusion Evidence Round-Up" publication. The remit of the Round-Up is to promote awareness of high-quality papers, facilitate training, education and networking opportunities for ISBT members, and to enhance the collaborative relationship between ISBT and the SRI.

Methods: Annually, a working group of ISBT and SRI staff meet to select four theme days. These days, which are spread across the year are selected to be representative of internationally relevant transfusion medicine issues, for example, World Blood Donor Day. For each theme, a 'Transfusion Evidence Round-Up' is produced. The round-up is a comprehensive curation of ten recently published papers from the SRI's Transfusion Evidence Library, chosen by a multidisciplinary ISBT panel. The panel is selected from ISBT members who have registered their interest in the project and new members work on each Round-Up. Round-ups are sent by email to all ISBT members and Transfusion Evidence Library subscribers and promoted through respective social media channels. The most recent two Round-Ups have been accompanied by live online journal clubs facilitated jointly by SRI and ISBT staff.

Results: The first Round-Up was disseminated in September 2021. To date there have been six round ups with themes including safer maternal and child care, World Thalassaemia Day, World Cancer Day, medication without harm, and World Haemophilia Day. Feedback and access analytic data has demonstrated that the Round-Ups have been equally well-received by both audiences. Specific measures of impact and interest: click rate and social media interaction have been consistent with an average click rate of 46% and an average of 200 views per Round-Up. Feedback from the webinars has been positive. ISBT members who have participated in the reviewing panels have also provided positive feedback on their experience.

Summary/Conclusions: The collaboration has had a successful start. Within ISBT, the Round-Ups have raised awareness of the importance of the role of evidence synthesis for transfusion medicine and have provided opportunities for young members and investigators to get involved in evidence synthesis. For SRI, the Round-Ups have begun to give them access to a wider multidisciplinary global audience.

In the longer term, we plan to develop a blog series focusing on gaps in the evidence base and to develop an education programme focused on systematic review methodology and evidence synthesis for to ISBT members.

P024 | Developing immersive virtual reality for transfusion laboratory training

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Background: Biomedical Scientists (BMS) working in the specialism of Transfusion Science perform diagnostic testing and provide blood products for transfusion. Provision of incorrect products is potentially fatal for the patient. In 2021, 18% of transfusion errors reported in the UK originated in the laboratory, six cases causing major morbidity. The red cell crossmatch has been identified as the only test that covers all key areas that can result in laboratory-based incidents. Staffing shortages are a reported obstacle for maintaining a service, creating difficulty for training, and allowing release to attend educational courses. Immersive virtual reality (VR) technology allows for more remote, independent, practical training, which does not rely on

consumables and patient samples. Furthermore, it has the potential to reduce the burden on the training lead, who can access data on trainee performance.

Aims: To develop a generic immersive VR training package for the crossmatch test that: can be used by trainees working in the UK hospital and reference laboratory environment ensures the delivery of practical and theoretical training in a safe and engaging environment.

Methods: A multidisciplinary team (RCI Senior Clinical Scientist; Trainee/Senior BMS; UK Regulatory, Guideline and Haemovigilance organisation representatives; Software Designer; Digital Learning Consultant) created a design document through observation of the crossmatch process and discussion with experienced BMS. Aspects of the design document included:

Target audience

Learning outcomes

Content walkthrough

Functional overview

The design document was used to develop a script that details the training package's simulated laboratory environment and voiceover instructions of use. Five review meetings of the content took place before the release of the final product.

Results: The design document identified the: Target audience as early career healthcare scientists and students on laboratory placements. Learning outcomes as: Identify when and why manual cross matching is required (e.g., presence of antibodies).

Selecting appropriate red cell units and understanding the importance of this for the patient, including consequences of transfusing the incorrect selection.

List each step in the cross matching process.

Describe the correct techniques required to complete the cross matching process successfully.

Read and interpret cross matching results to make the right decision for the patient.

Content walkthrough as a basic experience from start to finish

Aspects of the functional overview related to user interface e.g. language, platform, interaction.

A draft version of the script and final product was created via extensive review by the project MDT.

Summary/Conclusions: The project successfully created a VR training package through the engagement and collaboration of a broad range of stakeholders. This was achieved while overcoming obstacles in project development that included short deadlines, daily workload, and technical aspects in the VR environment for example, laboratory movements such as pipetting.

P025 | Pilot of an immersive virtual reality red blood cell crossmatching training package

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Background: Immersive virtual reality (VR) based training is becoming more widely used in the healthcare environment. This involves the participant wearing a headset to place themselves in a computer-generated environment where the real process is simulated. Staff of all levels of experience can train in their area of speciality before treating patients in the clinical environment, or investigating related samples in the laboratory environment. This has the benefit of reducing the risk to the patient that can be associated with training related errors. Currently there is no immersive VR platform that is routinely used by Biomedical Scientists (BMS)/trainees in the transfusion specialism for training. In 2021, 18% of transfusion errors reported in the UK originated in the laboratory, six cases causing major morbidity. Red blood cell (RBC) crossmatch has been identified as the only test that covers all key areas that can result in laboratory-based incidents.

Here we describe the pilot of an immersive VR training package, which was successfully developed to target the transfusion process for RBC cross matching.

Aims: To determine if immersive VR is effective for training for RBC cross matching by improving the participants:

- knowledge related to the process.
- application of practical skills related to the process.

Methods: Sixteen undergraduate BMS and healthcare science students with no prior experience in transfusion laboratories completed the immersive VR training package. The package allowed the user to complete the crossmatch investigation of three patient cases in the simulated laboratory environment. The impact of the use of immersive VR based training was assessed by comparison of pre and post results of a knowledge test, which was marked by an experienced BMS (paired *t*-test; level of significant result $p < 0.05$). A self-assessment was performed by participants to understand improvement in application practical skills.

Results: A statistically significant increase was observed in scores related to an improvement in knowledge ($p =< 0.05$), and overall improvement was observed from self-assessment scoring of practical skills.

Summary/Conclusions: Positive feedback and result outcomes from this pilot study demonstrate that immersive VR can be used as a training tool in Transfusion Science. Further work is required to compare this technology against current training methods to understand whether it comparable for achieving learning outcomes.

P026 | Implementation and evaluation of the satisfaction of online training program for the personnel of transfusion management office in Korea

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Background: Blood transfusion is common medical procedure performed within a healthcare organization, involving a multidisciplinary team of personnel. ABO-mismatched transfusions can be fatal, and safe and appropriate transfusions are crucial. In South Korea, the Blood Management Act requires medical institutions above a certain size to set up a transfusion management office starting in 2021 to ensure proper blood transfusions. Personnel working in the transfusion management office must complete 8 h of mandatory training per year. Accordingly, the Ministry of Health and Welfare has provided a training program for transfusion management personnel to help them complete the mandatory education.

Aims: We aimed to produce an education program for transfusion management office personnel required by the Blood Management Act.

Methods: The Korean Blood Safety Project Group, which was entrusted with the education project by the National Institute of Organ and Tissue Blood Management (NIOTBM), produced an online training program for basic education of transfusion management personnel. A total of six detailed contents were the role and task overview of the transfusion management office, appropriate transfusion of red blood cells, appropriate transfusion of platelets, appropriate transfusion of plasma, monitoring and response to adverse transfusion reactions, and collection, analysis, and evaluation of transfusion-related data. Six speakers produced the videos. The target audience of the training was extended to practitioners interested in transfusion management education in addition to personnel working in the transfusion management office of medical institutions. It was promoted by sending a letter to each medical institutions from the NIOTBM and emails from the Korean Society of Blood Transfusion to its members. The videos were streamed from September 5 to November 30, 2022. A satisfaction survey on the education was conducted in the form of an online survey after the training was completed, and participants could write down any difficulties in the free comments column.

Results: A total of 791 people registered for the course, with 703 (88.9%) completing the course. Those who completed the course were physicians 104 (14.8%), laboratory technologists 364 (51.8%), nurses 230 (32.7%), and others 5 (0.7%). In the satisfaction survey on education, the participants were most interested in monitoring and response to adverse transfusion reactions, followed by the role and task overview of the transfusion management office. Satisfaction ratings were above 4.1 (5 being most satisfied), indicating that participants were highly satisfied with the training. 84.3% of respondents said that the online course was as good or better than the in-person course. In the free comments column, some participants expressed their difficulties in work due to the lack of understanding from healthcare organizations and the need for help with computer programs. They also expressed the need for specific best practices and benchmarking. Through a total of six video lectures, 3 h of basic education for personnel at the transfusion management office were approved.

Summary/Conclusions: This online training program helped the personnel of transfusion management office to complete their mandatory education, and the appropriate combination of regular face-to-face and online training can help improve the quality of transfusion office personnel and transfusion safety.

P027 | The influence of the COVID-19 epidemic on the obligatory trainings for nurses and midwives in regional blood center in Poznań in years 2020–2022

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Background: According to the national regulations nurses and midwives in Poland need to undergo dedicated trainings in Blood Centers to be allowed to transfuse blood components. There are two types of trainings: the introductory training (2 days) and the continuing one (1 day). The trainings must be retaken every 4 years.

The WHO declared the state of pandemic for the infection of the SARS-CoV-2 on 11/03/2020. The declaration of pandemic in our country resulted in changes in the legal Directive of the Ministry of Health in relation to the trainings of nurses and midwives transfusing blood components. Revised regulations allowed for the trainings to be carried out using electronic means of communication i.e. on-line. Thanks to them, on-line trainings in Regional Blood Center in Poznań were started in April 2021.

P027 – Table 1

	2021	2022
Total number of trained nurses/midwives	3203	1815
Participants in the introductory training	947	577
Participants in the continuing training	2256	1238

P027 – Table 2

Online training	2021	2022
Average score	84.8%	87%
Average score–introductory training	85.49%	87.32%
Average score–continuing training	84.13%	86.85%

Aims: The aim is to analyse the influence of the COVID-19 epidemic on the obligatory trainings for nurses and midwives in Regional Blood Center in Poznań in years 2020–2022.

Methods: The analysis was performed using yearly training reports the final tests.

Results: In 2021, 17 trainings were organized: 6 introductory and 11 continuing ones. The total number of 3203 of nurses and midwives were trained. In 2022, 26 trainings were organized: 7 introductory and 19 continuing ones. The total number of 1815 of nurses and midwives were trained. Online trainings enabled us to reach a great number the hospital professionals without the interruption in their daily work. Every participant in the training was required to take final test. The average test result in 2021 was 84.8% of the total score, in 2022 it was 87%.

Summary/Conclusions: Online trainings proved to be an extremely useful and effective tool in the time of COVID-19 pandemic. Without the possibility of such training, a great number of medical professionals would not have been allowed to transfuse blood components which might have resulted in the risk of patients losing their health. Online trainings were positively welcomed by their participants and we believe they will remain as one of the option of the trainings for the medical professionals in the future.

P028 | Abstract withdrawn

Management and organisation

Risk models, standards and regulation

P029 | Abstract withdrawn

Management and organisation

Blood supply management and utilisation

P030 | Abstract withdrawn

P031 | Collaboration & communication: Key elements of implementing state-wide change to reduce O RhD negative red blood cell use

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Background: 2022 saw serious & prolonged shortages of O RhD negative (neg) red cells (RC). 16.2% of RC issued were O RhD neg (average to September 2022), only 8.7% of new Australian donors are O RhD neg.

Large numbers of O RhD neg RC held for emergency use are often transfused to non-group O RhD neg patients to prevent expiry. Reducing O RhD neg RC demand will improve ongoing supply sufficiency; lessening pressure on blood donors.

A National statement regarding use of emergency group O RC has been developed by the National Blood Authority working group.

Two large jurisdictions had previously implemented emergency use O RhD positive (pos)

Aims:

- Implement change to include emergency use O RhD pos RC for females >50 years & males >18 years in line with the National statement
- Reduce O RhD neg RC demand supporting ongoing availability for O RhD neg patients
- Prioritise risk of serious morbidity/mortality from traumatic haemorrhage over potential risk of alloimmunisation
- Improve communication between clinical & laboratory staff to ensure the most appropriate blood is available for transfusion

Methods: Consult & seek lessons learnt from jurisdictions who had implemented this change.

Highlight O RhD neg demand is unsustainable, seek feedback about potential enablers & barriers to practice change through extensive consultation & engagement with industry experts, pathology providers, Australian Red Cross Lifeblood (Lifeblood) & Safer Care Victoria (SCV) & associated clinical committees.

Following consultation develop practice change recommendations, tools & resources supporting the National statement, addressing enablers & barriers.

Seek resource pack endorsement from Lifeblood, SCV & associated clinical committees.

Pilot resource pack to ensure clear messaging & appropriate clinical language.

Conduct virtual information sessions about practice change & facilitate discussion.

Launch practice change & resource pack coinciding with the National statement release.

Results: Collaboration highlighted blood shortages severities had not reached bedside clinicians. In response, SCV Chief Executive Officer (CEO) cascaded Lifeblood CEO information to hospital CEO's.

Resources developed & piloted include:

- practice recommendations
- educational presentations
- emergency protocol guidance
- flowcharts
- compliance audit tool

Following early engagement some health services implemented use of emergency O RhD pos RC ahead of the launch.

Concerns exist about emergency use RC adverse events. Several sites are collecting alloimmunisation rates independently. Blood Matters

supports post-emergency transfusion alloimmunisation rates data collection.

Summary/Conclusions: Change requires careful planning & stakeholder engagement to gain acceptance & time to develop resources, undertake thorough consultation & incorporate feedback into material development.

Key messages:

- RhD neg RC are not universally compatible, alloimmunisation to other blood group antigens may result
- Prioritise risk of serious morbidity/mortality from traumatic haemorrhage over potential risk of alloimmunisation
- Obtain pre-transfusion sample as soon as possible, to minimise O RC use
- Use emergency use group O RC only in emergencies to save a patient's life if no valid pre-transfusion specimen.

Next steps:

- Post implementation follow-up
- Measure O RhD neg issues/demand
- Develop transparent governance of O RhD neg holdings

Undertake state-wide audit

P032 | Abstract withdrawn

P033 | Abstract withdrawn

P034 | O RhD negative demand in a regional aeromedical retrieval service

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Background: O RhD negative red cell demand is driven by the need to hold stocks for emergency transfusion. O RhD positive blood has been recommended first line for people not of childbearing potential of unknown blood group in order to preserve O RhD negative stocks but this policy will have minimal impact unless it translates into reduced demands stock holdings. Aero retrieval services carry an additional O RhD negative emergency stock outside of the transfusion laboratory.

Aims: To review retrieval service O RhD negative red cell demand from women of childbearing potential.

Methods: Data from the aero retrieval service registry were abstracted for all transfused patients for the period 2017–2020 with blood groups collected from pathology databases. People of childbearing potential were defined as females under 50 years of age.

Results: Over the four year period there were 2306 retrieval missions with 143 patients transfused 389 red cells (mean 2.8 and median 2 red cells per patient). There were 48 (33.5%) female transfused patients, of which 19 were under the age of 50 years. Blood groups were unknown in 6 patients due to retrieval to different destination or death. Of the remaining 13 patients, 3 were RhD negative, 1 of whom died. Only 2 RhD negative patients of potentially childbearing potential were transfused red cells. Both were retrieved from smaller hospitals, enabling utilisation of local hospital supplies. One of these patients was A RhD negative and received a large volume of blood products, exhausting the local supplies of group O and group A units, receiving RhD positive blood and developing anti-D.

Summary/Conclusions: The carriage of O RhD negative blood by this retrieval service did not prevent RhD alloimmunisation in any women of childbearing potential over a four year period. Carriage of O RhD positive blood for emergency use is feasible; however, should be considered in conjunction blood holdings in smaller hospitals.

P035 | Plasma self-sufficiency: Insights for the success of the action plan

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Background: Thousands of patients need plasma for their treatments, a figure that has doubled in the last decade and is expected to continue to grow. Currently, the volume of plasma donations in Catalonia covers just 39% of requirements for the manufacture of plasma derived-products (mainly immunoglobulins), so it has to be imported from other countries where donations are remunerated. Approximately 70% of the world's plasma comes from the United States. The low availability of plasma donors in the United States as a result of the pandemic and the steady growth in IgGev consumption has led to a worldwide shortage of this blood product. This makes self-sufficiency essential to avoid the effects of external factors and guarantee patients' health and safety.

Aims: To identify priority actions to meet annual plasma donation targets.

Methods: A benchmarking of international banks, interviews with internal staff, in-depth interviews with plasma donors, telephone interviews with former plasma donors and an on-line blood donor questionnaire were conducted.

Results: Banc de Sang i Teixits (Blood and Tissue Bank, BST) professionals play a key role in driving change and new habits among plasma and blood donors.

The plasma donor community values and trusts the BST brand; they are used to donating and participates in its voluntary and altruistic values.

Blood donors have little knowledge of plasma, although once it is explained to them they are favourable to donation, so they require information about the impact on patients, the process and the reasons behind the challenge of self-sufficiency.

Summary/Conclusions: Actions to increase donations must be approached as a collective challenge, in which health professionals themselves have a very important role to play, while also requiring the

involvement of all circles of influence: Public Health, patient associations, donors, the media and society in general, need to know the importance of plasma in the context of healthcare and the reasons that have led to taking on such an ambitious challenge.

P036 | Increasing efficiency and effectiveness by modifying blood bank cross matching policy: A single center experience

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Background: Reserving cross-matched blood units leads to excess waste, acquisition and use of personnel resources. Analysis of actual supply of units reserved revealed some departments had as low as 50% effective use. The current policy of ordering, and then reserving the cross matched units for 72 h, led to the afore mentioned problems.

This study hypothesised that by changing the current policy of reserving cross matched RBC units and start reserving blood units for patients with positive antibody screen only, will increase blood bank efficiency.

Aims: This study examined the effect of changing the standard operating policy in the blood bank on cross-matched blood units and waste.

Methods: Cross matches and RBC units supplied were analysed for 2 years previous to, and 4 years following the new policy implementation.

Results: 9.3% decrease in cross matches, 66% decrease in disqualified blood units, and 11% increased use of cross matched reserved units following the implementation of the new policy.

Summary/Conclusions: The new standard operating policy saved personnel hours of cross matching and unnecessary reservation of blood units, leading to increased monetary savings and more effective blood supply.

P037 | Abstract withdrawn

P038 | Platelet safety measures in Sweden: An assessment of current practices and comparison with international practices

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Background: In Sweden, platelet components (PCs) are produced by 28 blood centres. Bacterial control measures for PCs (donor skin preparation, diversion pouches) are based on European (EDQM) guidelines. Safety measures including pathogen reduction (PR) or large volume delayed sampling (LVDS) bacterial culture are widely used but are not required by national guidelines, except to extend shelf-life from 5 to 7 days.

Aims: Document PC safety measures in Sweden and assess LVDS methods used in Sweden against international LVDS practices.

Methods: All Swedish blood centres were invited to submit data from 2020 on PC production and bacterial safety measures via an electronic survey tool. Data were stratified by location, bacterial safety measure used (PR, LVDS, none), and PC shelf-life (5 or 7 days). Bacterial culture practices were stratified by sample volume, sampling delay and use of aerobic/anaerobic bottles. Consensus methods for LVDS were based on practices in the United Kingdom (UK), United States

P038 – Table 1

Table 1: Swedish LVDS Practices in 2020 ($n = 11$)

Swedish blood centres using LVDS *	Sampling delay (h)	Sampling volume (mL)	Aerobic	Anaerobic (facultative)
A, B, C	≥24–<48[a]	10	√	–
D	<24 or ≥24–<48[b]	8	√	–
E	<24[c]	8	√	–
F, G	≥24–<48[d]	8	√	√
H	<24 or ≥24–<48[e]	4	√	–
I	≥24–<48	4	√	–
J	≥24–<48	4	√	√
K	≥48	4	√	√

* Blinded for confidentiality; results reflect the majority of PCs screened. Exceptions described below.

a) Friday apheresis: <24 or ≥48 h.

b) ~50% in each category

c) Occasionally ≥24–<48 h.

d) Saturday apheresis: ≥48 h.

e) Apheresis PCs: <24 h. Pooled PCs: ≥24–<48 h.

P038 – Table 2

Table 2: International LVDS consensus practices

Country	Sampling delay (h)	Sampling volume (mL)	Aerobic (mL)	Anaerobic (mL)
United Kingdom (NHSBT)	≥36	16	8	8
Canada (Canadian blood services)	≥36	20[a]	8–10	8–10
Canada (Héma-Québec)	≥48	20	10	10
United States (FDA)[b]	≥48	16	8	8

a) For double apheresis PC, 40 mL volume, with 3 aerobic and 1 anaerobic bottle.

b) Apheresis PCs only. Each split apheresis PC should be sampled separately.

(US) and Canada. Comparative transfusion-transmitted bacterial infection (TTBI) risk data were compiled from the literature.

Results: Complete data for 2020 were received from all 28 Swedish blood centres. A Total of 52,497 PCs were produced of which nearly 72% were pooled PCs prepared either from buffy coats or interim platelet units (IPU) separated by Reveos®. Fourteen centers (50%) producing >24,000 PR PCs with 7-day storage (46% of national PC supply) had adopted PR with the amotosalen/UVA technology (INTERCEPT™ Blood System). Eleven centres (39%) used LVDS to extend PC shelf-life to 7 days. Three centres (11%) produced 5-day PCs with no supplemental safety measure. LVDS was performed for 12,747 PCs (45% of non-PR PCs). In 2020, 5 bacterial contaminations were detected with LVDS (1/2549 PCs screened). No TTBIs were reported to a voluntary haemovigilance system. Sampling volumes and timeframes differed widely among the 11 LVDS sites (Table 1). None of LVDS methods reported in Sweden matched consensus practices for sampling delay, volume and use of aerobic and anaerobic culture in the US, UK and Canada (Table 2). Haemovigilance data from countries that have converted from bacterial culture to PR or from standard culture to LVDS show the persistence of TTBI risk when culture is performed with smaller samples and earlier sampling.

Summary/Conclusions: Nearly half of PCs produced in Sweden are treated with a PR technology to protect against a broad spectrum of bacteria. However, most PCs are produced using sub-optimal LVDS methods compared to other industrialized countries. Increased TTBI risk has been associated with bacterial culture methods using smaller samples and shorter sampling delays.

P039 | Demographic change of blood donors in Taiwan

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Background: Blood transfusions are frequently utilised, particularly in cases of major bleeding, surgery, cancer and other haematological malignancies. Approximately 78.8 million (82.8%) of the total reported 95.2 million whole blood donations were reported as voluntary no reimbursed donations, according to the WHO Global Status Report on Blood Safety and Availability. Nearly 100% of the blood donated in Taiwan comes from willing and unpaid donors. However, as the whole society continuities ageing, the demographic trend is getting older and the eligible donor population is shrinking could be great impact on Blood donations and blood supply services.

Aims: The aim of this study is to investigate and analyse the age distribution and demographic data of blood donors in the past ten years in Taiwan.

Methods: The statistical data on blood donors and blood donations were compiled from the annual report of Taiwan Blood Services Foundation from 2016 to 2021. The donor rate was calculated by the number of donors in per 1000 population. Donor rates were calculated and separately by sex and age by dividing the number of each matching strata.

Results: The average annual donor rate in this study was 6.01%, while the donation rate from 2016 to 2021 was 7.62%.

The proportion of the overall donor population that was contributed by donors in the age ranges of 17–20, 21–30, 31–40, 41–50, 51–65, and 17 or >65 years was 9.84%, 23.80%, 25.10%, 21.06%, and 0.14%, respectively. The average percentage of first-time donors was 3.52% ± 0.36%. Between 2016 and 2021, over the total population. In terms of overall donors, men made up 58.5% of the total, while women made up 41.5% of the total. The annual donation rate fluctuated between 5.9% and 6.1% since 2016. However, the percentage of first-time donors and donors declining from 3.9% to 3.0%, along with donors under the age of 24 were from 9.5% to 6.3% throughout this time.

Summary/Conclusions: Due to a marked increase in the number of postponed or unmarried couples over the past 10 years, Taiwan's overall population has been steadily declining. One of the factors contributing to women's rising socioeconomic position and expanding work opportunities. The demographic panorama in Taiwan has drastically changed as a result of dropping marriage rates, low fertility rates, and rising life expectancy. The population of potential blood donors is expected to shrink as a result of the drop in birth rate, particularly among the young to middle-aged donors. The population of middle-aged to older people (aged 41–65) will expand in size as a result of the ageing process. The dwindling youthful generation and ageing will have a particularly negative impact on the possible donor pool. The prediction model for blood donors is a crucial awareness indicator that may have an impact on our healthcare system.

P040 | Abstract withdrawn

P041 | Experience with autologous and allogeneic serum eye drops

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Background: The dry eye syndrome (DES) is a common disease caused by abnormal secretion of the tear film. For patients, DES is the source of utmost discomfort. One of the therapeutic options for DES management are serum-based artificial tears, both autologous and allogenic. This blood-derived product is particularly recommended for patients unresponsive to standard eye drops and for persons unsuccessfully treated for DES. Serum is highly effective in dry eye treatment as it contains substances such as growth factors, vitamin A and E and various immunoglobulins.

In 1991, the Institute of Hematology and Transfusion Medicine (IHTM) was the first centre in Poland to start the production of serum-based autologous artificial tears. Motivated by the urge to increase the patients' access to serum eye drops, in 2019 IHTM started the production of allogeneic artificial tears from healthy AB

blood group male donors. In 2021, IHTM was first to start serum inactivation using riboflavin (MIRASOL system).

Aims: The aim was to analyse the experience of IHTM in the preparation of autologous and allogenic serum eye drops in the period between 1991 and 2022.

Methods: Statistical data for autologous donations from 1991 to 2022 were analysed. The main data source for allogenic patient's group were OSDI questionnaires and IHTM's internal surveys collected in 2019–2022.

Results: Between 1991 and 2022, 1493 autologous whole blood donations were collected and the number has been growing every year. In the first half of 2020, the number of patients who used autologous products was markedly reduced due to the COVID-19 pandemic, which however contributed to raising number of patients using allogenic eye drops. Between 2019 and the end of 2022, a total of 26 AB group whole blood donations was collected from donors for preparation of serum eye drops.

In the 'autologous' group (697 patients) the age at first visit ranged from 7 to 92 years (mean age 54). 71% of patients reporting to IHTM were between 30 and 70 years old. The gender ratio for women and men was 76% and 24% respectively.

In the 'allogenic' group (114 patients) age distribution was similar (mean age 64), as was the gender ratio: 79% and 21% for women and men respectively.

In the analysed group of patients, the most common disease entities were autoimmune diseases such as Sjögren's syndrome (also secondary to rheumatoid arthritis), glaucoma, cataracts, conjunctivitis, lacrimal gland or corneal damage due to trauma and/or surgery.

The benefits of allogenic serum eye drops were confirmed by patients in OSDI questionnaires. 51 patients from 2019 to 2022 studies returned completed OSDI questionnaires. The mean OSDI before using allogenic serum eye drops was 67.94. After applying non-inactivated allogenic eye drops it was 51.60. The OSDI after inactivated allogenic eye drops was 44.75. In all three cases eye drops were applied for one month.

Summary/Conclusions: The number of patients using serum-based artificial tears is growing every year. Currently, serum is used worldwide in DES treatment and the results are promising. As confirmed by lower OSDI values, the patients report better eye lubrication, less pain and burning sensation and, reduced eye redness. Allogenic serum eye drops are easily accessible and the product is safer due to inactivation. Inactivation has much contributed to strengthening the patients' satisfaction/safety.

Patients suffering from autoimmune disorders, who applied both autologous and allogenic serum eye drops during DES therapy, reported better effects for the latter.

P042 | Optimising WB component supply–A Monte Carlo simulation model

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Background: A recent UK study that piloted the use of a group ORhD negative Whole Blood (WB) component demonstrated that due to its short shelf-life (14 days) and limited use to pre-hospital trauma patients only, component wastage was high. Reducing component wastage is essential for the future feasibility of this component.

Aims: Examine the trade-off between component wastage due to time expiry and unmet demand using simulation methods.

Methods: A supply and demand model for a WB component was developed. The model was developed using data collected as part of a 2-year UK pre-hospital WB pilot study.

FIFO model: A First in First Out (FIFO) stock management model was created using MS Excel. Demand and supply variables were used to populate the FIFO model. The FIFO model was generated using the following parameters:

- 14-day WB shelf-life
- 3-day lead time on orders
- Units delivered at 2 days old
- 1-day lead time ad-hoc orders possible

Demand Variable: Daily demand was modelled as a discrete random variable using a Poisson distribution with a λ of 0.70 (mean pre-hospital WB component demand). The RAND function in MS Excel was used to generate different demand profiles sampled from this Poisson distribution.

Supply Variable: A total of 7 inventory management policies were tested. Heuristic methods were used to determine the algorithms used in each of the inventory management policies generated (Table 1). Each was inventory management policy was compared against a baseline.

Simulation: Each model had a run length of days with 1000 trials. Random demand profiles, following a Poisson distribution, were generated for each model. These demand profiles were used as input values for the demand element of the FIFO model to generate outputs of mean component usage; mean component wastage and mean unmet demand. All outputs were based on the average of 1000 trials.

Results: All of the models 1–7 outperformed the baseline model in percentage component wastage. The best performing model was model 6, with a mean wastage of 97.00 units, representing 16% component wastage. This model did however, have the largest mean unmet demand of 5.795, which overall represented 1.1% unmet demand over the 732-day run period.

Summary/Conclusions: The supply and demand model developed in this this study has demonstrated that by altering WB supply, component wastage can be reduced. However, there are trade-offs between unmet demand and component wastage that need to be considered. The risk of inventory levels falling to zero and opting instead for a potentially alternative component versus component wastage.

P042 Table 1: A table of the inventory management polices evaluated. No data is represented by (-).

	Basic order	Ad-hoc orders
Baseline	2 units 5 times a week (pilot study supply)	
Model 1	If inventory <10 order 3	If inventory ≤6 order 2
Model 2	If inventory <12 order 2	If inventory ≤6 order 2
Model 3	If inventory <12 order 3	If inventory ≤6 order 2
Model 4	If inventory <12 order 2	If inventory ≤8 order 2
Model 5	If inventory <14 order 2	If inventory ≤6 order 2
Model 6	If inventory <6 order 2	—
Model 7	If inventory <8 order 2	—

P042 - Table 2: A table representing the results of the inventory management policies evaluated.

	Component usage Mean [SD]	Component wastage Mean [SD]	Unmet demand Mean [SD]	% Wastage Mean [SD]
Baseline	515.79 [26.88]	682.81 [26.8]	—	57%
Model 1	515.97 [26.33]	278.02 [21.84]	0.086 [0.39]	35%
Model 2	516.49 [27.46]	249.36 [20.77]	0.025 [0.20]	33%
Model 3	516.71 [26.80]	340.31 [22.33]	0.026 [0.21]	40%
Model 4	518.06 [26.01]	263.48 [20.20]	0.009 [0.13]	34%
Model 5	517.27 [26.44]	308.59 [20.40]	0.005 [0.08]	37%
Model 6	509.25 [25.68]	97.00 [14.45]	5.795 [3.53]	16%
Model 7	512.71 [26.92]	153.95 [17.55]	1.024 [1.44]	23%

P043 | Insuring blood supply and financial sustainability with a new university-based blood bank

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Background: The Transfusion Medicine Department (TMD) of the University Clinic Erlangen (UCE) historically collected and processed at its premises only apheresis-derived platelet concentrates or PC ($n = 5686$)*. Red cell concentrates or RCC ($n = 19{,}192$)* and therapeutic plasma or FFP ($n = 8845$)* were purchased from other blood manufacturers. In response to increasing inconsistencies in the repository of red cell blood groups to supply medical institutions in the region of Nürnberg-Erlangen, along with increasing demands for specific blood cells for research, the UCE/TMD decided to expand its capacity to include whole blood collection and processing. A new institute in three levels with 850 m^2 was built to allow the execution of the project of self-sufficiency.

*Data from year 2021

Aims: To describe the project steps at the UCE that will lead to the development of a new self-sustainable blood transfusion service managing its own apheresis and whole blood donor's pool.

Methods: Apheresis collections continue to be executed with the Trima Accel device (Terumo BCT) using disposable 82,300 which enables the collection of single (15%), double platelets (85%) and concurrent plasma. Whole blood manufacturing is being introduced in gradual steps. In phase 1, validation of the full automated whole blood processing with the Reveos/TOMEs (middleware) combination system was performed. For this purpose $N = 26$ whole blood in 450 mL bags were collected. Quality of processed blood components were evaluated in accordance to the Paul- Ehrlich-Institut market approval guidelines, that is, RCC non-irradiated ($N = 14$) at Day 1, 7, 14, 21, 28, 35 and 42 post-processing; RCC gamma-irradiated ($N = 12$) at 30 Gy were evaluated at Day 1, 7,14, 21 and 28. Quality of FFP was controlled at the day of manufacturing, 1 month after storage at −30°C and at expiration (365 days). In this abstract, we will only show the quality results for non-irradiated RCC at Day 1 and 42 and FFP after 365 days of storage at −30°C.

Results: In the new building separate floors are dedicated for allogeneic and autologous collections and finally the third floor is dedicated to blood component processing. A full automated processing system enables rapid training and qualification of technicians to process whole blood ($N = 15$). At day of production RCC had in average 51.8 g haemoglobin/unit, a haematocrit of 59.3% and volume of 276 mL. At day of expiration (Day 42) average haemolysis rate was 0.05%. Plasma (FFP) after freezing and thawing at Day 365 post-fractionation showed in average 99.5% recovery of factor V, 97.4% recovery of Factor VIII, 89.4% recovery of factor XI with zero Leukocytes × 10e9/L and 6 Thrombocytes × 10e9/L.

Summary/Conclusions: A decision has been taken by the UCE to invest in a self-sufficient and sustainable blood collection and processing institute that will allow the regional management of blood donors and blood supply, as well as the support of investigational institutions with blood and blood-derived material in accordance to the regional demands. Automation of whole blood processing together with connectivity to blood bank central information system (BBCIS) guarantees full traceability and adherence to SOP. The preliminary data on quality of the manufactured components are very positive. We will continue with the full routine implementation in the next months.

P044 | Six-month experiences with platelet inventory management by RFID smart storage solution

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Background: Radio Frequency IDentification (RFID) Smart Storage kits for platelet Agitators (SST-A®; Biolog-id) have been implemented on three satellite depots of our Blood Establishment (Service du Sang, SFS) to supply hospital-based blood banks. RFID smart tags were put on platelet concentrates (PC) and encoded by SFS teams with data from barcodes on PC label before shipment to satellite depots. Dedicated software from Biolog-id captures real-time presence of the PC on SST-A® giving immediate inventories view. Data were exported into a data warehouse and a dashboard monitors platelet supply chain.

Aims: Evaluation of RFID technology after 6-month routine use for operational aspects and potential contribution to improve platelet supply chain.

Methods: SST-A® kits were integrated in four platelet agitators PF96i (Helmer) on three satellite depots and connected to a SFS server on which Biolog Data System (BDS) was installed. RFID tags were put on PC before shipment to satellite depots. Donation number, product code, blood group and expiry date (shelf life: 5 days) were automatically read from the label barcodes and encoded in RFID tags by a "One Step Encoder" (OSE) connected to BDS-Encoding software. PC were packed in dedicated transport containers (Delta-T) and shipped according to routine operating procedures. PC were put on SST-A® at reception in depots and BDS-Inventory software was used for real-time inventory monitoring on these distant sites. Data from BDS software were imported daily in data analytics and integration platform (QLK sense®) to create specific dashboards.

Results: 4553 PC were shipped to satellite depots (1 Aug 2022 to 31 Jan 2023). Data for 3721 PC (82%) were captured in BDS software. 5M analysis of root causes for missing data: Material 65%, Workforce 35%. Communication deficiencies between SST-A® and servers (Material), RFID tags not encoded (Workforce), malfunction of OSE (Material) or PC not placed on the agitator (Workforce). Average blood groups distribution in remote depots was 68% group O group and 32% group A. PC exit from SST-A® occurs 55% during morning shift (8 am to 1 pm), 23% during afternoon shift (1 pm to 6 pm) and 22% during evening and night shift (6 pm to 8 am). PC age was day 3

± 1 (mean ± SD) at the entry in SST-A® and day 4 ± 1 at the exit resulting in median storage time of 24 h. First 3 months of inventory monitoring with QLK sense dashboard detected 135 expired PC (6.9%). Detailed analysis of daily inventory levels identified overstock versus PC usage on Mondays (32 ± 9 vs. 23 ± 8; mean ± SD). Adjustment of ordering behaviour during next 3 months we observed 78 (4.5%) expired PC.

Summary/Conclusions: RFID Smart Storage solution evaluation confirmed potential contribution of the technology to PC supply chain improvement. Real-time platelet inventory monitoring in satellite depots associated with data historization warehouse and development of relevant dashboard allowed rebalance of daily inventory levels resulting in 2.4% expiry reduction of PC. Nevertheless, poor data integrity (18% loss of data) mainly caused by Material and Workforce errors biases data analysis and impairs implementation of strategies for real time inventory replenishment. Further optimisation of the process requires improvement of Biolog-id hardware downtime (communication with servers and OSE) and additional operators training. Objective should be >99% on time in real operations for further deployment of the system and reliable routine use.

P045 | Flex capacity: Multiple platelet concentrate preparation processes to maintain inventory level targets and respond promptly to crisis situations

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Background: "Flex capacity" is a production concept aimed at securing supply capacity by relying on more than one production process for a given product. Platelet concentrates (PCs) can benefit from a flex capacity production as they can be prepared by aphaeresis collection (AC_{PC}) or pooling multiple buffy coats from whole blood donations (BC_{PC}). Because these two processes involve different donor populations, their use can be modulated to achieve inventory targets.

Aims: To demonstrate how flexible capacity can address variations in the demand for PCs, such as a sudden and large increase in demand during a crisis situation.

Methods: The performances of three PC production processes (AC_{PC}, BC_{PC1} and BC_{PC2}) were compared. BC_{PC1} and BC_{PC2} use an automated process (Reveos, TerumoBCT, Denver, USA) to pool into one PC unit five ABO-matched, Interim Platelet Units (IPUs) obtained from whole blood (WB) donations. BC_{PC1} is one of the current blood bank production lines using four automated systems operated by fully trained lab technicians. BC_{PC2} represent a one-time response to a significant increase in demand for platelet products which were produced by unexperienced lab technicians operating one system after a 2-day training. For AC_{PC} and BC_{PC1}, performance indicators were extracted from production data generated during the past year at our blood bank. For BC_{PC2}, performance indicators were extracted from the production of 30 PCs. An average of 11 WB units per day were collected over 15 days (total = 165). WB units were processed within 24 h post-collection and two PCs were prepared by pooling ABO-matched IPUs into groups of five. Productivity results for all three processes were normalized to 100 PCs, to ease comparison. For all process PCs, quality markers (platelet concentration, pH, residual white blood cells and sterility) were compared.

Results: The quality control analysis revealed that AC_{PC}, BC_{PC1} and BC_{PC2} respected CSA standards for PC production. AC_{PC} requires approximately 2.25 h for the whole donation and separation process which can produce up to two PCs, for an average of 1.13 h/PC. Steps requiring lab technician assistance for BC_{PC1} and BC_{PC2}, respectively, are WB processing (0.6 and 0.2 h/WB) and pooling (0.3 and 0.3 h/PC). Since five WB units are required per PC, the average lab technician time required is 3.1 and 1.4 h/PC, respectively. To produce 100 PCs, BC_{PC2} is nearly as fast (144 h) per person and apparatus than AC_{PC} (113 h), despite lab technician being only newly trained. The gap between BC_{PC2} and BC_{PC1} (305 h) indicates that productivity is not limited by lab technician long term experience rather than other quality assurance or process documentation tasks.

Summary/Conclusions: AC_{PC} is the most efficient PC production method as it maximises the number of units per collection and the donation frequency. However, WB-derived PCs are more cost effective and can be rapidly scaled up in response to significant increases in blood product demands. The use of an automated system allowed inexperienced technical staff to prepare PCs of equivalent quality after a 2-day training period. This flex production capacity thus entails significant benefits despite the burden associated with maintaining two active production processes.

P046 | Analysis of appropriate indications and outcomes of patients receiving plasma-reduced platelet transfusions

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Background: Severe allergic reactions and anaphylaxis are transfusion reactions that are not uncommonly seen particularly with plasma and platelet products. Patients with prior anaphylactic reactions to plasma or platelet transfusion or repeated severe allergic reactions remain at risk of similar transfusion reactions in subsequent platelet transfusions. Evidence has shown that premedication prior to transfusion does little to prevent such reactions. In 2021, Blood Services Group, Health Sciences Authority, validated a process of preparation of plasma-reduced platelets from apheresis platelets and pooled-platelet. This new product was subsequently made available to hospitals from July 2021.

Aims: This is a quality review of the clinical use, efficacy and safety of plasma-reduced platelet transfusions.

Methods: Hospitals were informed of the availability of plasma-reduced platelets for patients who have a prior history of anaphylactic reactions to plasma and/or platelet transfusions, or recurrent severe allergic reactions to these blood components. Though the units had been plasma reduced, doctors were advised to monitor patients closely for potential allergic transfusion reactions as well as to monitor

post transfusion platelet count in view of risk of platelet activation during plasma reduction process. They were also advised to transfuse platelets within 6 h of preparation. Data on patient's diagnosis, transfusion indication, pre- and post-transfusion platelet count and transfusion reactions were collected for a period of 14 months.

Results: A total of 57 requests for plasma reduced platelets was received for 10 unique patients from 26/7/2021 to 23/9/2022. The patient age groups ranged from paediatric to adults. The patients had various primary diagnoses though a majority had underlying Haematological conditions and required more than one episode of plasma-reduced platelet transfusion. All patients for whom plasma-reduced platelets were requested had prior allergic reactions to platelet transfusions. Platelets were transfused for both prophylactic and therapeutic indications.

Pre-transfusion counts were documented for all 57 transfusions. Post transfusion platelet counts were documented for 50 transfusions. 42 out of 50 transfusions were noted to have post transfusion platelet increment out of which 93% had an increment of more than 10×10^9/L. For patients who had an increment of less than 10×10^9/L or a decrease in post transfusion platelet count, this could be related to underlying diagnosis or disease process. The same patients who had a decrease in post transfusion platelet counts also had good increments on other occasions. One patient had an equivocal platelet count after 2 out of his 3 transfusions, this patient had Myelodysplatic Syndrome and likely had underlying platelet refractoriness. 3 out of 57 transfusions were associated with mild allergic reactions. There were otherwise no reported anaphylactic reactions or severe allergic reactions. 1 patient was suspected to have a febrile non haemolytic transfusion reaction.

Summary/Conclusions: The results demonstrate that a majority of the plasma-reduced platelet transfusions had an acceptable platelet increment and there was no pattern of poor increment noted. Mild allergic reactions were seen in 5% of the transfusions and there were no reports of severe allergic or anaphylactic reactions. Plasma-reduced platelets are a safe and effective alternative for patients with prior anaphylaxis or severe allergic reaction to plasma products.

P047 | Transfusion of blood products according to NICE guideline thresholds: A clinical audit in a United Kingdom district general hospital

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Background: The NHS Blood and Transplant Group released an amber alert for supply shortages of blood products in the United Kingdom. Following this the Royal College of Surgeons England issued guidance for blood transfusion thresholds, usage of tranexamic acid, and in managing anaemia in the perioperative pathway for surgical patients. Frequently surgical patients are transfused blood products unnecessarily above published thresholds resulting in significant cost, risk of avoidable adverse events, and unnecessary increased nursing and administrative time.

Aims: To audit adherence of transfusion of blood products against NICE guideline thresholds. To review if alternative methods to reduce transfusion burden, such as administration of iron or tranexamic acid, were considered.

Methods: We retrospectively audited all patients receiving a blood transfusion consecutively across a 4-month period in a general surgery department at a district general hospital. The medical notes for each patient were assessed for their pre-transfusion haemoglobin, indication for transfusion, and whether transfusion was prescribed according to NICE guidelines. Data was collected on whether intravenous iron or tranexamic acid were administered, whether iron studies were measured, and whether any adverse blood transfusion reactions occurred.

Results: A total of 112 units of packed red blood cells were given across 42 patients. 45 units (40.2%) were prescribed according to NICE guidance and a total of two self-limiting transfusion-related reactions (1.79%) occurred, both in patients transfused above guidance thresholds. Iron studies were measured in 30 patients (71.4%), 6 patients (5.36%) received iron transfusions, and 17 patients (40.5%) received tranexamic acid over their admission. At a raw cost of approximately £135 per unit of donated blood, this corresponded to an additional £9045 over a 4-month period (£27,135 pa), not including nursing and administration costs.

Summary/Conclusions: Stricter adherence to clear transfusion thresholds has scope to help reduce blood transfusions, complications and hospital costs. Our unit is currently establishing a better pathway to identify, investigate and treat anaemia earlier, to minimise intraoperative blood loss with tranexamic acid, and to reduce above-threshold blood transfusions.

P048 | Abstract withdrawn

P049 | Demographics of transfusion recipients at Landspítali National University Hospital of Iceland in 2021

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Background: Blood transfusion is an essential part of modern medicine. Due to the fast-changing demographics of the Icelandic nation, it is crucial to map out the demographics of Icelandic blood recipients to better predict changes in the demand for blood components.

Aims: To carry out preliminary research to describe the demographics of blood recipients at Landspítali University Hospital, considering age, gender, medical department, and transfusion load.

P049 - Table 1. Summary of the age distribution of blood recipients categorized by Medical Field.

Medical field	Q1/Q3	Median	Mean (±SD)	% *n*
Paediatric ward	0/3	0	3.1 (±5.4)	4.7%
Emergency	60/83	74	69.7 (±17.4)	17.5%
ICU	53/75	66	60.8 (±21.4)	10.0%
Cardiology and vascular	67/82.5	76	73.8 (±13.1)	6.6%
Cancer care	59/76	67	65.6 (±15.1)	13.2%
Medicine	63/84	74	72.6 (±16.1)	8.9%
Surgical wards	65/84	75	72.2 (±16.0)	19.5%
Operating rooms	46/79	68	61.7 (±21.9)	9.5%
Geriatric care	77/91	86	84.0 (±7.8)	1.1%

Methods: Transfusion data from Landspítali in 2021 was collected via ProSang Statistics. The hospital's wards were sorted into descriptive medical departments. Blood recipients were categorized by age, gender, medical field and transfusion load.

Results: Most blood recipients were 50–80 years old, their average age was 62.8 years [± 23.5]. Women were 54% of red blood cell concentrates (RBC) recipients, 38% of plasma recipients and 34% of platelet recipients. Most recipients were transfused at surgical wards (19%) or emergency rooms (17%). Combining all blood components, 33% went to cancer wards, 12% to geriatric wards and 12% to medicine wards. Oncology- and cancer wards transfused 65% of all platelet units and medicine wards transfused 33% of all plasma units. Most recipients only received RBCs. The number of transfused RBCs was one (15%), two (35%), or three or more (50%). Only 3.34% of all blood recipients received all three types of blood components.

Summary/Conclusions: The average age of blood recipients was high. Many elderly patients received transfusions in the emergency room (average age 69.7 ± 17.4). The age distribution of blood recipients was the opposite of the Icelandic nation. Most blood recipients received two RBCs. Oncology and cancer wards used significantly more blood components than other medical departments.

P050 | Blood usage at Landspítali National University Hospital of Iceland in 2012–2022

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Background: Blood transfusion is an essential part of modern medicine. Due to the fast-changing population of Iceland, it is crucial to monitor blood usage to better predict changes in the demand for blood components.

Aims: To describe and analyse blood usage at Landspítali University Hospital during the years 2012–2022 regarding changes in population numbers.

Methods: Transfusion data from Landspítali in 2012–2022 were collected via ProSang Statistics. The data were categorized by type of blood component. Population numbers were collected from the Icelandic Bureau of Statistics. Transfusion rates were age-standardised using a direct method and a standard Northern European population. A Changepoint Analysis was then carried out in RStudio.

Results: Age-standardised transfusion rates of red blood cell concentrates (RBCs) decreased by 36% from 2012 to 2022, plasma by 66%, and platelets by 1%. Since 2020 the age-standardised rates for RBC transfusions have increased by 9%, plasma by 16%, and platelets by 43%. In all cases, a change point was detected in 2021 where the decrease in usage came to halt and a significant increase was detected. $p < 0.05$ in all cases (Student's *t*-test).

Summary/Conclusions: Blood usage decreased from 2012 to 2020 when there was a sharp turnaround and the usage increased, especially for platelets, for unknown reasons. The Icelandic Blood bank has responded to that increase by increasing the number of whole blood collections and apheresis platelet collections. Since 2020 the production of platelet units in Iceland has increased by 48%. Due to the ageing and growth of the Icelandic population, blood usage will most likely continue to increase.

P051 | Management of O-negative packed red blood cell stock before and after the COVID-19 pandemic: A report from the blood center of Sfax, Tunisia

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Background: Our blood centre is the only transfusion facility in the city of Sfax, the second largest Tunisian city (1 million inhabitants). We manage up to 30,000 blood donors per year from whom over 90% are replacement donors. We also manage a unique bloodstock (BS) for the whole region.

Aims: Our aim is to assess and discuss O-negative packed red blood cell BS (ON-BS) management in our setting.

Methods: We have prospectively monitored weekly, monthly and yearly BS indicators over 5 years (2018–2022). The years were, then, divided into four seasons. Total BS (TBS) and BS as per ABO/Rh D blood groups are monitored once a week on Mondays. Monthly TBS and ON-BS were calculated as means of weekly BS. The other assessed indicators were: monthly out-of-date PRBC units and monthly number of Rh-D negative patients transfused with RH D positive RPBC (O+ to ON). Statistics were performed on SPSS 20.0. Significance was set at 5%.

Results: The overall monthly average of TBS was 1644 PRBC, and varied from 988 during May 2020 to 2538 during May 2018. The

overall monthly average of ON-RPBC stock was 52 units and varied from 24 to 113 units or 3.3% of TBS (from 1.2% to 6.7%). ON-RPBC varied significantly with seasons. It was at its best during the summer of 2020 (mean = 5.1%; SD = 1.4) and at its minimum during the winter of 2018 (mean = 1.7%; SD = 0.3; $p < 0.0001$). It was significantly higher during COVID-19 years (mean = 4.2%; SD 1.2%) versus 2.7% SD = 1.1% during non-COVID-19 years ($p < 0.0001$). The overall out-of-date rate was 5% and monthly varied from 2% during January 2022 to 28% during Avril 2020. A total of 117 O+ to ON transfusions or 8.3% of ON-RPBC stock were performed (min = 0% during autumn 2021 and max = 22.9% of ON-RPBC minimal stock during winter 2018). Out-of-date rates and O+ to ON transfusions did not vary with any studied indicators (p from 0.1 to 0.5).

Summary/Conclusions: In our setting, blood is donated by random blood group replacement donors. To ensure the availability of rare blood groups including negative ones, we have to set up large TBS. During, the COVID-19 pandemic, large TBS and a decline in the health field resulted in very high out-of-date rates mainly during curfew peak (May 2020), but also in the unusual availability of ON stocks. So, Rhesus D transfusions weren't negatively impacted. We succeed to guarantee a minimal ON-BS for multi-transfused patients and young females (>10 units) at the price of a high rate of blood wastage. This minimal stock needs to be standardised. The rare group including ON donors should be retained as a matter of priority to minimise blood wastage.

P052 | Provision of HPA-1a negative platelets in Ireland: A national service evaluation

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Background: Fetoneonatal alloimmune thrombocytopaenia (FNAIT) is a rare but serious condition, occurring in up to 1 in 1500 pregnancies. (Kamphuis BJOG, 2010). The Irish Blood Transfusion service (IBTS) aims to provide HPA-1a negative platelets at all times for emergency treatment of suspected cases. To ensure supply, single donor donations are scheduled on a twice weekly basis that is, Mondays and Thursday or Tuesdays and Fridays. Platelets take approximately 2.5 days for processing (collection, irradiation, serological, microbiological testing). Platelet donations are not split into paedipacks as this gives a 6 h shelf. Although an effort is made to re-circulate surplus platelets that is, when two donations are on shelf, no official policy is currently in place.

24 cases of suspected HPA-1a were referred to the IBTS for investigation in Jan 2021–Jan 2022. Four of these were positive that is, 16% of referred cases. The results were confirmed by platelet antigen genotyping of mother, performed external to our institution.

Aims: This service evaluation reviews service efficacy and usage of surplus product.

Methods: Data pertaining to the HPA-1a negative donor panel was extracted from the Bleed Establishments electronic system, from January 2021 to December 2022. Variables reviewed included age, CMV status, donation frequency and site of distribution of platelets. Donations were reviewed using the electronic record system, eProgesa.

Results: 27 donors were listed on the panel between January 2021 and December 2022. The median age was 45 years (range 28–67) and the majority of donors (70%) were CMV negative.

There were 115 donations in 2021 and 90 donations in 2022. On 9 occasions in the 24 months, a donation could not be obtained. The reasons for unavailability were medical deferral, planned unavailability and unavailability at short notice. There are 110 scheduled donations per year. Therefore, scheduled donations took place as planned 96% of the time.

The mean number of donations was 4.03. Of note mean donation of standard apheresis platelets was 4.9 in December 2021 and 4.7 in 2022. The donation at interval was no less than 28 days. The range of donations given was 1–10.

The majority of donations were discarded (73%).

Summary/Conclusions: This study provided useful information concerning future improvements of our service.

Firstly, that donors are highly committed and that most planned donations took place. Secondly and consequently, the desired service is provided most (96%) of the time.

Thirdly, a large proportion of the donated platelets are discarded. The primary aim of the service is to treated affected neonates and foetuses, however we are committed to utilise the gift of the donors optimally. Blood is a finite and precious product, and a consequence of this audit, an effort will be made to formalise a policy on how to recirculate the donated units while maintaining the current level of service. Barriers to this may include the small donor panel, the duration of processing time of platelets (approximately 2.5 days) and the short shelf life of platelets meaning that the overlap period is typically of one day only. Possible solutions might include releasing on of a triple donation, or splitting the donations in the paedipacks when requested.

P053 | The COVID-19 pandemic and the demand for blood in South Africa

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Background: In response to the COVID-19 pandemic, South Africa instituted various levels of national lockdowns which restricted the movement of the populace, alcohol and tobacco consumption and mandated mask-wearing. These measures significantly impacted both the South African National Blood Service's (SANBS) ability to collect

blood as well as the demand for blood products by the two healthcare systems in South Africa. The extent of the impact on red blood cell utilisation (RBC) is not known.

Aims: To assess the impact of the COVID-19 pandemic on RBC demand in the public and private healthcare sectors in the eight provinces of South Africa serviced by SANBS.

Methods: We conducted a retrospective analysis of adult RBC products issued during two distinct periods; the Pre-COVID (the ~15 months prior to the initial COVID lockdown–1 January 2019 to 26 March 2020) and the COVID period (the ~18 months from 27 March 2020 to lifting on the national mask mandate on 17 August 2021). Analysis was stratified by healthcare sector. The public sector, funded exclusively by the National Department of Health, services approximately 85% of the population.

Results: Collectively, a total of 2,150,197 RBC units were issued over the two periods. Approximately 40% of the units were issued to the private sector. Compared to the same period in 2019, demand decreased by approximately 17% in both sectors in April 2020 (the start of the first lockdown and the first cases in South Africa), and was sustained through August 2020 in the public sector (−14%) with a more robust rebound over the same period in the private sector (−4%). The Beta wave was associated with an ~22% decline in RBC demand in January 2021, but only a ~9% decline in the private sector when compared to the same period in 2019. Similarly, demand showed a sustained decrease in the public sector during the Delta wave in July 2021 but with a smaller decrease in the private sector (−15% vs. −5%). Between waves, the private sector demand normalised rapidly while the demand in the public sector only fully normalised from March 2022 where after demand outstripped that of the pre-COVID period. Demand in the private sector fully normalised from December 2021.

Summary/Conclusions: The COVID-19 pandemic had a significant impact on RBC demand in South Africa. The well-resourced private sector was less affected than the resource-constrained public sector. The limited opportunity of the population utilising the public sector to safely isolate and their greater difficulty accessing health care may have contributed to this disparity. In addition, reports suggested that public sector healthcare workers were more affected by the epidemic than their private sector counterparts. SANBS's ability to successfully meet RBC demand during COVID was in part due to a concomitant decline in demand during the times our ability to collect blood was most severely constrained. This may not have been the case if the public sector had the same resilience displayed in the private sector.

Management and organisation

Quality management

P054 | The relationship analysis of thrombocyte concentrates storage duration with haemostasis function using thromboelastographic

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Background: Thrombocyte Concentrates (TC) during storage can undergo functional, biochemical, and structural platelet changes. One of the main functions of platelets is in the process of haemostasis. Thromboelastographic (TEG) is a method to assess the haemostasis process, and several parameters of TEG reflect the number and function of platelets.

Aims: The aim of the study was to analyse the relationship between storage duration of TC and haemostatic function using TEG. A prospective cohort observational study using TC product samples. Products were excluded if the platelet count per bag unit was $<50 \times 10^9$. The number of samples in the study were 14 samples. Platelet count was examined by haematology analyser, and haemostatic function was examined by TEG on storage day-1, day-3, and day-5. The data were statistically analysed by the Saphiro-Wilk test, Repeated ANOVA, Friedman and Pearson

Methods: The results showed that the K time, angle, MA value, and G value were not significantly different between storage day-1, day-3, and day-5 (p values were respectively 0.204; 0.265; 0.215; 0.236). Platelet count did not correlate with MA and G values on TC products stored on day-1, day-3, and day-5. It was concluded that the haemostatic function using TEG was not significantly different in TC products between storage day-1, day-3, and day-5. TC products are suitable for use up to 5 days of storage.

Results: The results showed that the K time, angle, MA value, and G value were not significantly different between storage day-1, day-3, and day-5 (p values were respectively 0.204; 0.265; 0.215; 0.236). Platelet count did not correlate with MA and G values on TC products stored on day-1, day-3, and day-5.

Summary/Conclusions: It was concluded that the haemostatic function using TEG was not significantly different in TC products between storage day-1, day-3, and day-5. Thrombocyte Concentrates products are suitable for use up to 5 days of storage.

P055 | Practice of computer system confirmation in Zhejiang blood center

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Background: ISBT has updated and released three versions of the confirmation specification for automatic system in blood institutions, which was first published in 2003, and again in 2010, and the latest version in 2022. It can be seen that ISBT has formed a relatively complete and perfect scheme for the confirmation of computer system in blood stations.

Aims: To learn from the requirements of "ISBT Guidelines for Validation of Automated Systems in Blood Establishments", and to conduct research and practice on the confirmation of the computer system of the blood bank.

Methods: According to the requirements of ISBT, the unit carried out the confirmation work of the computer system of blood bank. The confirmation team was established, which was composed of blood bank management personnel, business personnel, quality personnel, information technology personnel and product or service providers. The confirmation objects include new or changed processes, procedures, key equipment, key materials, methods and information systems, and so forth. The purpose of confirmation is to ensure that the intended use requirements are met prior to formal use. Taking the confirmation of blood collection and supply business system as an example, the main confirmation process is as follows: The business department (user) prepares the user requirements specification by referring to ISO/IEC, and prepares the confirmation plan, which defines the purpose, content, method of confirmation, and other departments or providers involved in the system. The department (personnel) or information technology personnel (including the supplier) are used to carry out the confirmation process from the qualification of the supplier, installation, operation and performance of the system. Finally, the quality management department will summarise and analyse the results of the implementation of each link, prepare the confirmation report, confirm the conclusions and existing problems clearly in the confirmation report, and propose solutions to the problems. The management personnel shall approve the confirmation plan and the confirmation report.

Results: Through practice, according to the requirements of "ISBT Guidelines for Validation of Automated Systems in Blood Establishments", it can be applied to the confirmation work of the computer system of the unit.

Summary/Conclusions: Validation of computer systems in blood stations is not only a technical problem, but also a management problem. In order to carry out the confirmation work effectively, it needs the full support of the top management of the unit, but also needs the participation and cooperation of all the staff. At the national level, a unified confirmation standard should also be established to further improve the computer management level of blood collection and supply institutions.

P056 | Measure less–save more

A temperature project

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Background: The Department of Clinical Immunology consists of six laboratories at five different hospitals in the Capital Region of Denmark. The department has refrigerators, freezers and rooms, and so forth that needs to adhere to specific temperature requirements. For that purpose, temperature sensors (sensors, loggers), either built-in or external, are used.

Control measurements of the temperature sensors opposite a certified thermometer is done yearly by employees at the University Hospital of Copenhagen–Rigshospitalet and external contractors at the other hospitals in the Capital Region. This is time consuming at Rigshospitalet and costly due to the external contractors used by the other hospitals in the region.

By reviewing historic data, we wish to examine if there is ground for an expansion of the time period between control measurements to three years instead of yearly measurements. This would give the employees more time to perform other tasks and ultimately result in cost savings.

Aims: The projects aim is to examine how many temperature sensors were adjusted or replaced in the period due to a direct result of the control measurements.

Methods: The project relies on a quantitative method to analyse data from the period 2018–2022. There must be at least three years of data for a sensor to be included in the study.

Results: Of the 235 sensors included in the study, only 10 were adjusted or replaced in the period (see Table 1) as a direct result of the control measurements.

Summary/Conclusions: Of the 235 sensors included in the study 10 was adjusted or replaced in the period 2018–2022 as a direct result of the control measurements.

The sensors are stable and the size of the deviations between the sensors and the certified thermometers do not accumulate year by

P056 - Table 1:

Rigshospitalet	93 sensors included, 2 adjusted or replaced
Herlev Hospital	53 sensors included, 1 adjusted or replaced
Hillerød Hospital	29 sensors included, 3 adjusted or replaced
Tissue Typing Laboratory	60 sensors included, 4 adjusted or replaced

Note: During a 10-year period, a saving of 1.2 million DKK is estimated and 211 workdays are liberated, if we control measure every three years as opposed to every year.

year even if the sensors aren't adjusted. So, there is no evidence to suggest that it is beneficial to control measure every year as opposed to every second or third year. The question ultimately comes down to whether the deviation is discovered within an acceptable timeframe?

The risk of control measuring every third year could be that refrigerators containing critical material would deviate from the specified and/or acceptable temperature requirements.

The relatively largest deviation found in the 5-year period is 1.3°C (refrigerator). A significant deviation for a refrigerator already close to the limits of your chosen temperature area. However, considering the possible savings in costs and workdays you would gain more than you risk by control measuring every third year as opposed to yearly. You can continue to control measure every year in places where you have sensitive reagents or instruments.

P057 | External quality assessment scheme for transfusion transmitted infections screening in Indonesian Red Cross Blood Transfusion Services in 2022

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Background: Indonesian Red Cross Central Blood Transfusion Services had already started the External Quality Assessment Scheme for Transfusion Transmitted Infections Screening since 2003 with the aim to raise the standards of quality of TTI testing in Indonesia. But the data had not been well evaluated and monitored.

Aims: The aim of the External Quality Assessment Scheme is to improve the quality of Transfusion Transmitted Infection Screening in Indonesian Red Cross Blood Services.

Methods: In 2022, blinded test panels, each consisted of eight panels with some samples reactive for HIV, HCV, HBV and Syphilis test were sent to participating Blood Services. All data was submitted through an online program managed by Indonesian Red Cross Central Blood Transfusion Services. The result from all the participants were analysed and evaluated with the reference result of Central Blood Transfusion Services.

Results: 116 (50%) of 232 Indonesian Red Cross Blood Services participated in the External Quality Assessment Scheme in 2022. One participant didn't send any data and was excluded. 19 (16.5%) participants tested the TTI Screening with rapid test, 4 (3.5%) with ELISA, and 92 (80%) with ChLIA. All rapid and ELISA test participants gave a concordant result, while 11 of 92 ChLIA participants reported aberrant results. Some of the aberrant results reported were either due to recording error or using the non-recommended reagent, while some others were unidentified.

Summary/Conclusions: The findings during External Quality Assessment Scheme should be evaluated and monitored. Some Blood Services with aberrant results should be monitored and if possible be consolidated with other Blood Services.

P058 | Evaluation of the haemocue plasma/low Hb system in central blood transfusion services Indonesian red cross

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Background: Haemocue Plasma Low/Hb System is one of the equipment used in blood component quality control testing, this equipment serves to determine the haemoglobin level that may be present in plasma, serum, fluid exposed to erythrocytes, to determine the level of haemolysis (free haemoglobin) in stored blood at random and to compensate for cloudy samples.

Aims: To evaluate Haemocue Plasma Low/Hb System using the Central Blood Transfusion Services–Indonesian Red Cross established protocol to verify whether it can be used for quality control testing at Blood Center. Furthermore, the evaluation results can be used as a reference for other Indonesian Red Cross Blood Center.

Methods: Run the sample with Control level 1 Plasma/Low Hb Control level 1.0, Control level 2 Plasma/Low Hb Control level 5.0, and Control level 3 Plasma/Low Hb Control level 20.0. Precision testing was done using 1 sample from each level, run 10 times within a run and run 5 days (between day). And we used 50 samples EDTA run comparing the results of Hb levels in plasma between the Haemocue Plasma Low/Hb and the haematology analyser (HA) as the gold standard in this case we using Sysmex XN-350. To the statistical data using linear regression test and the Bland Altman test.

Results: Based on the results of the precision test on within run on the Plasma Low/Hb control, it was found that the CV respectively from levels 1, 2 and 3 were 10.69%, 1.66% and 0.29%. Precision test results for repeatability with Plasma Low/Hb control at the three control levels 1, 2, and 3 were 0%, 0% and 0.22%. A comparison test of the Haemocue Plasma Low/Hb and the haematology analyser using Linear Regression which showed that there was a high correlation $R2 = 0.9879$. The Bland Altman test showed that the interval between the differences between the two methods was 0.15 (upper limit) and −0.04 (lower limit) and the mean difference between Haemocue Plasma Low/Hb and Sysmex XN-350 was 0.55 g/dL.

Summary/Conclusions: Based on the results of the Haemocue Plasma Low/Hb evaluation test, the equipment can be used as an alternative method in determining low Hb values in plasma. The Hb value in plasma is an indication of the occurrence of lysis in products that have entered their shelf life (expiration date) so that they can determine the percentage value of haemolysis of the blood product which is one of the specification requirements in the blood product quality test which is <0.8%.

P059 | Evaluation of a chemiluminescence immunoassay analyser and anti-HCV, HBsAg and Syphilis immunoassays in central blood transfusion services Indonesian Red Cross

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Background: In Blood Center, testing all blood donations for markers of infectious diseases plays an important role in maintaining the safety of blood transfusions. Mandatory serological testing in Indonesia is performed for HIV, HBV, HCV and Syphilis. Highly specificity and sensitivity tests with corresponding automation are essential for this purpose. The Mindray CL-2000I is a fully automated immunoassay analyser designed to generate up to 240 tests per hour and can be used infectious disease parameters to screen donor blood samples.

Aims: To evaluate Mindray CL-2000I and anti-HCV, HBsAg and Syphilis assays performance in terms of sensitivity and specificity using the Central Blood Transfusion Services–Indonesian Red Cross (CBTS–IRC) established protocol to verify whether it can be used for donor blood screening at Blood Center. Furthermore, the evaluation results can be used as a reference for donor blood screening checks at other Indonesian Red Cross Blood Center.

Methods: Total of 1.184 characterised samples (500 negative, 270 HBsAg positive, 164 anti-HCV positive and 250 Syphilis positive) were included for evaluation of which minimum 20% of total samples were included in Lot to Lot verification using three different reagent lots. Samples have also been confirmed by NAT testing. Precision testing was done using one positive sample from each parameter and run 10 times within a run. Precision results with the target acceptance criteria of CV ≤5%. Linearity was tested using characterised positive sample dilution. Sensitivity and specificity was calculated with acceptance criteria as follows: (1) HBsAg: Sensitivity >99.5%, specificity ≥99,8%; (2) Anti-HCV: Sensitivity >99.5%, specificity ≥99.8%; (3) Syphilis: Sensitivity >99.5%, specificity ≥99.8%.

Results: The clinical sensitivity and specificity for the evaluated three screening assays using one reagent lot was found to be (1) HBsAg: Sensitivity 100%, specificity 100%; (2) Anti-HCV: Sensitivity 100%, specificity 99.8%; (3) Syphilis: Sensitivity 100%, specificity 100%. Similarly, lot to lot verification was within acceptable limits. Precision (% CV) for the three screening assays (HbsAg 2.7%; Anti-HCV 4.53% and Syphilis 1.67%) was within the acceptance criteria of CV ≤ 5%. Linearity study showed good results.

Summary/Conclusions: In our evaluation, the Mindray CL-2000I was found to be suitable for blood donor screening for HBsAg, anti-HCV and Syphilis assays in Indonesian Red Cross Blood Center.

P060 | Quality validation of the new apheresis system for double dose platelet collection at Maharaj Nakorn Chiang Mai hospital

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Background: The new apheresis system (Fresenius Kabi) is a new device designed for the collection of leucocyte-reduced single-donor platelets. Leucocyte-reduced platelet apheresis (LRPA) with high platelet yield will help to provide superior quality platelets to the patients.

Aims: The aims of this study are to validate the performance and efficiency of the new apheresis system with software version 2.1J for collection of double dose platelets (DDP).

Methods: Thirty eligible repeat platelet donors were recruited for DDP donation. The targeted platelet yield was 6.6×10^{11} collected with platelet additive solution (PAS)–InterSol (Fresenius Kabi). The PAS to Plasma ratio in the final product was kept at 65: 35. Donor pre- and post-donation parameters and procedure characteristics were analysed. The platelet products were assessed twice (day 0 and day 1). Quality parameters measured included platelet volume, swirling and aggregation at day 0 together with platelet yield, swirling, aggregation and residual WBCs at day 1.

Results: All the recruited donors had an average pre-procedure platelet count of $312 \pm 45 \times 10^3$ μL and average post-donation platelet count $183 \pm 39 \times 10^3$ μL. The average collection time was 92 ± 15 min. No adverse reactions were reported during any of the 30 procedures. Platelet volume averaged 538 ± 1 mL. The DDP collections had an average platelet collection efficiency of 83.20 ± 6.33%. The average platelet yield was $7.55 \pm 0.60 \times 10^{11}$ and actual to targeted platelet yield ratios was 1.114 ± 0.09. All the DDP products had residual WBCs less than 1×10^6/unit (averaged $0.26 \pm 0.26 \times 10^6$ /unit) with quality swirling and no aggregation.

Summary/Conclusions: The new apheresis system provided satisfactory platelet yields and collection efficiency, with leucocyte-reduced platelets. The platelet products had acceptable characteristics for local and international standard requirements. This apheresis system is now routinely used in our hospital for collecting double dose platelets.

P061 | Abstract withdrawn

P062 | Using Taiwan laboratory indicator series to enhance the quality of laboratory services and patient safety

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Background: With the growth in technological developments, the need to control and improve quality in clinical laboratories has grown. Similar to its counterparts around the world, Taiwan's health care institution continually strives to improve quality by various means. Taiwan Society of Laboratory Medicine (TSLM) also developed Taiwan Laboratory Indicator Series (TLIS) to provide to various clinical laboratories as reference targets for improvement.

Aims: This study was aimed at using the TLIS to understand the differences of Quality Indicators (QIs) with peers and enhance the quality of laboratory services and patient safety.

Methods: A retrospective study was conducted on the data retrieved from TLIS platform during the years 2017–2022. Setup expected goal with below the average time of the peers for four QIs including rates of specimen identification errors (0.0121%), mean time of preliminary blood culture reports (31.8 h), mean time of final blood culture reports (73.3 h) and the 90th percentile time of completing monthly critical-valued reports in outpatients (113 min). Analyse the reasons for the poor performance of QIs and take improved actions such as using unique identification code labels, adjusting the positive blood culture smear from the afternoon to the morning and outpatient blood potassium samples are drawn with PST tubes and testing turn of samples after STAT and before general samples. We compared the data for two intervals between 2017–2019 and 2020–2022.

Results: Overall, the annual rates of specimen identification errors, mean time of preliminary blood culture reports, mean time of final blood culture reports, and the 90th percentile time of completing monthly critical-valued reports in outpatients issued from 0.0118%, 35.8 h, 78.2 h, 170 min between 2017 and 2019, to 0.0042%, 29.3 h, 67.7 h, 55 min between 2020 and 2022 (all $P < 0.01$).

Summary/Conclusions: Using TLIS platform can obtain difference of QIs information between own laboratory and peers and have a quick and significant impact on improving the quality of laboratory performance and enhancing the patient safety.

P063 | Abstract withdrawn

P064 | Longitudinal monitoring of median haemoglobin concentration reveals changes depending on external maintenance

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Background: Haemoglobin concentration of blood donors is among the mainstays of donor clearance. Its biological variation is accepted, but technical variation because of apparatus calibration, firmware updates or mechanical alteration is usually disregarded.

Aims: Our aim was to retrospectively determine the impact of technical variations on haemoglobin concentration.

Methods: We analysed all outpatient haemoglobin concentrations from four laboratories (Amedes Raubling, Fürstenfeldbruck, Munich, Raunheim) equipped with two types of analysers (Abbott Alinity h, Sysmex XN, all quality control approved and DAkkS-certified) from July, 1 2021 to June, 30 2022 ($n = 747{,}250$), calculated daily medians for each machine, rolling 7-day means thereof, and Z-transformed these. Loess regressions were used for evaluations.

Results: Haemoglobin concentrations varied by season, with higher values in winter and lower during Christmas and other holidays, most likely due to differences in patient composition. Device-related median values ranged from 13.0 to 14.4 g/dL. Variations of individual devices could be detected from seasonal variations after Z-transformation. Significant deviations (>2) were found in five of nine instruments (56 %) with smoothed differences of up to 0.5 g/dL. All cases had not been detected in internal and external quality controls.

Summary/Conclusions: Analytical quality control in haematology, by monitoring temporal trends in instrument daily medians, can detect abnormalities due to calibrations by external technicians, updating of instrument firmware, and gradual mechanical deterioration with drifts not revealed by quality control samples and interlaboratory tests.

P065 | Abstract withdrawn

P066 | Abstract withdrawn

P067 | Development of a total quality system in the institute for transfusion medicine in Republic of North Macedonia

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Background: Development and implementation of Quality Management System (QMS) is essential in order to increase the safety and quality of the blood and blood components.

Aims: Aim of this work is to show our experience in the development of the total quality system in the Institute for Transfusion medicine in Republic of North Macedonia (ITM-RNM).

Methods: The QMS includes policy, organizational structure, responsibilities, processes, procedures and resources established by the management to achieve and maintain quality.

Results: ITM-RNM is working according the actual national legislative, the law for blood safety (Official Gazette, September 2007) and its by-laws for safe and quality collection, processing and testing of blood, storage and distribution of the blood components that was made according the European Directives for blood. Reorganization and centralization of all transfusion services in the hospitals throughout the country was made in 2010, and the current structure of the Institute for Transfusion medicine-RNM is unified system of working standards, procedures and equipment. The results from the work of Quality Assurance and Quality Control (QAQC) department in ITM is represented with: total number of blood donations, list of processed blood components, incidence of TTI, number of recalled components, performed quality control of blood and blood components, number of reported serious adverse events and reactions, Quality manual (according ISO 9001:2015), revised standard operational procedures, registration of non-conformities, taken corrective and preventive measures, carried out internal and external audits, ISO 9001:2015 accreditation and reaccreditation, EDQM audits, and so forth.

Summary/Conclusions: Increased progress in transfusion medicine have imposed implementation of total QMS in Blood transfusion services. For all processes in the blood establishments, quality indicators should be defined, monitored, analysed, reported and finally implemented. A quality system should include complex management of the risk and safety in the blood establishments and should ensure that no part of the transfusion chain is lacking in quality.

P068 | Real-time comparative monitoring of daily QC in TTI laboratories

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Background: With assays as sensitive and specific as they have become, one of the greatest variables in donor testing is now in the laboratory itself. Ensuring the quality of the results is a critical component of the process. Laboratories tend to rely on checking the daily quality control results, usually plotting a Levy-Jennings graph, and cross-checking external quality assurance results to validate performance.

Aims: We wanted to look at the feasibility and utility of implementing a monitoring system that is widely used in other laboratories that facilitates monitoring the daily quality control samples and compares them in real time with other anonymised/peer group laboratories.

Methods: After reviewing the market, we implemented the Biorad Unity Interlaboratory program, based on the breadth of testing that it covers, with non-Biorad reagents included. Data is uploaded automatically. The results of daily quality control testing together with lot numbers of reagents, calibrators and individual instruments and sites are collated and the results can be presented graphically for review in real-time. Quality control (QC) results are compared with other peer laboratories, using the same reagents, instrument platform and lot of QC material.

Results: Implementation was relatively straightforward. The system has provided a central repository for multiple analysers, including from our organisation's sister laboratory. We have been able to follow how the QC material performs across multiple assays. Having readily accessible daily data has assisted in interpreting external quality assurance data, supporting accreditation by regulators and giving confidence in the results being produced. Monthly reports are comprehensive and easy to understand.

Summary/Conclusions: The implementation of real-time comparative quality monitoring system has improved the confidence in our results and given early warning signals to pre-empt problems through good selection of QC rules. The ease of use and visibility that the system has provided has led to better staff engagement with QC. This has increased the awareness amongst staff of good QC practice. It has also enabled streamlining of our QC program by facilitating selection of better QC materials. In addition, since our lab has a sister lab some distance away, we can see in real time how that lab is performing. The next step will be to engage management in oversight of laboratory performance.

On a wider scale we would like to share our QC performance with other blood transfusion services around the world through participation in affiliated reports that can show interlab performance between participants.

Management and organisation

Social, legal, ethics blood donation and transfusion

P069 | Changes in demography and infectious disease prevalence in US blood donors during the COVID-19 pandemic on behalf of the US TTIMS Program

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Background: The COVID-19 pandemic disrupted the operations of U.S. blood supply systems. The Transfusion Transmissible Infections Monitoring System (TTIMS) uses data from four major blood collection organizations (American Red Cross, New York Blood Center Enterprises, OneBlood, and Vitalant) to monitor demographic and infectious disease trends and represents approximately 60% of the US blood supply. We compared demography and infectious disease prevalence before and during the pandemic using TTIMS data.

Aims: To evaluate the COVID-19 pandemic impact on donor characteristics and infectious disease prevalence in the U.S. blood supply.

Methods: Data were categorized as before (March 2018–February 2020) or during (March 2020–February 2022) COVID-19; each period 24 months. Allogeneic, directed and COVID-19 convalescent plasma donations were included. Sex, age group, race/ethnicity, first-time donation status, blood type and U.S. Census region were compared for the two time periods; HIV, HBV and HCV prevalence was also compared. The chi squared test assessed heterogeneity in demographic characteristics and logistic regression predicted the odds of different infectious disease prevalence before versus during the pandemic.

Results: Overall, there were 26,804,093 donations; 13,430,595 before COVID-19 (2.32 donations/donor) and 13,373,498 during COVID-19 (2.64 donations/donor): a 0.43% reduction. There were significantly more donations from female donors (difference of 4.55%), those aged 40 and older (difference of 9.53%), white (difference of 4.68%), and those with repeat donations (difference of 3.76%) during COVID-19 versus before. The age difference likely reflects cancellations of high school drives during the COVID-19 period evaluated. There was a 22% decrease in the odds of HIV prevalence during COVID-19 compared to before (OR: 0.78, 95% CI: 0.66, 0.92) (Table). However, the adjusted odds ratio was not significant, indicating the decrease may have been driven by individual demographics (OR: 1.05, 95% CI: 0.89, 1.25). HBV prevalence was not significantly different between the two time periods (OR: 1.05, 95% CI: 0.96, 1.16). However, the adjusted odds ratio was significant, indicating individual demographics could be obscuring an actual increase in HBV during COVID-19 (OR: 1.22, 95% CI: 1.11, 1.35). There was a 35% decrease in the odds of HCV prevalence during COVID-19 compared to before (OR: 0.65, 95% CI: 0.61, 0.70). The adjusted odds ratio remained significant (OR: 0.63, 95% CI: 0.59, 0.68), indicating the decline in HCV prevalence during COVID-19 was not driven by individual demographics.

Infectious Disease Prevalence among TTIMS Donations before and during COVID-19

Summary/Conclusions: Some demographic and infectious disease prevalence changes occurred during the COVID-19 pandemic in the U.S. However, demographic changes appear to be relatively benign noting increased numbers of donations/donor during the pandemic allowing blood supply levels to be maintained. HCV prevalence will continue to be monitored to determine if the lower prevalence was specific to the pandemic (or other factors such as widespread ongoing availability of direct acting antivirals).

P069 – Table 1

	Before COVID-19 Prevalence /100,000 (95% CI)	During COVID-19 Prevalence /100,000 (95% CI)	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)*
HIV	2.56 (2.30, 2.85)	1.76 (1.54, 2.00)	0.78 (0.66, 0.92)	1.05 (0.89, 1.25)
HBV	6.50 (6.08, 6.95)	6.01 (5.60, 6.44)	1.05 (0.96, 1.16)	1.22 (1.11, 1.35)
HCV	17.97 (17.26, 18.70)	10.31 (9.78, 10.87)	0.65 (0.61, 0.70)	0.63 (0.59, 0.68)

* Adjusting for sex, race/ethnicity, region, age group, and first-time donor status.

P070 | The impact of plasma donation: A qualitative study of immunoglobulin recipients

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Background: Human plasma has been referred to as "liquid gold," invoking its lifesaving potential when turned into medicines. The plasma-derived medical product in highest demand is immunoglobulin (Ig), and uses have expanded significantly in recent years, driving up the demand for source plasma collected from donors. There is a need to understand how Ig recipients understand and experience this treatment within the broader political landscape of the production and distribution of Ig. This can help to promote awareness of the impact of Ig and encourage plasma donation.

Aims: (1) Document Ig recipients' lived experiences of illness and treatment within social, cultural, familial, and institutional contexts; (2) Investigate Ig recipients' knowledge, perceptions and values related to the origins of the therapeutic products they have received, the processes that determine which products are available, and the connection to a plasma donor.

Methods: This is a qualitative study using a narrative approach to understanding the lived experience of the participant. We conducted narrative interviews with recipients of Ig products in Canada, aiming to understand the recipient's illness experience, the story of how treatment affected them, and their awareness of the product and the connection to a donor.

Results: We completed 46 narrative interviews with 23 recipients of immunoglobulin, each interview lasting 40 to 90 min. Investigators collaborated with participants to identify a chronological pattern to their story of illness and treatment, then identified themes within each stage. Prior to diagnosis, participants described their first symptoms and the referral process. They discussed fear of the unknown, the lack of available evidence about their rare condition, and feeling overlooked or ignored by health professionals. During diagnosis, participants described the tests they had to undergo to receive a diagnosis, the different health professionals engaged in their care, and the relief they felt upon receiving a diagnosis. During the treatment phase, participants described attempting to understand Ig, it's uses and limitations, reaching out to other recipients and advocates for more knowledge about Ig, and how Ig affected their quality of life. They were aware that the product came from a donor, but their primary objective was to understand their illness and get treatment.

Summary/Conclusions: The sparse literature on patient experiences with Ig use does not address the social relations of how the treatment has an impact on the patient's life, or how the recipient understands their role in a broader healthcare system and the connection to a donor. We explore how the production, supply and deployment of plasma is socially mediated. Our findings suggest that immunoglobulin has a significant impact on the life of the recipient. Further, that recipients of Ig struggle to receive a diagnosis, obtain reliable information about their condition, and the treatment, and find support for managing illness. Addressing these systemic issues in the healthcare system could allow recipients to access a community that can support them, and raise awareness about the impact of plasma products. Findings indicate that when they are well, Ig recipients have a desire to express their appreciation to a donor and to promote plasma donation.

Blood donation

Blood donor recruitment and retention

P071 | Post donation call back in Singapore: A review of cases from 2020–2022

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Background: To further enhance blood safety, blood donors in Singapore are informed to call the blood bank call back hotline if they feel that their donated blood should not be used by patients, forgot to declare pertinent information during medical interview or if they develop signs and symptoms of infection within 2 weeks of donation. Donors are not required to provide a reason when making a call back. The hotline uses an automated call-back Interactive Voice Response System which helps with the different options. The automated call back (ACB) system has an option which enables the donor to instruct the blood bank not to use their donated blood without talking to blood bank staff and giving a reason. The hotline also has a non-automated call back (N-ACB) option for donors to talk to the staff regarding their donated blood, enquiry option on the blood bank opening hours and an option to speak to a staff for other general enquiries. Blood components associated with the donation implicated in all call backs are recalled, quarantined and discarded.

Aims: The aim of the study is to review the post donation call back cases received by the Health Sciences Authority Blood Services Group from 2020 to 2022 as well as the number of discarded blood components associated with call back cases.

Methods: The records of donors who called the call back hotline from January 1, 2020 to December 31, 2022 were analysed and the reason for the call back if provided were reviewed. The collection and inventory records were checked to find out how many blood components were discarded due to call back cases.

Results: A total of 2743 call back cases from donors were reviewed (1681 or 61% from ACB and 1062 or 39% from N-ACB) which is 0.72% of the total number of successful donations during the study period. The top three reasons provided by donors when calling the hotline (N-ACB) were COVID infection (362 or 34%), flu/fever (282 or 27%) and rashes (164 or 15%). 13 donors informed our staff on their next visit that they previously called the hotline using the ACB system to make an enquiry but ended up making an error when keying in their response which resulted in their donation being discarded. A total of 5377 blood components were discarded due to call back of which 2319 or 43% were red cell units.

Summary/Conclusions: Most of our donors prefer using the automated call back (ACB) system than talking to our staff when they call the hotline. The call back hotline has helped improve blood safety as we were able to recall and prevent the transfusion of remaining blood components in the inventory from donors who informed us that they were sick or had an infection within 2 weeks of donating blood. Blood safety can still be improved through donor education by emphasizing to donors to call the hotline as soon as they are diagnosed or develop signs or symptoms of an infection. To prevent mistakes when using the ACB system resulting in donated blood being discarded, we recommend that a review be performed to make it more user friendly and that the hotline should be dedicated to call back purposes only, and not include other options for general enquiries.

P071 – Table 1

	2020	2021	2022
Number of call back (ACB & N-ACB)	659	704	1380
Number of successful donations	126,890	124,417	127,585
Number of discarded components due to call back	1383	1489	2505
Top three reasons given by donors for call back (N-ACB)	Flu/Fever (96) Rashes (40) Close contact with a COVID infected person (18)	Flu/Fever (78) Rashes (63) Covid infection (49)	Covid infection (309) Flu/Fever (108) Rashes (61)

P072 | Assessing unintentional creation of bias against men who have sex with men as a function of exposure to blood donor screening questionnaire: A national randomised controlled trial

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Background: Canadian Blood Services (CBS) historically screened and deferred donors based on group membership (e.g., being in the group of men who have sex with men, MSM) instead of on the basis of participation in specific, high-risk sexual practices (e.g., occurrence of condom less sex with a new sexual partner). The MSM screening and deferral question was embedded in a cluster of questions about stigmatised attributes such as history of imprisonment, trading sex for money, and illicit substance use. This juxtaposition of the "MSM question" and stigmatised attributes may have unintentionally caused blood donors by association to perceive MSM more negatively.

Aims: The aim of this research was to determine whether the CBS donor eligibility questionnaire generated negative bias against MSM.

Methods: A national, randomised online study of 903 current Canadian Blood Donors was conducted. Participants completed either the existing blood donor eligibility questionnaire or a modified donor questionnaire that repositioned the MSM question among neutral questions. After completing the existing or modified questionnaire, bias against MSM was measured using the sexuality implicit association test (IAT) and the Modern Homonegativity Scale – Gay Men (MHS-G). Lastly, participants estimated prevalence rates among MSM of certain stigmatised behaviours

Results: Participants who completed the existing donor eligibility questionnaire more strongly associated gay men with negative attributes on the IAT (p one-tailed = 0.045), indicating that question position within a cluster of stigmatizing items generated implicit negative bias toward MSM. Responses to the MHS-G (pone-tailed = 0.506) and prevalence estimation task ($p = 0.443$) indicated that question order had no significant impact on explicit bias.

Summary/Conclusions: Positioning the MSM screening question among stigmatizing questions creates implicit negative bias against MSM. Policy makers should be mindful of question positioning when designing donor questionnaires. Discussion will consider how the findings of this study are related to the development and evaluation of an alternative approach to donor screening, based upon questions about specific risky behaviours, not group membership, that the Canadian Blood Services has recently adopted.

P073 | Alternatives to blood donor deferral of gay, bisexual, and other men who have sex with men: Acceptability of screening the sexual risk behaviour of all blood donors

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Background: Blood operators screen donors to reduce the risk of transfusion transmitted infections (TTIs). Many are evolving screening procedures from those that defer all who have had a sexual interaction with gay, bisexual or other men who have sex with men (gbMSM) to an approach that assesses individual donors' recent sexual risk behaviour with any partner.

Aims: The current study aimed to assess the acceptability and feasibility of a donor screening approach that focuses on individual participation in risky sexual behaviour with any partner, compared to an approach that screens out all who have participated in sexual activity with a man who has sex with men.

Methods: A representative sample of current Canadian Blood Services blood donors ($N = 1194$) was recruited online and randomised to complete either the existing (at the time of the study) donor questionnaire (DQ) that screens out those with recent gbMSM sexual experience, a modified donor questionnaire (MDQ) that assesses individuals' recent sexual behaviour with any partner, or an MDQ that assesses individual sexual behaviour with any partner and explains why these questions are asked. Respondents were asked for their perceptions concerning difficulty, comfort and acceptability of these screening questionnaires.

Results: Across experimental conditions, current donors regarded screening questionnaire difficulty to be low; discomfort in responding was minimal; screening questionnaires were perceived to be relatively inoffensive and justified, and very few donors would cease donating if the screening questionnaire they responded to became the one in general use. Some minor sex differences were observed, and in some cases, perceptions of the MDQ with explanation were somewhat more positive than those of the DQ and MDQ without explanation.

Summary/Conclusions: An individual risk behaviour screening approach appears to be acceptable to current blood donors as an alternative to screening out all who have recently engaged in gbMSM sexual interactions. Discussion will focus on the social and political climate and research that is relevant to the introduction of this alternative approach to screening blood donors on the basis of individual sexual behaviour. Next steps for studying the acceptability of this approach among first time donors and gbMSM donors will be discussed as well.

P074 | Abstract withdrawn

P075 | A study on the donor motivation and recruitment strategies of voluntary blood donor organisations in Malabar region of South India

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Background: Voluntary blood donor organizations form the backbone of the transfusion system as they provide blood centres with donors and camps. As the national target is to achieve 100% voluntary non-remunerated blood donation, they play an integral role in the same. Often many blood centres fall short in voluntary blood donation and rely on replacement donors to fulfil their needs. The current literature mainly focuses on the individual donor rather than the organizations they support. VBDO play an important role in transforming a non-donor into a first-time donor and in turn transforming them into a regular donor. A survey of the voluntary blood donor organization to understand how they recruit and retain their donors followed by the challenges faced will provide a fresh outlook on the pressing matter.

Aims: To understand the donor motivation and recruitment strategies of VBDO.

To identify the challenges faced by the VBDO.

Methods: A survey form was given to all organizations and the data was collected after ethics committee approval. The data were analysed in SPSS with a chi-square test.

Results: A 27 VBDOs filled out the survey out of 35 organizations included in the study. 33% of the VBDO had member strength of 500–100 followed by 22.2% with 100–500, and the least 7.4% for both <50 and >5000 member strength. When considering the geographical distribution of the work, 33.3% concentrated on an institutional level and 25.9% worked at the district level. 70.4% were primary VBDOs which worked only for blood donation purposes and all of them had branches across the geography. Out of them 88.9 % were government registered and the remaining were unofficial support groups.

The median number of donors arranged by a VBDO in the past 12 months was 150. All organizations had the same strategy for donor recruitment like phone calls, direct contact and social media. Source of funding included Contributions from members (73.3%), Donations (20%) and crowdfunding (6.7%). More than 75 % of VBDO has conducted donor awareness and motivational classes either virtually or offline. The donor pool comprised students (96.3%), government employees (48.1%), those employed in the organized sector (44.4%), employed in the unorganised sector (44.4%).

When comparing the issues faced in organizing camps, 37% had financial constraints, 25.9% had difficulty in setting up camps, 14.8% had the inability to arrange venues for camps, 11.5% reported non-cooperation from blood centres, 11.1% had difficulty in transporting men and material to the blood centre and venue. The donor recruitment concerns include Knowledge deficit (33.3%), Unwilling to dedicate time (29.6%), gender bias (18.5%), and skew towards government and private blood centres (18.5%). Donor retention concerns included laziness/inconvenience from the donors (40.7%), donor motivation (33.3%), inability to dedicate time (33.3%), anxiety and stress (22.2%), previous deferral (15.4%) fear of adverse reaction (7.4%).

Summary/Conclusions: VBDO forms the pillar of Blood transfusion services and plays a crucial role in blood supply. It is the duty of the government/Blood transfusion services to bridge the gap in knowledge and provide training, manpower & material to the VBDO to help recruit and retain the donor population.

P076 | Impact of temporary deferrals on return behaviour

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Background: To ensure a safe blood supply to the patients, a strict blood donor selection is conducted to check the eligibility that may lead to temporary or permanent deferrals. Temporary deferral from blood donation may impact donor return.

Aims: Try to understand the impact of the temporary deferrals on return behaviour.

Methods: A retrospective study has been conducted, which includes all attempted donations in the two donor centres (Al Ain and Abu Dhabi) in the emirate of Abu Dhabi during the period from 1 January 2016 to 2 March 2021 ($n = 163{,}347$) to determine the impact of temporary deferrals on blood donor return behaviour. This study includes all types of donation (W = whole blood donation, C = Plateletpheresis, P = plasmapheresis.)

The donations can come from the fixed centres (one in Abu Dhabi and one in Al Ain) or mobile campaigns set up at people's workplace or at university campuses.

We are interested in the return behaviour from the day the donor is eligible to donate again.

We look at the details of the target donation (gender, blood type), donor experience (first time versus repeat donor), number of deferrals, probability of donors returns in the next 24 months from being eligible to donate again and the number of days' donors take to come back since eligible to donate again

Results: After a deferral, male donors have 36.4% chance to come back in the next 24 months (from the date they are eligible again) and 48.3% chance to come back in the next 24 months after a successful whole blood donation.

Female donors have 20.9% chance to come back in the next 24 months (from the date they are eligible again) and 34.6% chance to come back in the next 24 months after a successful whole blood donation.

After a deferral, first time male donors have 24.6% chance to come back in 24 months' period while 63.5% of repeat male donors come in the 24-month period.

After a successful donation, 33.1% of first time donors come back in the 24-month period while 74.6% of repeat time donors come back after successful whole blood donation.

Summary/Conclusions: Donors who are being deferred are less likely to come back than donors who made a successful whole blood donation.

Males come back more than females regardless if first time or repeat donors.

This study does show that the more experienced donors are more likely to come back to donate than the unexperienced donors.

P077 | To reward or not to reward? The effects of a loyalty program on plasma donor retention

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Background: In 2020, a proof-of-concept plasma-only donation centre was opened in the Netherlands. An important goal of Sanquin (the Dutch blood supply organization) with this centre was to test new ways to retain plasma donors. Part of the retention strategy is the use of a special loyalty program: Donordruppels ('donor drops'). Upon (voluntary) registration, donors get points for donating and bonus points for special occasions (e.g., eighth donation within a year). After at least three donations, donors can redeem their points for items such as gift cards, gadgets, a health check or charitable gift to Sanquin's research fund.

Aims: To explore how users and non-users perceive the Donordruppels loyalty program and to assess the effect of the loyalty program on retention and donation frequency.

Methods: We combined survey data about how donors use, perceive and appreciate Donordruppels with donation information. We invited 1500 donors to fill out the survey. The survey assessed use, attitudes, perceptions of usefulness, social influence, and intentions to use Donordruppels on 5-point Likert scales ranging from 1 (totally disagree) to 5 (totally agree). Open-ended questions assessed benefits and possible improvement for Donordruppels. For all survey participants, we retrieved donation information from Sanquin's donation registry from 1 year prior to until 1 year after registration with Donordruppels. For donors who did not register for Donordruppels, we calculated from the date of their first donation at the new centre.

Results: Of the 376 responses, 360 donors finished the survey, and their data could be linked to their donation information. 324 respondents participated in Donordruppels (90%). They indicated that the program made them feel appreciated or that it felt like a nice little extra. About 15% of donors participating in Donordruppels would prefer more diversity in gifts offered in the program (e.g., sustainable goodies, or Sanquin merchandise). Donors who participate in Donordruppels had more positive attitudes, perceived Donordruppels as more useful and had higher intentions to keep using it than non-participants (see Table 1). A between-subjects ANOVA showed that donors participating in Donordruppels had a higher donation frequency than non-participants, $F(1358) = 11.48$, $p < 0.001$, $\eta 2 = 0.007$ (see Table 1). Additionally, a repeated measures ANOVA amongst Donordruppels participants comparing donation frequency before ($M = 7.4$, $SD = 7.5$) and after registration showed higher donation frequency after registration for Donordruppels, $F(1323) = 5.97$, $p = 0.015$, $\eta 2 = 0.007$.

P077 - Table 1. Means (*SD*) for Donordruppels participants versus non-participants

	Participants	Non-participants
Attitude	3.7 (1.0)*	2.3 (1.3)*
Usefulness	3.0 (1.1)*	1.8 (1.0)*
Social influence	2.7 (0.9)	2.5 (1.1)
Self-efficacy	4.8 (0.6)**	4.5 (0.9)**
Anxiety	1.3 (0.9)**	1.7 (1.3)**
Intention to keep using	3.6 (1.1)*	2.6 (1.5)*
Donation frequency (follow-up)	8.5 (4.6)*	5.8 (3.9)*

* Significantly different at $p < 0.001$ ** Significantly different at $p < 0.05$.

Summary/Conclusions: Our results show that the loyalty program does have a small effect on their donation frequency. Remarks in open text fields showed that donors felt that this program was a little extra that made donating even more fun. As one donor said: "I feel the service of the staff and being pampered with coffee and cookies is more of a token of appreciation than Donordruppels is. I once received yellow shoelaces from an employee out of the blue. I liked that much more than what any online loyalty program could ever offer me". Thus, a loyalty program can be beneficial to donor retention, but should be used to complement (and not replace) other retention strategies.

P078 | The impact of public policy for combating the COVID-19 pandemic on sociodemographic characteristics of first-time blood donors in Taiwan

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Background: The coronavirus disease 2019 (COVID-19) was first discovered in Wuhan, China, and rapidly spread worldwide. The COVID-19 pandemic had impact on blood supply due to significantly reduced blood donation for blood transfusion services. Subsequently, the emergence of alpha variant (B.1.1.7), delta variant (B.1.617.2), and a more virulent mutant, as known as omicron poses a threat to public health. Central Epidemic Command Center establishes epidemic alert level to prevent sustained community transmission.

Aims: This study analysed the changes in sociodemographic characteristics of first-time blood donors during the crucial policies for combating COVID-19 in Taiwan.

Methods: Data were collected from the Taiwan Blood Services Foundation blood donor database between Nov. 1, 2019–July 6, 2021 and April 4, 2022–July 31, 2022, and a total of 232,252 first-time blood donors were enrolled. We classified four periods: Period 1: pre-COVID-19 pandemic, Period 2: COVID-19 Alert levels 1 and 2, Period 3: COVID-19 Alert level 3 (zero severe cases policy) and Period 4: coexisting with the virus and effective epidemic control and management policy. This study compared the differences in distributions of sociodemographic factors, including donation sites, age, gender, and occupation for first-time blood donors during these four periods. The Chi-square tests for categorical variables were used for analysis. All statistical analyses were performed using the statistical analysis system (SAS) software, version 9.4.

Results: The number of first-time donors was 35,094, 156,670, 9234, and 31,254 among Periods 1–4. The mean age of the first-time donors was 25.42 ± 11.27, 27.13 ± 11.89, 30.47 ± 11.79 and 29.78 ± 11.45 years old for Periods 1–4, respectively. Compared with Periods 1, 2, and 4, Period 3 had the highest proportions in fixed sites of blood donation location (61.12% vs. 20.62–36.05%), age group of 25–40 years (38.62% vs. 20.72–33.43%), female gender (56.48% vs. 49.69–53.54%), and service occupation (17.91% vs. 8.36–12.14%), furthermore, Period 3 had the lowest proportions in age group of 17–24 years (40.87% vs. 45.93–66.80%) and students (26.47% vs. 30.37–46.45%). All differences were significant (all $p < 0.001$).

Summary/Conclusions: Young donors and students are major sources of first-time blood donors. Our study demonstrates that proportions of young people and students decreased during period of COVID-19 Alert level 3, that is, zero severe cases policy period. To improve long-term sustainability of young age and students to donate is an important issue for future public health emergency.

P079 | Donation drivers and brakes: A qualitative approach to the human condition

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Background: The Banc de Sang i Teixits (Blood and Tissue Bank, BST) is the health system player responsible for correct supply of blood and tissues in Catalonia. In order to perform its task, it needs a donor base capable of providing 1000 donations per day.

The pandemic has had an impact on the recruitment of new donors, as well as on the frequency of donation from regular donors. There is also an upward trend in average donor age. As things stand at present, it is essential to deploy a well-focused communication strategy that can change these trends.

Aims: The BST's communication strategy seeks to ground itself on real and objective insights. We now need information and data on the factors that motivate and curb people from donating blood.

We also want to define the narrative's focus and the key messages on which the campaigns should be based.

On a quantitative level, the aim is to increase the number of donors, the number of donations per donor and the yield obtained from the campaigns.

Methods: A qualitative research was carried out consisting of six focus groups, with the following profiles of men and women equally represented.

1. Non-donor profile: young people between 18 and 30 years old.
2. Sporadic and new donor profile: profiles between 20 and 35 years old.
3. Loyal donor profile: profiles between 30 and 50 years old
4. Passive donor profile: profiles between 30 and 50 years old.

Results: This research offers a very valuable analysis for categorizing and providing a true picture of four types of profiles, taking into account:

- The dominant emotions associated with the blood donation time-experience.
- The concepts and values associated with donation.
- The brakes and drivers on blood donation.

The fact of donating blood (or not donating blood) has such a personal, experiential, internal and even psychological charge that it is a reflection of the human condition itself. People live with an internal duality and contradiction that clearly affects their behaviour in relation to donation:

- People who are fearful… but also committed
- People who are courageous… but also uninformed
- People who are aware…but also too busy

The BST's positioning map is built on these insights. A positioning that addresses two key elements:

- One that seeks **to generate traction**: that mobilizes citizens and elicits a positive behaviour toward donation.
- Another that generates a **positive experience:** not only to compensate for the act of donating, but also to connect us to a scenario and an experience worth having; what we do together matters and is crucial.

Summary/Conclusions: As BST we have the duty to awaken citizens' awareness. We have to activate people's responsibility. Promote the act of donating blood as an act of collective responsibility. Because societies and individuals only move forward when we all play our part.

P080 | Impact of WhatsApp on donor invitations

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Background: Instant messaging channels have impacted on how we relate with our environment. As a society, we expect the information

we receive to be up-to-date, brief, concise and, most importantly, two-way.

In the era of over-information, and given the need to obtain donations every day, it is essential to develop a communication strategy that puts the donor at the centre and studies which communication channels have the greatest conversion rate.

The Banc de Sang i Teixits (Blood and Tissue Bank, BST) must guarantee the supply and proper use of blood components, interacting with more than 300,000 active donors. To date, the main messaging channels have been email and SMS. Now WhatsApp enters the scene, as 9 out of 10 people use this channel to interact on a daily basis.

Aims: Analyse the behaviour of donors to WhatsApps from BST. Until now, we had only used this social messaging network as a one-way communication channel, between donor and BST, but not from BST to donor.

The main objective is to notify donors that they can now donate again through WhatsApp, using a more personal, friendlier tone and channel.

Methods: The first step before incorporating this communication channel as another option in inviting donors to donate is to analyse whether receiving WhatsApp and not SMS or mail causes a change in the donor's behaviour.

That is why we have based our work on an A/B TEST, taking two representative samples of donors in Barcelona. Some have been invited by WhatsApp and others by SMS. In addition, to compare with each other, we have made variables of these tests, changing times, communication tone and content wording. Also, in some cases we have sent an opt-in, a message giving consent to send via WhatsApp, and depending on their replies, we have been able to analyse their willingness to donate.

Results: The A/B TEST has allowed us to compare donor responses according to the invitation channel used. We have detected that although the invitation made through WhatsApp, in an initial phase, does not bring more donations, it does create favourable conditions for a conversational environment that SMS or email does not have.

In addition, people who answered YES to the opt-in have a profile that is more favourable to donation, since the conversion rates were higher compared to those who answered NO or did not answer at all.

Summary/Conclusions: With this new use of the application, we can adopt a more personal approach in our contacts with the donor, creating favourable conditions for a conversational environment that enables doubts to be answered before the donation. This is an advantage over mail and SMS.

P081 | Connecting with donors: Empathy maps

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Background: The Banc de Sang i Teixits (Blood and Tissue Bank, BST) has set itself the ambitious challenge of reaching 50,000 plasma donations by 2025. Achieving this target of self-sufficiency in selflessly donated plasma, and thus avoid being affected by external factors that might impede the guarantee of health and safety in Catalonia, means increasing the donor base and frequency of donation.

Aims: To explore the emotions and rationale of current plasma donors to identify actions that will help increase their donation frequency.

Methods: Focus groups with loyal and sporadic plasma donors, to develop empathy maps that help understand donors and adapt the value proposition to their profile and needs.

Results: This analysis has helped answer the question, *"What do donors think and feel?"* In other words, what really matters to them? What do they believe and what emotions do they feel? *What do they hear?* What does their environment say about donation? *What do they see? What do they say and do? What obstacles and what benefits do they find?* The aim is to match our communication as closely as possible to their own.

Summary/Conclusions: Donating plasma is a satisfying and rewarding experience for the donor that makes them feel useful by dedicating time to help sick people in need. They do not want to receive anything material in return, or to be differentiated from blood donors in any way.

Comfort, peace of mind and having time for oneself are positive elements associated with plasma donation. Having the time, proximity to donation centres and the duration and effects of the donation are aspects that affect the act of donating.

P082 | The relationship between blood donation and subjective well-being: A randomised trial

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Background: Blood transfusion is a lifesaving procedure that blood can only come from blood donors (BDs). BD recruitment remains an important worldwide challenge.

Aims: This study aims to clarify whether blood donation can bring benefits in terms of the donors' levels of subjective well-being (SWB) in order to encourage more blood donation in China.

Methods: A single-centre, single-blinded, parallel randomised trial involved 601 BDs and 1, 127 non-donors (NDs) was performed in Guangzhou, China. Blood donor group (BDG) completed baseline Questionnaire A immediately after they donated whole blood. Then they were randomly assigned to two groups that they received two different questionnaires on the day when their donating blood were used. Three hundred BDs in BDG1 completed Questionnaire B1 which contained a thank-you note at the beginning with a picture of a patient undergoing a blood transfusion; whilst 301 BDs in BDG2 completed Questionnaire B2 which did not include such note. Non-donor group 1 (NDG1) completed two different baseline questionnaires that 517 NDs were asked to recall a previous prosocial behaviour (NDG1) in the Questionnaire C1, and 526 NDs (NDG2) were not in Questionnaire C2.

Results: The BDG scored significantly higher than the NDG1 on negative affect in the baseline questionnaires, but no significant difference

was found between these two groups on neither the positive affect nor the life satisfaction. The SWB in the BDG was slightly lower than in the NDG1, whereas higher than in the NDG2, but the differences were neither significant. When BDs were thanked, SWB was improved in BDG1, that the scores of SWB were ranked from highest to lowest as: BDG1, NDG1, BDG2 and NDG2.

Summary/Conclusions: The present research should be viewed as a first step in understanding the relationship between blood donation and SWB.

P083 | A comparative policy analysis of blood donor eligibility criteria across hospitals in Lebanon

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Background: Blood donor eligibility criteria are purportedly intended to protect the health and safety of both the donor & recipient of the blood transfusion through unified standards of healthy donors & safe procedures. However, in Lebanon, the same donors considered eligible in one hospital could be deferred in others for various reasons, including, for example, removing their hair via laser treatment, & being of a particular nationality, among other reasons. This is problematic for a country such as Lebanon, which is experiencing a severe economic crisis whereby the Lebanese currency has lost more than 95% of its value between 2019 and 2023. This means that fees spent on transportation, time off from work, and all other costs inflicted on donors to make it to the donation location only to be rejected make it extremely challenging to retain blood donors.

Aims: This study examines the reasons behind the variations in blood donor eligibility criteria across 17 major hospitals in Lebanon. The study engages with the following research questions: What affects the "donatability" of blood in Lebanon? To what extent are blood donor deferrals across hospitals in Lebanon medically valid and evidence-based deferrals?

Methods: This study draws on fieldwork conducted in Lebanon between June and September 2022. The datasets include (1) the blood donor eligibility criteria of 17 major hospitals and (2) the pre-donation/ blood donor history questionnaire of seven hospitals in Lebanon. The criteria for blood donor eligibility vary by hospital, and these are usually checked before the potential donor is allowed to take the donor history questionnaire. The donor history questionnaires vary based on standards set forth by the hospital's unique accrediting body. For example, an American-identifying hospital with American accreditation will use a donor history questionnaire that complies with American standards. I conduct a comparative analysis of each of the two types of documents.

Results: Findings suggest that the hospitals set the blood donor eligibility criteria; however, blood bank personnel decide who gets to donate blood irrespective of policy guidelines. The main differences between hospitals for blood donor deferrals in Lebanon are due to medically valid and miscellaneous reasons. The top reasons include: (1) the time period required (a) between doses of the different COVID-19 vaccines; (b) between blood, platelet, and plasma donations; (c) after receiving certain medications; (d) after experiencing certain medical conditions; (e) after travelling to certain countries; (2) miscellaneous conditions (e.g., removal of hair by laser, having children, staff working hours); and (3) specific identity markers of potential blood donors, such as their gender, marital status, and nationality; for example, in some hospitals, persons with Iraqi, Egyptian, Chinese, Philippines, and African nationalities can never donate. Many of these exceptions for donation are based on discriminatory practices rather than scientific evidence.

Summary/Conclusions: Not all deferrals of potential blood donors in Lebanon have a scientific rationale; deferrals can be based on medically invalid and scientifically unbacked reasons. Much of the deferrals are for miscellaneous reasons, negatively impacting the country's ability to ensure sustainable blood donation practices. The translation of policy into practice by blood bank personnel deserves critical attention since they are the ones who eventually accept and reject donors, irrespective of policy guidelines. Even donors with an active history of donating blood become less likely to re-donate due to frequent deferrals that the donor only knows of upon arriving at the donation station.

P084 | Abstract withdrawn

P085 | Abstract withdrawn

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P087 | Platelet transfusion to patients with CD36 isoantibody

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Background: CD36 type I deficiency enables the formation of CD36 isoantibodies as a consequence of immunization by pregnancy or platelet transfusions in patients originating from Africa, East Asia and Arabian countries. Because in middle European individuals CD36 deficiency is virtually unknown, CD36 negative platelet donors usually are not available and therefore platelet transfusion to CD36 immunised patients is a challenge.

Aims: We describe our strategy to provide platelet concentrates for immunised patients with CD36 isoantibodies.

Methods: Within two months we identified anti-CD36 in two patients by a commercial bead based Luminex assay (Immucor Lifecodes PAK Lx, Dreieich, Germany). Patient 1 of presumable African origin is suffering from de-novo AML and showed thrombocytopenia during his first induction chaemotherapy before high dose chaemotherapy and allogeneic HSCT. Patient 2 of presumable Arabian origin with a glioma

shows refractoriness to platelet transfusions with 0–13 platelets/nl. CD36 expression on the patients' platelets and monocytes was tested by flow cytometry using anti-CD36 FITC (clone FA6-152, Stem Cell Technologies, Vancouver, CAN), anti-CD42b PE (clone HIP1, Biolegend, San Diego, CA, USA), and anti-CD14 PE (clone M5E2, Biolegend). However, it is complex to define a definite cut-off for median MFI values if only few samples from CD36 immunised patients are available as reference. For patient 2 platelet crossmatch was performed by MASPAT assay (Sanquin, Amsterdam, NL).

Results: Both patients showed a CD36 type 1 deficiency with negative platelets and monocytes. Therefore, no compatible donors were available within our regular apheresis donor cohorts. As a result of an earlier research project 5 years ago, we identified some whole blood donors with CD36 negative platelets who predominantly originated from Syria. In the meantime, most of these donors are not available for blood donation any longer, either because they had moved, contact tracing was not possible, or because of medication. For patient 1 we could address one donor with a borderline CD36 expression who agreed to give platelets by apheresis during the patient's next cycle of induction chaemotherapy. For patient 2 we identified one potential heterotransplant donor and luckily, some family members were identified as CD36 negative with a negative MASPAT crossmatch, too. Fortunately, both patients did not show additional HLA class I antibodies, which would further aggravate platelet supply.

Summary/Conclusions: Supply of CD36 negative platelets to immunised patients is a challenge for middle European blood services. To keep rare donors that have been identified as CD36 negative requires constant efforts. For some patients searching within closely related kins can be promising. To have a higher number of active CD36 negative platelet donors, we intend to continue our study on identification of donors originating from African and Arabian countries.

P088 | Increased tourist activity and the safety of blood and its components

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Background: Blood service in Poland is based on voluntary and non-remunerated donations. Regional Blood Donor Centre in Poznan is one of the largest blood centres in Poland with the total number of donations exceeding 100,000 per year. Due to substantial decrease in costs of travelling in recent years we have observed a massive increase in tourist activity, which bears the risk of potential exposure to various tropical diseases and can have a negative impact on the safety of blood and its components. The current policy requires a temporary deferral of 28 days for donors returning from locations with the risk of WNV infection and a temporary deferral of 12 months for donors returning from malaria-endemic countries.

Aims: The aim was to analyse the trend in the number of temporary deferrals due to the potential exposure to malaria and West Nile Virus and possibly work out some recommendations for the future.

Methods: The analysis was made using the data obtained from the computer system 'Blood Bank' in the years 2010–2019. We have analysed the total number of deferrals of donors returning from locations with potential exposure to WNV and malaria. Data from years 2020–2022 were not included due to worldwide travel restrictions related to COVID-19.

Results: We have recorded a substantial increase in number of deferrals due to potential exposure to malaria from 17 in 2010 to 301 in 2019 (+1671% in general; men +2866%, women +1018%), peaking with 395 in 2018. This trend remained the same for the group of men and women. Whereas the number of deferrals was relatively equal for these two groups in years 2010–2016, we can observe a shift towards more men than women being deferred in years 2017–2019 (ca. 60% men vs. 40% women). In terms of number of deferrals due to potential exposure to the WNV a massive increase has been observed: from 26 in 2010 to 604 in 2019 (+2224% in general; men +3675%, women +1840%). In the analysed time period the majority of deferrals was placed in the group of men (between 60% and 70% depending on the year). At the same time look back analysis recorded no post-transfusion infections with malaria or WNV in years 2020–2019.

Summary/Conclusions: The policy of temporary deferrals of donors returning from locations of potential exposure to malaria and WNV proves to be a reliable method of increasing the safety of blood and its components. At the same time donor deferral especially in the seasons known to be problematic for the blood supply (e.g., summer months) might have further negative impact manifesting in longer absence of donors, loss of donors' satisfaction and/or motivation to donate blood. Hence, it might be worth to consider some measures to counteract these phenomena. One of them could be introducing obligatory NAT testing for the infection of malaria for the donors returning from countries with endemic malaria which would allow for the time of deferral to be shortened to 4 months. The other measure would regard WNV infections which in a way affects more directly the situation of blood supply in our region as infections have been reported all across Europe (e.g., France, Italy, Hungary, Balkan countries etc.). The solution might be to introduce the NAT testing for the WNV (some countries across the world such as Canada have already implemented this on a regular basis or at least as seasonal routine testing e.g., US) instead of keeping blood donor deferral procedures as they are. Further analysis taking into consideration the balance between the cost side and potential benefits is necessary. One is certain, with environmental factors such as climate change and increasing mobility of people, continuous surveillance is important for the safety of the blood supply.

P089 | Removal of UK-residence deferral for variant Creutzfeldt-Jakob disease: Impact on donor sufficiency in Australia

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Background: Until recently, Australian Red Cross Lifeblood indefinitely deferred people with prior residency in, or extended travel to, the UK during the risk period 1980–1996 ('UK donors') to mitigate the potential vCJD risk to blood safety. Regulatory approval to remove the deferral was underpinned by published (McManus, Vox Sanguinis 2022) mathematical modelling that concluded its removal would not increase the risk of vCJD transfusion-transmission beyond levels considered tolerable for blood safety. The modelling predicted a substantial sufficiency benefit, estimated at approximately 17,000 donors and 58,000 donations annually. The deferral was removed on 25 July 2022.

Aims: To analyse the sufficiency impact (additional donors and donations) of removing the deferral by tracking the donation metrics of the cohort of newly eligible UK donors, in the first 6-months after the policy change.

Methods: Lifeblood's donor questionnaire retained the UK deferral screening question until a version update effective from February 12, 2023. This permitted the identification of the cohort of newly eligible donors. Their donations were tracked for the period between 25 July 2022 and 24 January 2023 (6-month study period) and compared with baseline Lifeblood donation metrics and the modelled sufficiency predictions.

Results: A total of 38,462 UK donors attended to donate 78,762 times. Of these, 32,358 donors (females = 19,456, males = 12,902) successfully donated 67,916 times during the study period, representing 8.4% of the 804,830 total Lifeblood collections, at an average collection success rate of 86.2%. Of the 67,914 total donations excluding discards and autologous ($n = 276$), 40,108 were fresh blood and 27,530 were plasma donations for further manufacture. The trend of weekly donations by newly eligible UK donors showed an initial peak in the first 2 weeks (4020 and 3064 for weeks 1 and 2, respectively) with a subsequent stabilisation, maintaining high donations rates with a weekly average of 2866 in the last 2 weeks of the study period.

Summary/Conclusions: Cessation of the UK residence deferral for Australian donors has resulted in sufficiency gains well exceeding modelled predictions in the continuing absence of vCJD in Australia. In the first 6-months after the deferral was removed, the 67,914 additional fresh blood and plasma donations is more than double the predicted number, and if the trend at the end of the study period continues, will result in excess of 100,000 additional donations in the first year. The model assumed that UK donors would donate at rates proportional to that of the current donor base. However, the higher than modelled number of additional donations was expected because UK donors have factors associated with a higher rate of blood donation, such as higher education status. The additional donations occurred at a time when blood supplies were low due to ongoing COVID-19 pandemic impacts, and they helped avoid a potentially more significant blood shortage. If these newly eligible donors had not donated, donations would have been 88% of the inventory target; the additional collections allowed 96% of the inventory target to be achieved. The cohort of new and returned donors appear to be highly committed and have already made a substantial contribution to supporting both plasma for fractionation inventory, as well as local red blood cell and platelet inventories.

P090 | Abstract withdrawn

P091 | Blood donor deferral in COVID-19 pandemic (2 years of Polish experience)

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Background: The COVID-19 pandemic had significant impact on blood services worldwide and brought about a variety of new challenges–among others the necessity to introduce additional criteria for blood donor deferral. One important consequence of the COVID-19 pandemic observed in many countries was the shortage of blood and blood components. The volume of blood issued for clinical use may be closely related to donor deferral. On the other hand, the safety of donors and recipients can only be ensured through restrictive adherence to eligibility criteria as specified in the legal regulations.

Aims: The study aim was to investigate the prevalence and reasons for donor deferrals in the Blood Establishments (BEs) of the Polish public blood transfusion service under pandemic conditions.

Methods: A retrospective analysis of 2020–2021 data on donor deferrals based on annual reports from 23 Polish BEs.

Results: In 2020, a total of 653,467 persons volunteered to donate blood, but only some (569,914) were found eligible for donation. In 2021, 703,958 persons came to donate blood, and 615,784 were qualified for donation. In 2019 these values were respectively 719,627 and 614,579.

Donor deferrals:

- In 2020, a total of 9537 permanent deferrals were applied as well as 214,049 temporary deferrals of 176,854 persons. The most common cause (65,892 cases) was low haemoglobin level. Temporary deferral was applied to 2303 people for a variety of reasons related to the COVID-19 pandemic (disease itself, quarantine, contact with infected person/persons).

- In 2021, a total of 9637 permanent deferrals were applied as well as 223,836 temporary deferrals of 186,206 persons and the most common cause (69,758 cases) was low haemoglobin level (just like in the previous years). Temporary deferral was also applied to 2080 persons for a variety of reasons related to the COVID-19 pandemic. Additionally, 1802 temporary deferrals were related to vaccination against COVID-19.

Summary/Conclusions: In the 2020–2021 period, blood transfusion service worldwide was forced to face new problems mostly related to the COVID-19 pandemic. According to the study data, the COVID-19 pandemic impact on blood donation in Poland was relatively weaker in 2021 than in 2020. Differences between BEs were reported as regards the number of deferrals applied which may indicate the need for implementation of more transparent and standardised eligibility criteria. Temporary deferrals directly or indirectly related to the COVID-19 pandemic accounted for a relatively small portion of all reasons for deferral.

P092 | Abstract withdrawn

P093 | How to ensure a memorable donation experience

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Background: Ensuring a memorable donation experience is one of the main strategic pillars of the Banc de Sang i Teixits (Blood and Tissue Bank, BST).

In Catalonia, the experience of blood donation begins when the person first comes into contact with the donation and/or our organization, and ends when the person stops donating.

It is vitally important to take into account all those aspects that determine this relationship in order to guarantee an optimal experience and thus recruit, retain and lock in the existing and new donor community.

The BST is the health system player responsible for correct supply of blood and tissues in Catalonia. Each year it recruits between 20,000 and 30,000 new donors and it interacts with more than 300,000 active donors.

Aims: Design and structure a memorable, personalised experience matched to the different donor types and profiles in their relationship with the BST, that is, before, during and after the donation process.

Methods: Qualitative research

A qualitative research was carried out consisting of six focus groups, 2-h group discussion sessions with different donor profiles (new, sporadic and regular donors) and non-donors, aged between 18 and 50 years, with equal proportions of men and women.

Customer journey, paint points and performance indicators

Assessment of the blood donation experience by the donor and non-donor: perceptions, pros and cons and brainstorming of ideas for a better experience.

Results: Based on the qualitative analysis performed, **2 groups of essential actions** are identified to ensure a memorable donation experience.

The first refers to a set of actions focused on the functioning and day-to-day aspects of the relationship with the donor. If we focus on the day-to-day aspects, it is essential to work on aspects such as:

- **Visibility:** boost our presence on all types of communication media and channels to reach a broad spectrum of the population, especially young people.
- **Capillarity:** donation centres that are close to most of the population.
- **The donation experience:** what happens while donating.
- **Engagement:** The connection with users is a determining factor in generating a certain regularity.

The second focuses on a set of actions that are more structural and essential for influencing this experience. We include aspects such as:

- Creating a donation **culture and awareness**
- Establishing a **permanent message** that the population can engage with.
- Defining our **identity and the role** we play in the public's mind.

Summary/Conclusions: The relationship with blood donation goes far beyond the moment of donating and requires, on one hand, working on the entire **donation value chain** and, on the other, doing so using a two-speed system: one speed that **caters for day-to-day needs** and another speed that enables construction of an **example, a narrative and a worldview** around donation that is **credible,** that **connects,** that **supports** and that **engages** the entire population.

P094 | Abstract withdrawn

P095 | Abstract withdrawn

P096 | Influencing first time donors to return

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Background: Over 6 months the Welsh Blood Service has measured its post-donation interaction with donors giving blood for the first time to explore how engagement methods relate to donor retention. 1641 first time donors were included in the study. 456 were successfully called, 642 received a text message (SMS) within one month of successfully donating blood. A further 543 received no additional interaction as the 'control group' of the study.

Aims: To measure retention rate of first time donors based on communication method used post-donation.

Methods: Three 'groups' were created to measure which communication method had the highest retention rate amongst first time donors.

- Group one–post-donation 'phone call' group.
- Group two–post-donation 'SMS' group.
- Group three–post-donation control group, no additional communication.

Results: Within the first 6 months following a donation:

456 first time donors were successfully called. In total, 27% of this group donated blood within 6 months of their first blood donation. 642 first time donors received an SMS congratulating the donor for making their first donation. In total, 17% of this group donated blood within 6 months of their first blood donation. 543 first time donors received no congratulatory message for making their first donation. In total, 14% of this group donated blood within 6 months of their first blood donation.

Within the first 12 months following a donation:

- Overall, 59% of those called returned within 12 months followed by 47% who had received an SMS and 46% who had no 'thank you' interaction.

Summary/Conclusions: Congratulating donors had a positive outcome to retaining donors following their first donation. Donors who were successfully called after they donated for the first time were almost twice as likely to return within 6 months of their first blood donation. Furthermore, over 12 months, the likelihood of returning at all increased from 43% to 59%.

Sending an SMS to donors after their first donation had a marginal impact, 3.13% higher than those who did not receive a congratulatory message. Although, over time, SMS had little impact (+1%) against the control group. This suggests, it would influence to come back sooner, but not necessarily to keep donating.

Future considerations:

review the process with 'emailing' functionality.

consider impact of changing style of messaging to increase retention.

increase size of sample contacted to monitor retention.

P097 | COVID-19 and its impact on the activity of donors in blood center in Poznan, Poland

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Background: The first case of Covid-19 infection in Poland was confirmed on 3 March 2020 in a hospital in Zielona Góra. As a result Polish national authorities introduced special regulations due to the pandemic situation.

Aims: The aim of this abstract is to present the impact of the Covid-19 epidemic on the number of donors (including plasma donors).

Methods: The analysis was conducted using data from the computer system, "Blood Bank". The number of donors registering for donation in years 2020–2022 was analysed in comparison to the year 2019.

Following data were analysed: number of donors registering for donation, number of donors that were qualified for donation for the clinical use; number of plasma donors (apheresis procedures), number of deferred donors.

Results: In 2020 the decrease in the number of donors by 16% was observed in comparison to 2019, in 2021–by 10%, in 2022–by 6%.

The number of first-time donors registering for donation in 2020 decreased by 38% in comparison to 2019, in 2021 by 13%, whereas in 2022 an increase of 32% was observed.

The numbers of first-time donors that donated blood and its components in 2020 was smaller by 25%, in 2021–by 10%, whereas in 2022 there was an increase by 51%.

The general number of donors that donated blood and its components decreased by 3% in 2020, by 6% in 2021, and only by 1% in 2022 in comparison to 2019.

The number of deferrals (temporary and permanent) remained stable and totalled 29% of all donors in 2019, 29% in 2020, 27% in 2021 and 28% in 2022.

A massive increase in the number of plasma donors (aphaeresis procedures) was observed in 2020-increase by 67% compared to 2019. Despite difficulties related to the pandemics and special sanitary measures the number of plasma donors in 2021 was even greater–increase by 135%. In 2022 a light decrease by 21% was recorded in comparison to 2019, which was due to the need for the supply of the convalescent plasma.

Summary/Conclusions: The analysis has proved the enormous impact of the pandemics on the activity of donors.

P098 | Ferritin testing as a risk mitigation strategy to protect frequent whole blood donors at Health Sciences Authority, Singapore

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[1]*Blood Services Group, Health Sciences Authority, Singapore, Singapore*

Background: Frequent whole blood donors risk iron depletion as 200–250 mg of iron is lost per donation. They may become iron deficient without anaemia and experience symptoms such as fatigue, dizziness, breathlessness, and so forth. This is an emerging risk.

To minimise iron deficiency among regular blood donors, we started a qualitive improvement initiative based on ferritin levels of frequent whole blood donors who donated five times per calendar year which is our maximum allowed donation frequency.

Aims: This project aimed to detect and mitigate iron deficiency without anaemia among frequent whole blood donors.

Methods: Frequent donors in 2020 and 2021 were identified using eProgesa. Ferritin was tested when they returned for donation the following year. Data entry and chi-square analysis were carried out using Microsoft Excel. Depending on the ferritin level, extension of inter-donation from the routine 12 weeks was instituted. Donors with low ferritin (<30 μg/L) were sent a letter about their ferritin results, deferral period, education material and supplemental iron in addition to the ones given post-donation.

In 2021, frequent donors' blood samples were tested (First ferritin). Donors with ferritin <30 μg/L were deferred for different durations based on 2 cut-offs (<15 or 15 to 29 μg/L). When they returned to donate, a second testing was carried out (second ferritin) and they were deferred based on the same criteria. A review was carried out to improve the algorithm in January 2022 and all donors with ferritin <30 μg/L were deferred for 24 weeks (Table 1).

P098 - Table 1

Ferritin level (μg/L)	2021	2022
≥30	Routine inter-donation interval	Routine inter-donation interval
15–29	Defer 16 weeks	Defer 24 weeks
<15	Defer 24 weeks	Defer 24 weeks

P098 - Table 2

	<30 μg/L		≥30 μg/L	
First ferritin	**2020**	**2021**	**2020**	**2021**
2020 ($N = 464$)	226	260	213	338
2021 ($N = 630$)	(48.7%)	(41.2%)	(45.9%)	(53.7%)
	$p > 0.05$		$p > 0.05$	
Second ferritin	**2020**	**2021**	**2020**	**2021**
2020 ($N = 226$)	117	68	82	130
2021 ($N = 260$)	(51.8%)	(26.1%)	(36.3%)	(59%)
	$p < 0.05$		$p < 0.05$	

Results: Data from frequent donors in 2020 were tabulated based on the <30 or ≥30 μg/L cut-offs for comparison with frequent donors in 2021 (Table 2). There was a total of 464 and 630 frequent donors in 2020 and 2021 respectively. Iron depletion in frequent donors was common with almost half of them having ferritin <30 μg/L on first ferritin. About 5% of frequent donors in 2020 and 2021 did not return for first Ferritin and 11.9% of frequent donors in 2020 and 23.8% in 2021 did not return for second Ferritin.

The interventions and longer inter-donation interval of 24 weeks for ferritin <30 μg/L in the revised algorithm resulted in (1) less donor with ferritin <30 μg/L and (2) more donors with ferritin ≥30 μg/L in the second ferritin for the frequent donor group in 2021 compared to 2020 and these were statistically significant ($p < 0.05$). The longer inter-donation interval was a more effective intervention and yielded an improved outcome for donors' ferritin level.

Summary/Conclusions: Blood Transfusion Services need to manage the emerging risk of iron deficiency without anaemia among frequent donors. Ferritin testing is useful to mitigate iron deficiency risks among frequent donors. From our experience, the intervention was generally well received as donors felt it was more personalised. This was a donor-centric approach with increased donors' involvement in the management of their iron stores.

P099 | Abstract withdrawn

P100 | Improving the quality of services is a vital solution for blood donor recruitment and retention

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Background: Cho Ray Blood Transfusion Center (CRBTC) belongs to Cho Ray hospital-Vietnam was built in 2002. Our main responsibilities are to collect whole blood, platelet and supply safe blood components to served hospitals in Ho Chi Minh City and South-Eastern area of Vietnam for over 10 million population. The average of whole blood collection was yearly recorded approximately 130,000 units of 350 mL. Most of whole blood collection came from mobile sites (99.8%); the rest came from fix site of CRBTC (0.2%). Therefore, the fix site has been overlooked for along time. During the COVID-19 pandemic happened in Ho Chi Minh City, we realized the vital role of the fix site. Therefore, a strategy was established to improve the quality of services at fix site for more blood donor recruitment and retention.

Aims: Improving the quality of services at fix site of Cho Ray Blood Transfusion Center for more blood donor recruitment and retention.

Methods: Cross-sectional descriptive study. The strategy has been run with four main solutions from December, 2021 included: 1. Adopting social media for introducing the fix site on blood collection of CRBTC to potential donors in Ho Chi Minh city-Vietnam; 2. Training and improving the well-communication for staff at fix site; 3. Well-donor care at pre-donation, during donation and post donation. 4. Monitoring opinions of blood donors to have the right adjustments and improvement on blood donation campaign.

Results: 12 months later, the number of blood and platelet donation at fix site was surprisingly changed in 2022, as follow:

Number of blood donation at fix site took 7174 (5.35%) of 134,552 units of total blood donation. It was impression if we compared with 0.2% of blood donation in previous years.

The percentage of blood donor retention has monthly risen and got 50.88% in December, 2022. The repeat donor has overcome the first time donor at fix site.

Number of platelet donation took 383 units for single dose and double doses compared with 30 units in average in previous years at fix site.

The percentage of platelet donor retention got 100% in December, 2022.

The percentage of satisfied donors for the quality of services at fix site got 98.9%.

Summary/Conclusions: The strategy on improving the quality of services at fix site of Cho Ray Blood Transfusion Center is going on the right way. The number of blood and platelet donations has monthly increased. Therefore, continuously stepping forward to this strategy and mainly focusing on improving the quality of services is a vital solution for blood donor recruitment and retention.

P101 | Abstract withdrawn

P102 | Blood donors' profile in a Costa Rican regional hospital from 2015 to 2019

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Background: Despite the National Blood Bank (NBB) existence as part of the public health system, blood availability and distribution in Costa Rica is not fully organized by this haemocenter. As such, many public hospitals have blood donation units to satisfy their own needs, and the NBB supplies the rest of the required blood.

San Vicente de Paul Hospital (HSVP), located in Heredia, attends a population of approximately 500,000 inhabitants. At this hospital, there is no data about blood donor's population characteristics.

Aims: To describe the characteristics of blood donors in HSVP to improve annual blood donation rate.

Methods: This study is a descriptive analysis of data extracted and analysed from the eDelphyn statistics module, considering effective blood donations from 2015 to 2019.

The blood donor characteristics available were monthly number of donations, gender, age and type of donation (voluntary or family replacement). Statistic tests (t and ANOVA) were calculated to assess the existence of significative differences in number of donations over time, and significative differences related to gender, age and type of donation.

Results: There were 8366 whole blood donations in HSVP from 2015 to 2019. There were not seasonal variations in blood donation rates over time (2.35 donations per 1000 people).

There was significative difference among the donor age groups ($p < 0.05$). Most of donors were 30–41 years old. The youngest group of donors (18–29 years old) was the less frequent every single year and during the whole period.

There was significative difference related to gender ($p < 0.05$). 60% of donors were male ($n = 4942$) and 40% were female ($n = 3424$).

There was significative difference between family and replacement donors ($p < 0.05$). Replacement donors constituted 80% ($n = 6760$), while voluntary donors accounted for 20% ($n = 1606$).

Summary/Conclusions: Blood donors aged <29 years were underrepresented and should be targeted by HSVP because they are candidate for long donor career.

To increase female's donor's assistance, some authors recommend educating donors and reducing the frequency of adverse reactions during donation.

Suggestions were made on how to motivate replacement donors to become voluntary donors. For example, establishing increased and flexible schedules for donors, optimizing efficiency of the process, and educating population to raise awareness.

P103 | Blood donations in Iceland 2012–2022

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Background: It is important to monitor the demographics of the blood donor pool to ensure a steady supply of blood components. During the COVID-19 pandemic 2020 and 2021, the Icelandic Blood bank focused on reaching out to active donors to maintain the blood supply and decreasing the outreach to new donors and young people. Increased demand for blood components was noted around the same time the pandemic slowly stopped, and it became crucial to register new donors to keep up with the demand.

Aims: To look at the age distribution and gender proportions of the Icelandic donor pool over 2012–2022, regarding active donors and newly registered donors, and to observe changes in whole blood donations and platelets donated via apheresis.

Methods: Donation data from the Icelandic Blood bank in 2012–2022 were collected via ProSang Statistics. Donors were categorized by age, gender, donor type and type of donation. A Changepoint Analysis was then carried out in RStudio.

Results: Whole blood donations decreased by 9% from 2012 to 2022 but increased by 14% from 2020. Males donated on average 74% [72%–77%] of all whole blood units. The average age of male donors was 42.3 years [Q1: 27.3–Q3: 56.8] and female donors 39.2 years [Q1: 23.9–Q3: 53.7]. The gender proportions of newly registered donors were pretty even throughout. Newly registered male donors were on average 36.3 years old [Q1: 21.8–Q3: 49.2] and newly registered female donors were 34.5 years old on average [Q1: 20.5–Q3: 46.6]. A significant increase in whole blood donations was detected in 2021 and in new donor registrations in 2022. Apheresis platelet donations increased by 23% over the study period, but by 30% from the year 2020. Around 98% of all platelet apheresis donors are male.

Summary/Conclusions: Due to the increased demand for blood components, whole blood donations have increased since 2020. Maintaining a proper number of blood components on hand has been a success. Most donors are males in their 50 s, but due to tremendous efforts in new donor recruitment in 2022 with people under the age of 30, the Blood bank has hopefully succeeded in maintaining the blood donor pool after a big hit in recruitment during the COVID-19 pandemic.

P104 | Abstract withdrawn

P105 | Abstract withdrawn

P106 | Participation of women in voluntary blood donation in North Macedonia

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Background: Gender studies are very limited in the transfusion field, whether considered broadly or with specific regards to the selection, management and retention of donors. Therefore, it seemed important to compare the presence of women among blood donors in different parts of our country and examine the roles that gender is reported to play in the donation of blood in order to identify possible implications for communication and management of donors.

Aims: Evaluation of the percentage of female blood donors in the voluntary blood donation process in different parts of North Macedonia in 2020 and 2021.

Methods: Retrospective analysis was performed on data collected from the annual blood donation reports and Information system E-Delphyn at Institute for Transfusion Medicine of RNM.

Results: There were in total 45,084 blood donation in N. Macedonia in 2020, of which 16.7% (7520) were females. According to the different parts of the country where they donate blood, their distribution was as following: 18% (4508/25,014) in the Institute for Transfusion Medicine–Skopje, 16.7% (1105/6627) in Regional Center for Transfusion Medicine (RCTM)–Stip, 16.5% (1358/8234) in RCTM–Bitola and 10.5% (549/5209) in RCTM–Tetovo. There were in total 50,906 blood donation in N. Macedonia in 2021, of which 17.71% (9013) were females. According to the different parts of the country where they donate blood, their distribution was as following: 19.7% (5804/29,366) in the Institute for Transfusion Medicine–Skopje, 17.6% (1304/7392) in RCTM–Stip, 15.7% (1302/8286) in RCTM–Bitola and 10.3% (603/5862) in RCTM–Tetovo.

Summary/Conclusions: Presented data showed that the percentage of women blood donors is around 17% in general and only in RCTM–Tetovo the percentage of women blood donors lower than in other parts of our country (around 10% in both years). More targeted blood promoting activities are needed to motivate women donors and to encourage this particular category to donate blood.

P107 | Seroprevalence of anti-SARS-CoV-2 among blood donors in Sfax, Tunisia

C Arbiya[1], L Nour[2], G Saba[1], R Taicir[2], J Jihene[3], C Amel[1], B Lamia[1], G Jalel[2], H Hela[1], I Ben Amor[2], A Wajdi[4], M Hela[2]
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Background: Accurate and large-scale serological tests that include the detection of anti-SARS-CoV-2 antibodies are essential to assess the pandemic's spread.

Aims: To assess the SARS-CoV-2 antibodies seroprevalence among blood donors (BD) in south Tunisia, and its correlations with gender, age, geographical origin, and blood groups.

Methods: SARS-CoV-2 seroprevalence was determined in a random sample of voluntary and replacement BD from the Blood Center of Sfax during June and September 2021. All BD underwent a medical examination before donation and were asymptomatic at the time of evaluation. The presence of antibodies against the SARS-CoV-2-Nucleocapsid (N) protein (anti-N IgG) was assessed by an Enzyme-Linked Immuno Assay (ELISA) in the virology unit at microbiology laboratory in Habib Bourguiba hospital of Sfax.

For the correlation analysis, we studied blood donation period, age groups ([18–20 years], [21–30 years], [31–40], [41–50 years], [51–60 years], >61 years), gender, type of donation, blood groups, and place of residence (Sfax city, the delegations of Sfax and southern Tunisian cities).

Statistical analysis was performed using SPSS version 20.0.

Results: We included 1320 BD (1 277 replacements and 43 voluntaries BD) from the Blood Center of Sfax: 601 during June and 719 during September 2021. The anti-N IgG seroprevalence increased significantly from June to September 2021 (29% vs. 38.7%; $p < 0.001$). This increase was significant in BD originating from Sfax city (27.9% vs. 42.6%; $p = 0.024$) and the delegations of Sfax (28.8% vs. 36.9%; $p = 0.01$), in BD aged between 31 and 40 years (28.7% vs. =39.3%; $p = 0.002$), in replacements BD (29.1% vs. 38.5%; $p < 0.001$), and in A+ and B+ BD (A+: 32.4% vs. 42.7%; $p = 0.03$, B+: 18.1% vs. 33.3%; $p = 0.023$).

Gender ($p = 0.67$ and 0.11), originating from Southern Tunisian cities ($p = 0.018$), and other age groups ($p = 0.29$; 0.33; 0.1 and 0.86) didn't significantly impact seroprevalence between both periods.

Summary/Conclusions: Our results are in line with data from the literature: the increasing seroprevalence over time, the higher prevalence among the youngest and in crowded cities.

Our BD, who has developed anti-N IgG antibodies after SARS-CoV-2 infection, can be recruited as plasmapheresis donor. In fact, their convalescent plasma can be an efficient tool to treat COVID-19 infection.

P108 | Abstract withdrawn

Blood donation

Blood donor health

P109 | Causes of blood donation deferrals in Armenia among patients' relatives: Analysis of 5 years of data from Armenia

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Background: Blood donation is a life-saving procedure in medical practice. There are three types of donors in Armenia: volunteers, patients' relatives and paid donors. About half of donations are from patient relatives who meet eligibility criteria.
Aims: The aim of our study is to evaluate the common causes of blood donor rejections among patients' relatives in Armenia between 2017 and 2021.
Methods: The data of patients' relatives who visited the blood bank of the Hematology Center after Prof. R. H. Yeolyan (the only haematology centre in Armenia) within the past 5 years (2017–2021) were extracted from medical documents and were retrospectively reviewed.
Results: 32,939 people came for donation in the past 5 years. There were 2533 deferrals among patients' relatives during that period. Of them, 12.59% (319) were rejected in 2017, 25.23% (639) in 2018, 17.49% (443) in 2019, 24.91% (631) in 2020, and 19.78% (501) in 2021. The highest average percentage of rejections was due to the detection of hepatitis B antibodies (12.20%). The second main cause of deferral was low haemoglobin level (10.33%), followed by cardiovascular/lung diseases (8.80%), elevated alanine transaminase (ALT; 8.68%), and being underweight or overweight (8.49%). In addition, there were 5 (0.78% of 639 deferrals) cases of congenital heart diseases detected only in 2018. The least frequent causes were food intake (1 case in 2018, 0.06%) and absence of identification documents (ID) (2 cases in 2020, 0.3% of 631 deferrals). Moreover, 2.73% of rejections were because of diagnoses mentioned in military ID cards. Other causes were surgical/dental procedures (5.23%), other infections (4.23%), gynaecological conditions/childbirth/breastfeeding (3.50%), neurological disorders (3.47%), vision problems (3.47%), endocrine disorders (3.39%), chole-/urolithiasis (2.97%), varicose veins (2.83%), gastrointestinal disorders (2.34%), risky sexual behaviour (2.31%), medication intake (2.14%), skin disorders (2.07%), rheumatic diseases, allergy and vaccinations (1.86%), fainting/headache (1.42%), benign tumours (1.08%), difficult venous access (1.05%), recent travel (0.95%), age (0.9%), trauma (0.83%), alcohol intake (0.79%), otorhinolaryngological disorders (0.66%), self-recusal/fewer sleep hours (0.62%), tattoo (0.41%).
Summary/Conclusions: The most common causes of rejections differ from that published in literature from different countries. There is a need for increasing the population's knowledge about blood donation eligibility. Also, further research is needed to estimate the prevalence of the above-mentioned causes among the remaining donors and the prevalence of hepatitis B in Armenia.

P110 | Performance evaluation of a point of care haemoglobin analyser in a blood donation setting

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Background: Blood donors are required to fulfil various conditions before a donation, both for their safety and for the safety and quality of the final components. During the pre-donation medical evaluation, beyond the relevant medical history and physical examination, the haemoglobin level of the donor is checked. For Whole Blood Donors, the European Directive 2004/33/EC Annex III and the Council of Europe Guide to the preparation, use and quality assurance of Blood Components state that haemoglobin levels must not be lower that 125 or 135 g/L for females or males, respectively, and this should be evaluated preferably before the donation and always in donors who were previously deferred due to insufficient haemoglobin levels or anaemia. Point of care haemoglobin analysers emerge as a preferable option since they spare the donor from an additional venepuncture, provide immediate results, require sparse physical space, require minimal operator training and present a very affordable cost per analysis. These portable point of care systems also facilitate the organization of mobile blood collection units.
Aims: To compare the haemoglobin determined by a point of care analyser with a reference haematological analyser in a blood donation setting.
Methods: A retrospective evaluation of the haemoglobin results from blood donations from January 1 to December 31 2021. Point of care testing was conducted using a fingerstick point of care method (CompoLab TM, Fresenius Kabi GmbH). Blood samples were collected and analysed on a Beckman-Coulter LH780 Hematology Analyser (Beckman Coulter, California, USA). Statistical analysis was conducted using IBM SPSS® Statistics version 26 (IBM, New York, USA). The data distribution was tested for normality using the Komolgorov-Smirnov test. A Bland-Altman plot was constructed to analyse the agreement between the two measurements. XLSTAT (Addinsoft, Paris, France) was used to construct a Passing-Bablok regression analysis to further assess the agreement and to check for any possible systematic bias.
Results: A total of 6988 haemoglobin determinations were evaluated, ranging from 125 to 185 g/L on the point of care testing and 93.8 to 183.7 g/L on the reference haematology analyser. The Komolgorov-

Smirnov test revealed that the difference between the two methods does not follow a normal distribution. As such, the limits of agreement were calculated using the 2.5 and 97.5 percentiles with a confidence interval (CI) of 95%, resulting in a mean difference between the two methods of 0.29 [−1.73025; 2.42050] g/L. Passing-Bablok regression analysis revealed an intercept of 11.215, 95% CI [8.732; 13.550] with a slope of 0.916, 95% CI [0.900; 0.934].

Summary/Conclusions: From a statistics point of view, the two methods cannot be used interchangeably, as there is not sufficient agreement. The Passing-Bablok regression analysis reveals an inconstant difference between the two methods and a proportional bias for the lower levels of haemoglobin. However, the impact of these results should interpreted at the clinical level. The maximum difference of 2.4 g/L at the confidence interval at the 97.5 percentile means that a donor with 125 g/L could have, at a minimum, 122.6 g/L, which is highly unlikely to cause harm to the donor that is thus accepted for donation and the whole blood unit collected should still fall into the appropriate quality standards. Given the advantages of the point of care system, we consider it appropriate for blood donor haemoglobin screening.

P111 | Abstract withdrawn

P112 | Abstract withdrawn

P113 | A comparative & beneficial analysis of use of ACD-A versus MMW HES (130/0.4) in granulocyte apheresis using spectra optia: Retrospective single centre study at a tertiary care oncology centre

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Background: In Prolonged neutropenic patients with severe bacterial/fungal infections, granulocyte transfusion (GTX) is one of the best therapeutic modalities. Granulocytes harvest using conventional Acid Citrate Dextrose (ACD-A) anticoagulant by apheresis is not satisfactory in comparison to use of hydroxyethyl starch (HES) but later is associated with various adverse events, especially with high-molecular-weight (HMW) HES.

Aims: To compare the two granulocyte harvest methods with use of ACD & HES by apheresis using spectra optia. To assess the beneficial impact of MMW-HES over ACD in granulocyte apheresis.

Methods: In this retrospective study, donors who received ACD were included in ACD group and others who receive HES were grouped as HES during granulocyte harvest. Inj. G-CSF 10 µg m/kg given 10–12 h before and tablet dexamethasone 8 mg given with breakfast for both the groups donors, before harvest. Numbers of adverse incidents observed and were noted, if occurred. Donor/procedure parameters were compared using Mann–Whitney U test/unpaired t test.

Results: Granulocyte yield (mean 3.29×10^{10}/unit-ACD vs. 4.5×10^{10}/unit-HES, p value =< 0.0001), is significantly better in HES group. The collection efficiency is also better in HES group (mean 15.86%-ACD vs. 26.70%-HES; p value =< 0.0001) in ACD and HES groups respectively). There was no occurrence of any adverse events between ACD and HES groups.

Summary/Conclusions: In our study, granulocytes with optimum yield can be easily harvested with Spectra Optia cell separator by using 6% HES (MMW) and tri-sodium citrate combination with standard interval gap between mobilization to harvest. This strategy also has no extra cost burden to patients.

P114 | 'Ironing out the risk': Assessing the effect of apheresis donation frequency on iron stores in Asian donors

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Background: Published research has established that whole blood donors experience a progressive decline in iron reserves as the frequency of donation increases. Although apheresis donors lose fewer RBCs than blood donors, they may be at risk for iron depletion because of the higher donation frequency and chronic, small-volume red cell losses (blood samples for lab tests and residual losses in the apheresis kit ≈80–100 mL per procedure). Consequently, this may result in an annual blood loss of 2400 mL (about 4–5 blood donations) if they donate at the maximum allowable frequency of 24 times per year.

Aims: To study the correlation between donation frequency and iron/haematological indices, and assess the predictive capability of haematological indices to detect iron depletion.

Methods: 150 Repeat donors and 30 First time donors (FTD) were recruited for a prospective observational study. Based on their donation frequency in the preceding year, repeat donors were categorized into Repeat Infrequent Donor (RID) and Repeat Frequent Donor (RFD) groups. A structured enrolment questionnaire was administered to all participants which collected information regarding their platelet donation history, smoking habits, dietary and medication history. CBC, Reticulocyte Indices, Iron Indices, Vitamin B12 and Folate assays were conducted on the samples collected from the donors.

Results: Enrolled donors were stratified into three groups based on their Serum Ferritin (SF) Levels- Donors with Iron Replete (DIR) status, Donors with Low Ferritin (DLF) and Donors with Absent Iron Stores (DAIS). Our study showed the highest proportion of DLF and DAIS in the RFD group and notably, a few donors in the FTD group also had low/absent iron stores.

P114 - Table 1 – Pearson correlation coefficients (CBC & iron indices with lifetime & recent donations)

Indices	Lifetime donations	Recent donations (Last 12 months)
HCT	−0.204	−0.208
RDW	0.149	0.013^
PLT	0.115^	0.162
Reticulocyte%	−0.042^	−0.082^
Serum Iron	−0.169	−0.195
Ferritin	−0.196	−0.346
TIBC	0.209	0.235
Transferrin Saturation	−0.225	−0.265

^ represents non-significant results, (Significant @ $p < 0.05$).

P114 - Table 2 – Area under the ROC Curve (AUC) using SF as the reference standard

	SF cut-off values	
Haematological indices	**<15 ng/mL**	**<30 ng/mL**
Hb	0.744	0.693
HCT	0.732	0.66
CH	0.743	0.689
RDW*	0.667	0.644
%Hypo*	0.685	0.646
CHr	0.667	0.59
CHm	0.69	0.619

* RDW & %Hypo were inversely related to SF.

- FTD: 30 donors, 22 (73.3%) DIR, 5 (16.7%) DLF, and 3 (10%) DAIS
- RID: 75 donors, 47 (62.7%) DIR, 16 (21.3%) DLF, and 12 (16%) DAIS
- RFD: 75 donors, 16 (21.3%) DIR, 35 (46.7%) DLF, and 24 (32%) DAIS

We tested the Iron and CBC indices for correlation with recent and lifetime donations. All Iron indices showed significant correlations with both recent and lifetime donations but only a few CBC parameters showed statistically significant correlations.

Further, we also used ROC analysis to evaluate the ability of haematological indices to predict iron depletion. SF was used as the gold standard reference, and cut-off values of 15 and 30 ng/mL were used in the ROC analysis. Our findings showed that Hb and CH indices were the most reliable in predicting iron depletion, and using a SF cut-off value of 15 ng/mL enhanced the predictive performance of all haematological indices.

Summary/Conclusions: Regular apheresis donation can lead to different degrees of NAID, which can vary depending on the geographic region. Thus, the present frequency of 24 donations/year may not be advisable for maintaining normal body iron levels considering the genetic, environmental and dietary factors in our population. Alternatively, donor centres can opt to regularly monitor donor SF levels and ideally maintain them >30 ng/mL so as to safeguard the health of altruistic individuals.

P115 | Simulated effects of ferritin screening on hs-CRP in recruited donors

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Background: Too frequent blood donation can deplete body iron stores, causing adverse health effects. To address this, some blood services have adopted ferritin screening as part of their donor management policies. While ferritin is a commonly used marker for iron stores, ferritin levels can also be elevated by inflammation, positively correlating with C-reactive protein (a serum marker of acute phase response to infection/inflammation).

Aims: We hypothesize that a ferritin-based blood donor selection policy might increase the average level of inflammation in a blood donor population. Using Finnish general population cohorts, we aim to quantify this effect by measuring CRP levels as a surrogate for inflammation. The study's goal is to estimate the impact of implementing a ferritin cut-off on the proportion of donors with hs-CRP over 3 mg/L.

Methods: We analysed ferritin and hs-CRP measurements in two Finnish national health survey cohorts, FINRISK 1997 and Health 2000, including only potential blood donors who met eligibility criteria, to simulate selection of new blood donors from general population. Participants were divided into subgroups based on sex and menstruation status. To determine the impact of ferritin filtering on the proportion of potentially inflamed donors, we examined hs-CRP levels over the commonly used threshold of 3 mg/L for low-grade inflammation in subpopulations filtered by ferritin lower bounds ranging from 0 to 50 µg/L, with a step size of 1 µg/L. We estimated 95% confidence intervals using bootstrapped normal approximation (sample size 10,000) and evaluated if the proportion of inflamed potential donors differed significantly between no ferritin filtering and 15, 30, and 50 µg/L ferritin filtering.

Results: Our results show statistically significant differences in the proportion of potentially inflamed potential donors at various ferritin screening levels. For instance, a ferritin cut-off of 30 µg/L for menstruating women would result in a 2.3%–6.3% point increase in the proportion of individuals over the threshold for low-grade inflammation based on the FINRISK 1997 cohort. Similar increases were observed at ferritin screening levels of 15 and 50 µg/L, and for non-menstruating women. The increases for men were smaller but still significant.

Summary/Conclusions: Our simulation suggests that using a ferritin cut-off to screen blood donors from the general population will result in an increase in the proportion of individuals with low-grade inflammation. These donors may not be able to tolerate the physiological stress of blood donation and maintain long donation careers. Additionally, research suggests that high iron status may also be associated

with higher disease risks, further exacerbating the issue. Countries that have implemented ferritin measurement policies are in a perfect position to study this hypothesis and determine if a hs-CRP measurement should be used alongside ferritin measurement to address these concerns.

P116 | Impact of plasma unit weight and donor features on post-donation citrate level: From an experimental study towards the development of a personalised donation program

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Background: Although plasmapheresis has been proved to be safe and well tolerated for donors the effect of anticoagulant reinfusion on ions chelation, and consequently on bone resorption and deposition, remains not completely elucidated. The amount of anticoagulant (volume of ACD-A) delivered to the donors and to plasma units depend on haematocrit (HCT), total processed blood, amount of plasma collected, as well as on donor characteristics.

Aims: The study presented here aim at assessing: (i) The possible effect of the unit weight setting on plasmapheresis efficiency and ACD-A distribution. (ii) The differences between male and female donors and the effect of the donors characteristics, differentiating between male and female donors groups, in the citrate levels post donation. Based on the obtained results it is developed a statistical model for a personalised donation approach.

Methods: To reach the first aim, male donors donating 700 and 720 g of plasma in two different sessions, 56 men donating 720 g and, 12 months after, 700 g of plasma, were enrolled in the study. For the second aim, 46 women donating 700 g of plasma were also recruited in the study. The parameters evaluated were: the measured citrate (through aspectrophotometric citrate kit) post/pre-donation (Δ), the ACD-A volumes and the general donors' features (e.g., HCT, age, etc.).

Results: Herein we proved that the whole blood processed by the machine increased of 44 mL shifting from the 700 g to the 720 setting, while the donors in the 720 g group were reinfused with 12 mL more ACD-A. Similarly, the mean post/pre-donation Δ citrate concentration in the 720 g was 1.5 times higher (12 mM) and with a larger variability; Moving to the second aim, we observed higher (and highly variable) Δ citrate and re-infused ACD-A in women, concomitantly with lower anticoagulant in the unit. Increased post/pre-donation Δ citrate is inversely associated to HCT and age in men, and with HCT and triglycerides in women. Re-infused ACD-A correlates with HCT in women but not in men. Based on these elements we built a statistical model to predict whether a subject might have a high probability to slowly metabolize citrate.

Summary/Conclusions: The results presented pave the way for the development of personalised donation settings aimed at maintaining similar safety profiles for all donors.

P117 | Abstract withdrawn

P118 | Prevalence of iron deficiency among Swedish platelet and plasma donors

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Background: As standard regimen, the iron levels of Swedish whole blood donors (WBD) are monitored and when necessary donors are offered oral iron supplementation. The same routine do not apply to apheresis donors (AD) donating plasma or platelets although it is associated with a concomitant loss of red blood cell volume. The volume lost during apheresis donation is marginal compared to the loss during whole blood donation. On the contrary, AD are allowed to donate at a considerably higher frequency compared to WBD. Therefore, the cumulative losses over time might have to be considered when regarding blood donor health.

Aims: The main purpose of the study is to assess the prevalence of iron deficiency (ID) among AD in the region of East Gothia (Sweden) as part of a quality follow-up.

Methods: This is a retrospective single centre study regarding AD. From existing data the following parameters were extracted; plasma ferritin levels, number of donations, concomitant whole blood donation and when applicable also haemoglobin levels. We defined a period of six-month for data retrieval.

Results: The parameters described above were identified from 118 apheresis donors; 93 unique platelet donors and 25 unique plasma donors. The total frequency of ID among the apheresis donors was 11% ($n = 13$); platelet donors 12% ($n = 11$); plasma donors 8% ($n = 2$). Mean ferritin value among donors with ID was 27 (11–33) ug/L. None of them had anaemia. No significant difference in ID-frequency was observed between platelet and plasma donors. For platelet donors with ID 91% ($n = 10$) also committed to whole blood donation whereas 9% ($n = 1$) did not. There was no significant difference between these groups regarding ID-frequency, however mean ferritin values were significantly lower ($p = 0.0004$) among those who also gave whole blood compared to those who did not (61 vs. 89 µg/L). Among plasma donors there was no significant difference regarding ID-frequency or mean ferritin levels between those who also gave whole blood donations and those who did not. We found a significant moderately negative correlation between the number of AD and ferritin values for all apheresis donors that did not also commit to whole blood donation during the follow up period ($p = 0.02$).

Summary/Conclusions: The prevalence of ID was 11% among our apheresis donors where the majority of these also gave whole blood donations concomitantly. We found a negative correlation between the number of apheresis donations and ferritin levels for apheresis donors that did not also commit to whole blood donations. As a part of the improvement of blood donor health, this study shines a light on the need to acknowledge and evaluate the possibility of iron

deficiency among apheresis donors and especially those who also commits to whole blood donation.

P119 | Features of iron metabolism in regular blood donors in a dry hot climate

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Background: Despite the implementation of the State program for the development of voluntary unpaid blood donation in the Republic of Tajikistan, there remains a shortage of donors, especially regular unpaid ones. The main reason for the temporary suspension of regular donors from blood donation is low haemoglobin (Hb) level associated with the widespread prevalence of latent iron deficiency, especially in female donors. The study of iron metabolism disorders in blood donors remains relevant, since the most common cause of donations withdrawal is anaemia in condition of a dry and hot Republic of Tajikistan climate, where natural iron losses are higher.
Aims: To identify iron metabolism features in regular blood donors, and to develop recommendations for the iron deficiency and anaemia prevention in regular blood donors.
Methods: The serum ferritin (SF) concentration was determined in 1800 regular blood donors (600 men, 1200 women). 1000 of them received iron-containing drugs in capsules and tablets (Fenyuls, Ferrum Lek, Tardiferon, Maltofer) in a single dose of 250–300 mg after each blood donation as an anaemia prophylaxis (group I), 800 did not receive prophylaxis (group II). In donors withdrawn from blood donation due to low Hb—in men <130 g/L, in women <120 g/L ($n = 400$; 150 men and 250 women)—in accordance with the national standards of the Republic Blood Service of 2005, the levels of SF (radioimmune analysis), erythropoietin (EP) and soluble transferrin receptors (sTfR) (chemiluminescent immunoassay technology) were examined. The donors' age ranged from 18 to 65 years.
Results: The SF content in regular male donors was 60–130 ng/L, in women 40–95 ng/L. In group I an increase in the SF level compared to baseline indicators was revealed in 851 regular blood donors, in 102 donors SF did not change significantly, in 47 donors decreased. The SF decrease was detected only in female donors. In group II—donors who did not receive iron supplements after 4–8 blood donations, an increase in the level of SF was noted in 45 donors, mainly male donors, a decrease in 544, mainly women, unchanged in 211 donors (mainly men). In male donors withdrawn from donation, the Hb level was in the range of 114–126 g/L, in women—92–116 g/L. SF decrease was detected in 311 out of 450 examined donors, the average value in men was 1.75, and in women 4.3 times lower than that in the control group (healthy primary donors). In 215 donors, SF did not exceed 14 ng/mL. An increased level of sTfR was detected in 145 out of 450 donors, which was also combined with a low SF concentration. The sTfR values ranged from 28.12 to 52.44 nmol/L (norm 7.89–29.51 nmol/L), the median exceeded the control level by 2.62 times and was 40.64 nmol/L, respectively, versus 15.80 nmol/L in the comparison group. The EP concentration was higher than the threshold value in 220 of 450 donors with low SF values. In 58 cases, this was combined with a high sTfR index. The remaining individuals had EP within normal values, but the median EP in donors was 2.42 times higher than the median of the control group.
Summary/Conclusions: The preventive therapy with iron-containing drugs in regular blood donors, especially women, is an effective measure to minimise the risk of withdrawal from blood donation associated with the development of iron deficiency and iron deficiency anaemia.

P120 | Abstract withdrawn

P121 | Abstract withdrawn

P122 | Donor notification, responses, and follow-up: Status in India

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Background: Notification, counselling, and follow-up of seroreactive donors are an essential part of the donor management. Not only it helps such donors by seeking early investigations and management, but also prevents them from future donations. Thus, reducing the risk of transfusion transmissible infections (TTI) and the efforts/resources directed for its identification. In India, large proportion of donors are identified as sero-reactive (1.5%–2%) every year. Although donor notification process is mandatory, the data regarding notification rates, their responses or counselling, visits to the referral facilities and their follow-up treatment are unclear.
Aims: The aim of this study was to evaluate the donor notification process, counselling and follow-up of seroreactive donors among various observational studies reported from India. We also evaluated the challenges faced in the process and possible strategies to improve it.
Methods: Of 25 articles identified in search on PubMed, Scopus and Google Scholar, 16 were included for assessment of notification mechanism, notification rates, response rates to notification, reasons for non-responsiveness, assessment of high-risk behaviours during counselling and follow-up of referred sero-reactive donors for HIV/HBV/HCV and syphilis.
Results: Overall sero-prevalence for TTI marker was 1.52% (6888 donors), this included 0.15% HIV, 0.78% HBV, 0.37% HCV and 0.22% sero-reactives for Syphilis. Repeat testing, prior to notification was done for 24.3% (1673) donors. Notification using both phone and letters was the commonest method (50%), followed by only telephonic notifications in 43.8% studies. This resulted in overall notification rate of 71.2%. Despite this, only 33.2% (2292) donors responded by attending the post-test counselling at blood centres. The response

rate for HIV sero-reactivity (42.1%) was higher compared to HBV (31.5%), HCV (31.7%) or syphilis (35.8%). Common reasons for low responses included wrong/missing contact details, distance or busy schedule of the donor.

History of risk factors for TTI reactivity was available for 6 (37.5 %) studies with 631 donors being counselled, of which 76.9% (485) donors gave significant history for an increased risk of TTI sero-reactivity.

Data regarding follow-up of seroreactive donors was evaluated amongst 1452 referred donors, which resulted in 64.9% (943) donors attending the referral centres. Of these, 55.5% (606) were receiving treatment whereas, others were either found to be negative or were lost during follow-up visits.

Summary/Conclusions: Blood centres are often viewed as community health resources. To improve the overall donor's health and the transfusion safety, perseveration for care of sero-reactive donors is required. Inclusion of pre-donation information and education TTI notification, mode of communication, importance/impact on the donors (household contacts) and the transfusion services should be made mandatory and monitored regularly. Alongside a network of referral facilities or setting-up of donor health clinics at the transfusion centres should be encouraged. It will also help in reducing the loss of donors due to false positive sero-reactive donors and enable framing of re-entry algorithms for blood donors into the eligible donor pool.

P123 | Controlled task shifting in the blood donor eligibility assessment experienced by senior trained nurses at the Finnish Red Cross Blood Service

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Background: The Finnish Red Blood Service (FRCBS) is a national blood establishment in Finland. FRCBS operations cover donor recruitment, blood collection, manufacturing and distribution of blood units and labile blood products needed in Finland.

In 2014, the FRCBS introduced a new operating model for assessing the eligibility of blood donation also known as controlled task shifting in the blood donor eligibility assessment; ten experienced nurses were trained as the first senior trained nurses and became primary consultants instead of physicians. The senior trained nurse model in assessing the eligibility of blood donation has reduced the number of consultant calls to physicians but has not affected the safety of blood donors. This operational change has helped to develop a more appropriate division of labour between physicians and senior trained nurses in the FRCBS.

Aims: The purpose of the study was to describe and explain the experiences of senior trained nurses from the task shifting. The aim was to interpret and understand the connections between experiences and find out what kind of development targets emerge from controlled task shifting.

Methods: The study was made by using quantitative methods. In November 2021 all the senior nurses who have been trained for the blood donor eligibility assessment since 2014 and who still work in the FRCBS were invited to complete study questionnaire ($N = 23$). 18 nurses responded; the response rate was 78%. The statements in the questionnaire covered the following topics: (1) initial orientation and further training received for the task shifting, (2) development at work, utilisation of competence, (3) working conditions and (4) compensation as the demand for work increases. The results were analysed by statistical methods. The background data of the subjects were used in cross-tabulation.

Results: Age, education or work experience were not relevant to the experiences of senior trained nurses. The duration of employment in the FRCBS or in which unit the senior trained nurse works also did not affect the experience.

The results of the study show that working as a senior trained nurse improves work motivation. It also increases the interest and demanding nature of the work. Senior trained nurses perceive the task shifting as a mainly positive development when training and orientation are appropriate and sufficient. Work experience or age of a senior trained nurse do not determine whether a senior trained nurse experiences mental strain or insecurity in her work. This is partly explained by the fact that the position of senior trained nurse is applied for according to one's own interests and has not been prescribed by a supervisor, for example.

Summary/Conclusions: In conclusion, the controlled task shifting in the FRC Blood Service has been successfully completed and the senior trained nurses are satisfied with their new role. However, continuous further training is important. The special factor allowance for senior trained nurses should also be evaluated and possibly increased at regular intervals.

P124 | Abstract withdrawn

P125 | Post donation information in haemovigilance: The French experience

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Background: Post donation information (PDI) is defined as the knowledge of information about the donor or his donation, occurring after it, which challenges quality and/or safety of the blood components (BCs) stemming from the current or previous donations. Depending on the nature of the PDI, BCs are recalled or not for destruction. PDIs are mostly provided by donors themselves and are managed by blood establishments (BEs) (PDIs notified to BEs). If at least one BC has been released and the information of the recipient is required (prescriber,

P125 – Table 1

	2013	2014	2015	2016
Number of PDIs notified to BEs	14,776	16,076	16,825	18,549
Number of PDIs reported to ANSM (e-FIT)	1570	1565	1730	1911
Percentage of PDIs reported to ANSM	10.6	9.7	10.3	10.3
Incidence of PDIs reported to ANSM per 100.000 donations	55.0	55.4	58.7	66.4

P125 – Table 2

2017	2018	2019	2020	2021
18,945	19,038	19,244	18,775	17,745
1888	1860	1955	1972	1918
10.0	9.8	10.2	10.5	10.8
66.9	64.3	67.9	70.7	70.1

Note: Data for 2022 will also be shown.

manufacturer of plasma derived medicinal products), a reporting to the ANSM (French National Agency for the safety of medicines and health products, the national competent authority) is necessary, via e-FIT (French national haemovigilance tool for the reporting on line of adverse reactions, events and PDIs) (PDIs reported to ANSM). The analysis of PDIs allows also to figure out the future of the donor (e.g., temporary deferral, permanent deferral for donation).

Aims: To present the organisation and the evolution over the years of the management of PDIs in France. To develop the characteristics of PDIs over the years (source and nature of the PDI, part of BCs recalled, transfused and follow up of the recipients of BCs).

Methods: Overview of the evolution of the management of PDIs over the years. Analysis of PDIs notified to the BEs and PDIs reported to the ANSM (via e-FIT) over the period 01/01/2013 to 12/31/2022, per year.

Results: PDIs have been reported to ANSM via e-FIT since late 2012. From 2013 to 2021, 159,973 PDIs have been notified to the BEs. Among them, 16,369 PDIs (10.2%) have been reported to the ANSM, representing an incidence of 63.9 PDIs per 100,000 donations. The majority concerns an infectious risk (infection in the donor, like gastro enteritis, or donor at risk of infection, such as a history of transfusion). Following PDI, about 25% of the BCs have been recalled. Regarding BCs that have been transfused, look back investigations showed an adverse reaction (AR) in recipients in less than 1% of the PDIs. The analysis by year of the characteristics of the PDIs will be presented.

Summary/Conclusions: The reporting system of PDIs was put in place in 2003 between BEs and ANSM. The management of PDIs has been carried out continuously since 2003. It shows that the donation may have been infectious or that any other information not disclosed during the pre-donation interview and examination may render the previous donations at risk for transfusion. It also allows appropriate measures to be taken to mitigate the risk for recipients.

P126 | Ferritin concentration in repeat donors in South Western part of Slovenia: Prospective study

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Background: Our Blood Transfusion Centre located in South Western part of Slovenia has a double responsibility; we secure safe blood products for patients and perform blood banking for four hospitals in the region, on the other hand we have a responsibility to assure enough blood donors and at the same time to diminish the risk of developing health problems related to frequent whole blood (WB) donations.

Aims: We wanted to determine how frequent donations influence blood donors' potential iron deficiency.

Methods: The study was performed form March till September 2022 in Blood Transfusion Centre of Izola. During this period of time approximately 2500 blood donors donated WB; some of them donated WB more than once. We designed a prospective study, which was approved by Slovenian National Ethical Commission in December 2021 (No. 0120-519/2021/3). Inclusion criteria were: ≥45 donations for male (M), ≥25 donations for female (F) donors, frequent donations, drop in Hb for more than 20%, and trend in lowering of Hb concentration. Despite the wider initial inclusion criteria we decided to enrol donors mainly according to number of donations. We enrolled 114 blood donors within 7 months; 75% male and 25% female, who fulfilled the inclusion criteria. On a day of donation a blood sample was taken for total blood count, ferritin and CRP determination. In case of anaemia donors were deferred and informed by a physician. If necessary donors were referred to family physician for further treatment. 14 of mentioned donors who returned to our centre after 3-months period were re-tested for all above mentioned tests. Two of them were excluded from the study because of high CRP level (possible infection) and iron supplement intake.

Results: Average age of blood donors was 54.6 years (for M 54.2 years and F 55.5 years). Average number of donations were 69.3 (for M 77 times, F 46.2 times). Average Hb concentration was 150.1 g/L (for M 153 g/L, F 141.2 g/L). Average ferritin concentration was 46.6 μg/L (for M 50.9 μg/L, F 33.7 μg/L). Lowest ferritin within male donors was 8.43 and 6.23 μg/L for female donors. Highest observed ferritin was 222.37 μg/L for a male blood donor who had 3.5 years of no-donation interval and 64.61 μg/L for a female donor. 12% of all blood donors had ferritin concentration below 15 μg/L (WHO threshold). Lower level of ferritin concentration (M < 30 μg/L) had 34.7% of male donors, and (F < 20 μg/L) 20% of female donors.

We noticed ferritin lower than 10 μg/L in 7% of blood donors (4 M & 3 F), who were in average 49 years old and have donated in average 64 times. The youngest blood donor was 32 and donated 51 times. His ferritin concentration was 9.88 μg/L.

Summary/Conclusions: Iron deficiency was noticed in an important part of frequent blood donors. The most important reason for lower ferritin concentrations was a frequency of donations and not the overall number of donations. Transfusion centres in Slovenia do not propagate the iron supplement intake. But our study shows that very frequent blood donors should have benefit form iron supplement. We wonder if it's ethical for someone to take dietary supplements just to be able to donate blood.

P127 | Increasing the upper age limit for blood donation: Perspectives from older donors

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Background: To protect the safety of both blood donors and recipients, various blood establishments have instated an upper age limit for donating blood. Nonetheless, evidence of safe donations at higher age is slowly building up, donors seem willing to keep donating after their 70th birthday and retention of these, often very loyal donors, is of importance to blood banks. In the Netherlands, as of April 2018, the upper age limit for blood donation has been raised from 69 to 79 years, providing an opportunity to study the safety of blood donation at higher age, as well as donor perspectives regarding continuing to donate.

Aims: This study aims to explore whether older donors agree with the increase of the upper age limit, if they feel obliged to continue donating, to identify their motivators and barriers for donating blood, and self-reported donation related symptoms.

Methods: An online survey was distributed among Dutch blood donors aged 68–73 year. The survey contained questions about opinions regarding the increase of the upper age limit, motivations and barriers for donating, experiences with donation-related symptoms and obligatory feelings to continue donating.

Results: A total of 662 donors (55%) were included in the analyses, of which 40 (6%) stopped donating. The majority of donors (92%) agreed with the increase of the upper age limit. Approximately 63% of participating donors felt obliged to continue donating, especially women (OR 2.6; CI 95% [1.7–3.9]) and donors with lower education (OR 0.4 (CI 95% [0.2–0.7]) and OR 0.6 (CI 95% [0.4–0.9]), for middle and high education, respectively). Donors indicated they felt healthy enough to keep donating (95%), and 72% of donors indicated they thought it is good for their health to keep donating. Only 5% of donors reported that they found it hard to keep donating blood or plasma and 3.4% indicated that they did not feel healthy enough to donate blood or thought it was not safe for them anymore. The most self-reported negative donation related symptoms were feeling thirsty (4.3% often/always, 18.0% sometimes), bruising (1.6% often, 10.4% sometimes), and scarring (2% often/always, 7.9% sometimes).

Summary/Conclusions: Older donors agree with the increase of the upper age limit for blood donation and feel healthy enough to keep donating blood. However, a large number of donors feel obliged to keep donating. Therefore, blood banks should be aware that donors keep donating blood completely voluntarily and do not feel pressured to continue, as donors might have doubts about safety or health issues.

P128 | Investigating donor and donation characteristics influencing prediction error of haemoglobin and ferritin trajectories for blood donors

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Background: Whole blood donors with low haemoglobin (Hb) levels are currently deferred from donation and donation intervals are extended based on ferritin measurements. Donor deferral is demoralizing for the donor, costly for the blood bank, and potentially threatening to the blood supply. Inviting donors based on prediction models has the potential to prevent deferral from donation.

Aims: In this study the accuracy of predicted Hb and ferritin trajectories and the contribution of donor and donation characteristics to the prediction error of Hb and ferritin values are analysed.

Methods: Our prediction model was applied to whole blood donors with ferritin measurements available at the start of their donor career and at least once thereafter. The model input consists of ferritin at first donation, the average of the first two Hb measurements, sex, weight, height, date and volume of donations. The prediction error is calculated as the difference between measured and predicted Hb levels in mmol/L and the ratio of the log10 measured and predicted ferritin levels. We calculate the average absolute prediction error and standard error per donation number. The contribution of donor and donation characteristics on the prediction error is analysed using coefficients of linear regression models and SHapley Additive exPlanations (SHAP) values from a random forest model.

Results: We predicted Hb and ferritin trajectories for 29,405 donors with 224,749 donations. The model can predict Hb with a slowly increasing prediction error (0.40, SE 0.31–0.59, SE 0.40; Table 1). There is no clear trend in the average prediction error of ferritin (0.96, SE 0.41–1.74, SE 0.91) as the number of donations increases. The linear regression model reports the strongest association between the prediction error of Hb and the measured Hb value (0.48, $p < 0.05$), difference with the first donation (−0.18, $p < 0.05$), difference with the previous donation (−0.48, $p < 0.05$). For log10 ferritin, we observe

the strongest association with the measured value (2.80, $p < 0.05$), difference with the previous donation (−0.53, $p < 0.05$), donation number (0.11, $p < 0.05$), and female gender (0.13, $p < 0.05$). The SHAP values identify the same important factors for the prediction error of Hb and ferritin, respectively: difference with the first donation (0.1; 0.05), difference with the previous donation (0.05; 0.01), the measured value (0.03; 0.01). Donation number is also important for Hb (0.04).

Summary/Conclusions: This study shows that our model can predict Hb values of whole blood donors up to well after the initial measurement with a modest increase in average prediction error. The prediction error is mainly influenced by donation characteristics, such as the measured values and change over time. Our model may therefore in future be used to derive optimal personalised donation strategies.

P129 | Abstract withdrawn

P130 | CD4+ lymphopenia among French cytapheresis donors

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Background: Studies have shown a profound and durable CD4+ lymphopenia among cytapheresis donors and that donors using an LRS chamber were at increased risk of immunosuppression-related and common bacterial infections in a dose-dependent manner. In France, two separators are being used, with or without a LRS chamber, providing the opportunity to compare the relative frequency of cytapheresis associated lymphopenia.

Aims: To assess the frequency of profound CD4 lymphopenia among cytapheresis donors in France.

Methods: French donors are allowed to donate up to 12 times/year. In general, repeated CP donations by a given donor are with the same separator. The mean number of CP donations/donor/year is 2.4, with no difference whatever the separator used.

Lymphocyte counts (LC) were performed on all cytapheresis ($n = 4251$) and randomly selected whole-blood (WB; $n = 1232$) and plasmapheresis (PL; $n = 986$) donations collected between 06/01/2022 and 06/20/2022.

Lymphocyte counts are performed on all cytapheresis (CP) donations in France since 05/2021. LC results from 05/2021 to 12/2022 were analysed according to the type of separator used for CP collection. From 03/2022, leucocyte immunophenotyping was performed on all CP with a LC < 0.8 g/L in a single laboratory, except for those collected on Fridays, due to transportation durations from collection sites to the laboratory. All donors were eligible and found healthy at time of donation.

Results: From 05/2021 to 12/2022, 139,329 CP involving 49,895 donors were collected on Amicus or Trima. 1044 CP (0.75%) were found with LC < 0.8 g/L: 425/50,170 Amicus CP (0.85%) versus 619/89,159 Trima CP (0.69%; $p < 0.01$). Corresponding donations were from 625 different donors (1.25%): 256/18025 Amicus donors (1.42%) versus 369/31,870 (1.16%) Trima donors ($p = 0.01$). 494 immunophenotyping results were available from 335 donors (135 Amicus; 200 Trima). 24/335 (7.2%), 117 (34.9%), 189 (56.4%) and 5 (1.5%) donors had CD4+ lymphocyte counts <200, 201–300, 301–500, and >500/mm^3 respectively. Among donors with immunophenotyping results, 11 Amicus and 13 Trima donors had CD4+ <200/mm^3, which was not statistically different. Their median number of CP donations was 18 (range: 1–146; 16 for Amicus donors; 23 for Trima donors), with a median time since their 1st CP donation of 6.5 years (range: 0–30.8; 8.24 for Amicus; 4.55 for Trima).

Summary/Conclusions: Lymphopenia seems to be associated with apheresis donations. We found that lymphopenia <0.8 g/L was more frequent with the use of Amicus separators compared to Trima separators using a LRS chamber. However, CD4+ lymphopenia occurred in cytapheresis donors whatever the type of separator used.

Blood donation

Blood collection including apheresis

P132 | Donor thrombocytapheresis in the institute for transfusion medicine of the Republic of Srpska, Bosnia and Hercegovina

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Background: With the procedure of donor thrombocytapheresis, a healthy person donates blood through an automatic blood cell separator, which is programmed to separate and collect the desired number of platelets while simultaneously returning all other blood components.

Aims: The objective is to present data on collected apheresis platelets at the Institute for Transfusion Medicine of the Republika Srpska in the period from February 2021 to January 2023.

Methods: Separation of apheresis platelets was performed on the TRIMA ACCEL 7 automatic blood cell separator, using sets (LRS, Platelet/Plasma) for single use and collection of leukoreduced platelets.

Results: In the analysed period, 136 donor thrombocytapheresis were performed from 56 voluntary donors, in which the platelet

concentrates obtained had a yield of 3–4.5 × 10^{11}. The apheresis procedure was performed once in 25 (44.64%) donors, more than once in 31 (55.36%) donors, during the legally prescribed period of every 15 days. Out of the total number of performed apheresis, during 120 (88.24%) procedures two units of platelets were separated, and in 16 (11.76%) one unit. The average age of the donors was 36 years, the average haematocrit value before the procedure was 44.02%, after the procedure it was 42.68%, average haemoglobin value was 150.81 g/L, platelets before the procedure 239.15 × 10^9/L, after the procedure 190.74 × 10^9/L, processed volume was 2522.4 mL, used AC 273.12 mL. The procedure lasted for 43 min in average, with an average of 392.3 mL separated of the final product with leukocytes below 1 × 10^6. We pathogenically inactivated 39 (28.7%) platelet concentrates collected in nutrient solution (SSP+) with a volume of over 300 mL on the INT100 INTERCEPT Illuminator.

Summary/Conclusions: Platelet concentrates obtained by the procedure of donor thrombocytapheresis meet all quality requirements in accordance with the latest recommendations from the Council of Europe. Considering the great clinical importance of apheresis platelets, our plan is to increase the number of apheresis procedures and expand our existing register of voluntary platelet donors.

P133 | EFS first evaluation of a new platelet apheresis system

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Background: The French Blood Establishment (EFS) has the monopoly in France over the collection, testing, preparation and distribution of labile blood products. Approximatively 80,000 platelet apheresis procedures are performed each year including the use of the Amicus since the 2000 s. The AmiCORE (Fresenius Kabi) device is the new generation of apheresis platelet separator intended to replace the current Amicus.

Aims: The objective was to perform a destructive evaluation of the platelets stored in PAS & Concurrent Plasma (cPlasma) obtained with AmiCORE to support the first step application for regulatory approval by French authorities (ANSM).

The quality of the platelets and cPlasma were evaluated according to the French regulatory requirements. Additionally, platelets needed to be compliant to the quality recommendations for pathogen inactivation (Intercept).

Methods: Two AmiCORE devices (SW 2.1G) collected a minimum of 30 platelets and cPlasma products (EFS Occitanie). 15 platelets were stored in InterSOL and 15 in SSP+ with a PAS/Plasma ratio of 65/35%. A sample of both platelets and cPlasma has been taken for analysis. All platelet products have been treated with the pathogen inactivation method (Intercept) within the next 18 h after the end of collection. Plasma was frozen within the next 18 h after the end of collection.

Before and after donation, a sample has been taken from the donor to measure cytological and haematocrit parameters. Donor reaction to citrate has also been followed.

Results: A total of 34 apheresis donations were performed on AmiCORE with a ratio of 18 men and 16 women. No adverse effects other than those typically associated with an apheresis procedure and related to citrate injection had been observed.

Summary/Conclusions: The quality results obtained with AmiCORE separator show the possibility to obtain leukoreduced platelets stored in PAS complying with the French regulatory requirements and the recommendations for the Intercept treatment.

The cPlasma collected with the AmiCORE separator also complies with the French regulatory requirements applicable to category one plasma for fractionation intended for the extraction of labile proteins.

The results were submitted to ANSM which issued favourable notice to initiate the second routine evaluation phase targeting final approval for AmiCORE apheresis system.

P133 – Table 1

	Parameters	Mean (SD)	Requirements or intercept recommendations
Procedure	Procedure time (min)	66.2 (7.9)	/
	Target yield (10^{11}\U)	4.8 (0.8)	/
	Actual yield (10^{11}\U)	4.34 (1.06)	/
	Ratio target yield/actual yield (%)	91 (17)	/
Platelet $n = 34$ before inactivation	Volume (mL)	370 (31)	300–420 mL
	Yield (10^{11}/U)	4.34 (1.06)	2.5–8.10^{11}/U
	Residual WBC (10^6/U)	0.13 (0.07)	≤1.10^6/U
	% Plasma	36.08 (2.42)	32%–47%
	pH	7.23 (0.08)	/

P133 – Table 2

	Parameters	Mean (SD)	Requirements or intercept recommendations
Platelet $n = 34$ after inactivation	Volume (mL)	325 (29)	≥ 100
	Platelet concentration (G/L)	1204 (223)	≥ 600 G/L
	Yield (10^{11}/U)	3.94 (0.90)	≥2,0.10^{11}/U
	pH	7.08 (0.12)	≥6.4
	Amotosalen	0.38 (0.08)	≤7.5 µM
Plasma $n = 34$ before freezing	Volume (mL)	452 (137)	≥150 mL
	Residual WBC (10^6/L)	0.22 (0.08)	≤1.10^6/U
	Total proteins (g/L)	55.99 (3.44)	≥50 g/L
	Factor VIII (UI/mL)	0.98 (0.25)	≥0.7

P134 | Blood donation in regional center for transfusion medicine in Tetovo

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Background: Although the municipality of Tetovo has a great tradition in blood donation, the last few years were very challenging due to COVID-19 pandemic.

Aims: The purpose of our study is to present and compare blood donation in 2020 and 2021 in Tetovo.

Methods: This is a retrospective study and data was obtained from the blood donations reports in the Regional Center for Transfusion Medicine – Tetovo (RCTM-Tetovo) and informatics software e-Delphyn from 2020 to 2021, including donations with mobile teams.

Results: There were 2239 blood donations in 2020–1724 (76.99%) in the RCTM-Tetovo and 1178 (25.13%) with mobile teams. There were 1762 (78.69%) voluntary and 477 (21.30%) family blood donors, 2058 (91.91%) males and 167 (7.45%) females, 1916 (85.57%) employed, 219 (9.78%) not employed, 49 (2.18%) high school students, 46 (2.05%) university students and 7 (0.31%) retired. According to ethnicity the most of them were Albanians 1107 (49.44%), followed by Macedonians 961 (42.92%) and others 38 (1.69%). There were 137 (6.12%) deferred donors. There were 2448 blood donations in 2021–1785 (72.91%) in RCTM-Tetovo and 663 (27.08%) with mobile teams. There were 1966 (80.31%) voluntary and 482 (19.68%) family blood donations, 2181 (89.09%) males and 267 (10.90%) females. According to social status they were employed 2022 (82.59%), not employed 175 (7.14%), high school students 117 (4.77%), university students 122 (4.98%), retired 12 (0.49%). According to ethnicity there were 1493 (60.98%) Albanian blood donors, 926 (37.82%) Macedonian and 29 (1.18%) others. There were 187 (7.63%) deferred donors.

Summary/Conclusions: The blood supply in RCTM-Tetovo was stabile with slight increase in 2021. There was an increase in the percentage of Albanian blood donors and both, high school and university students in 2021, as well. This was achieved with great effort of our personnel, motivation on many fronts including lectures, presentations, sticking posters in many frequent places in the city and so on. We should continue with our positive work with different targets groups in order to enlarge the blood donors' pool.

P135 | Effect of standard mobilization protocol on yield of granulocyte concentrate collected by apheresis method and it's impact on healthy donors: A prospective study from a tertiary care oncology centre

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Background: To collect optimal therapeutic dose, the granulocyte donors must be stimulated with standard mobilization protocol.

Aims:

- To analyse the correlation between post-mobilization total leucocyte count (TLC) of granulocyte donor and yield of apheresis granulocyte concentrate.
- To analyse the effect of standard mobilizing agent on haematological parameters of the granulocyte donor.
- To analyse effect of apheresis on haematological parameters of mobilized granulocyte donors.
- To analyse mobilization and procedure-related adverse events.

Methods: This was a prospective observational study conducted over a period of 6 months after approval of Institutional Ethics Committee (IEC) and registered under Clinical Trial Registry - India (Registration No: CTRI/2022/08/044779). Total 44 donors were enrolled for the study after obtaining the written informed consent, of which 42 granulocyte harvest procedures were conducted. The mobilization of donors was done using standard mobilization protocol (Inj. G-CSF and Tab. Dexamethasone) and granulocyte concentrate was collected using the apheresis equipment. The Complete Blood Count (CBC) of donors at different stages (i.e., pre-mobilization, post-mobilization/pre-apheresis and post-apheresis) were analysed. Granulocyte donor's demographic details, product-related parameters were analysed. Appropriate statistical tools were applied for analysis and 'p' value of <0.05 was considered as statistically significant.

Results: A total 42 granulocyte harvest procedures were conducted on 42 male donors. Mean age and mean weight of donors were 34 ± 7.66 years and 70.93 ± 11.27 kg, respectively. Tables 1 and 2 illustrates the comparison of the CBC parameters pre-mobilization, pre-apheresis and post-apheresis.

P135 - Table 1: Comparison of baseline CBC with post-mobilization CBC parameters

Parameters	Pre-mobilization [Baseline CBC] (Mean ± SD)	Post-mobilization [pre-apheresis] (Mean ± SD)	*p*-value
WBC ($\times 10^3/\mu L$)	7.78 ± 1.75	32.41 ± 10.07	<0.001
NEUT ($\times 10^3/\mu L$)	4.05 ± 1.25	30.19 ± 9.88	<0.001
PLT ($\times 10^3/\mu L$)	296.57 ± 62.64	314.64 ± 83.83	0.033
RBC ($\times 10^6/\mu L$)	5.19 ± 0.49	5.29 ± 0.42	0.201
Hb (g/dL)	14.44 ± 1.29	14.82 ± 1.19	0.010
HCT (%)	45.49 ± 4.33	46.02 ± 2.85	0.371
LYM ($\times 10^3/\mu L$)	2.58 ± 0.71	0.83 ± 0.84	<0.001
MXD ($\times 10^3/\mu L$)	1.19 ± 0.76	1.15 ± 0.82	0.552

P135 - Table 2: Comparison of pre-apheresis (post-mobilization) and post-apheresis CBC parameters

Parameters	Pre-apheresis (Mean ± SD)	Post-apheresis (Mean ± SD)	*p*-value
WBC ($\times 10^3/\mu L$)	32.41 ± 10.07	32.52 ± 9.61	0.385
NEUT ($\times 10^3/\mu L$)	30.19 ± 9.88	30.04 ± 9.84	0.807
PLT ($\times 10^3/\mu L$)	314.64 ± 83.83	233.29 ± 60.21	<0.001
RBC ($\times 10^6/\mu L$)	5.29 ± 0.42	5.10 ± 0.51	0.001
Hb (g/dL)	14.82 ± 1.19	14.35 ± 1.61	0.003
HCT (%)	46.02 ± 2.85	43.97 ± 4.07	<0.001
LYM ($\times 10^3/\mu L$)	0.83 ± 0.84	1.11 ± 0.87	0.004
MXD ($\times 10^3/\mu L$)	1.15 ± 0.82	1.41 ± 1.08	0.003

Note: A positive correlation was noted between post-mobilization TLC and yield of the granulocyte concentrate ($r = 0.29$). Out of 42 donors, 24% experienced mobilization-related adverse events, primarily myalgia, and 37% experienced procedure-related adverse events, primarily citrate-related, which were mild and self limiting.

Summary/Conclusions: The standard mobilization protocol resulted in a significant increase in pre-apheresis TLC of the granulocyte donor; hence, granulocyte concentrate had optimal granulocyte yield to provide therapeutic benefit in the management of patients with febrile neutropenia. Though there were variations in pre-apheresis and post-apheresis haematological parameters, they were within the normal limits. All the granulocyte donors tolerated the procedure well, without any significant mobilization and procedure-related adverse events.

P136 | Is platelet pheresis donation of anaemia risk? A study in the blood center of Sfax, Tunisia

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Background: Iron deficiency is a well-known adverse event among whole blood donors. However, it remains unclear whether this incident can be observed on platelet apheresis donations.

Aims: We aim to analyse the effect of platelet apheresis on blood haemoglobin (Hb) levels in platelet apheresis donors in order to detect anaemia.

Methods: This is a retrospective exhaustive study of apheresis donor's haemoglobin (Hb) levels carried out at the Blood Center of Sfax, Tunisia over 5 years (2018–2022). Assessment of anaemia signs and Hb levels were based on clinical examination and blood cell count performed before each apheresis donation eligibility check and then the variation of the Hb level between two sessions was analysed. Platelet apheresis donation was initially performed on a discontinuous flow cell separator. Since January 2021, they were either on continuous or discontinuous flow cell separators. In addition to the cell separator type, age, sex, blood group records and Hb variation between two platelet apheresis sessions were studied.

Results: Ninety-nine donors gave 447 platelet apheresis concentrates from whom 44 were regular, the donor's median age was of 40 (Range: 20 to 62 years). The sex ratio was 2.8. Blood groups were: O+ in 36, A+ in 28, B+ in 19, AB+ in 6, A- in 5, O- in 4 and, B- in one donor(s). Regular donors donated a total of 392 platelet apheresis concentrates with an average of nine times per 5 years (extremes 2–44) and of 1.78 times per year (extremes: 0–11). The average Hb variation was equal to −1.19 (extremes: −0.46, −1.95). One patient had developed anaemia before his thirteenth donation or 1% of total donors and 2.3% of regular donors. He was a 41-year-old man with no medical history who provided a total of 44 donations (the average rate was of one donation every 41 days; extremes: 18–89 days). The etiological investigation concluded to iron deficiency due to a low iron diet and repeated donations. He normalized his Hb level after 2 months of iron supplementation and dietary rules. He continued his platelet donations 1 month after the disappearance of anaemia without subsequent relapse.

Summary/Conclusions: Platelet apheresis is considered to be a cause of Hb levels decrease based on the analysis of the average Hb level before and after each donation. Hb levels variation in our study was higher than that reported in the literature (−0.80 g/dL; extremes: −0.75, −0.86 g/dL). And one of our regular donors (1%) developed anaemia whereas studies have shown a decrease in Hb levels without reaching the stage of anaemia.

P137 | Abstract withdrawn

Blood donation

Donor adverse events

P138 | Predicting who is at risk of experiencing vasovagal reactions using pre-donation anticipated emotional states

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Background: Vasovagal syncope reactions range from feeling a little unwell to completely passing out and are often associated with increased anxiety and needle fear. Blood donors systematically underestimate the impact of anxiety and overall emotional states of the blood donation; thus, some physical symptoms cannot be easily explained by biological mechanisms, but manifest from excessive arousal and anticipated emotions.

Aims: The aim of this study is to measure blood donors' somatosensory amplification, anxiety sensitivity, interoceptive awareness and emotion regulation strategies prior the blood donation and predict whether the donor is going to experience low or high levels of VVRs during and after the donation.

Methods: We included three groups of blood donors: (1) a control group, who never experienced VVR in the past ($N = 83$), (2) a 'sensitive' group, who experienced VVR at their last donation ($N = 63$), and (3) new donors, who are at increased risk of experiencing VVR ($N = 164$). All participants completed anxiety sensitivity, somatosensory amplification, emotion regulation and interoceptive awareness questionnaires before the blood donation and self-reported their physiological and psychological vasovagal reactions at several stages during the donation. We split blood donors into a low ($N = 220$) and high VVR group ($N = 90$) based on their self-reported VVR levels during the blood donation and used machine learning techniques with questionnaire scores as features to predict low or high VVR scores of the groups. The dataset ($N = 13$ features) was split into training and testing on which four machine

learning models—decision tree, random forest, XGboost and artificial neural network–were applied. To select the best hyper parameters and estimate the performance nested k-fold cross-validation with GridSearchCV were applied.

Results: The best performance using questionnaire data prior to the donation was achieved using a neural network with an F1 (=the weighted average of precision and recall) score of 0.81. The most important features of this model were scores of physical concerns from the anxiety sensitivity scale, scores from the somatosensory amplification scale, and cognitive concerns from anxiety sensitivity. Both higher anxiety related to physical concerns and higher somatosensory amplification were associated with high VVR scores during and after the blood donation and lower anxiety related to physical concerns and lower scores on the somatosensory amplification scale were associated with low VVR scores.

Summary/Conclusions: This study demonstrates that subjective experience of somatic sensations prior to the blood donation is a highly predictive feature of VVR symptoms during blood donation, confirming the need to reduce anxiety in a timely manner to avoid or reduce adverse reactions.

P139 | Abstract withdrawn

P140 | Novel risk patterns of vasovagal reactions in NZ blood donations complicated by COVID-19 restrictions

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Background: Vasovagal reactions (VVRs) are common but complex donor adverse reactions (DAEs) in blood donations. VVRs have been extensively studied, with a multitude of risk factors identified including young age, female gender and first-time donor status. However, it is not clear how the risk factors interplay.

Aims: This retrospective study aimed to examine VVR risk factors and their interactions using NZ-wide blood donations and DAEs reported between 2011 and 2021.

Methods: Data of blood donations and DAEs collated by NZ Blood Service were extracted from dedicated databases and integrated after proper quality control. In total, 1,984,116 blood donations and 60,028 DAEs, including 27,952 immediate VVRs (iVVRs) and 1365 delayed VVRs (dVVRs), were initially characterised for known risk factors. Concentrating on iVVRs, three age groups were defined (age <= 22 years, 22 < age <= 40, and age > 40) and used in subsequent multivariate logistic regression analyses, where donations associated with iVVRs were considered as cases and those free of DAEs as controls. In each regression analysis, stepwise selection was used to identify the best model with risk factors carrying significant main effects and/or interactions. Identified interactions were examined closely and used to inform in-depth analyses to dissect iVVR risk patterns and identify the vulnerable donors.

Results: We observed a surging demand of plasmapheresis in recent five years and sharp increases of DAEs in 2020 and 2021 coincident with the Covid-19 pandemic. Comparing to male donors, female donors outnumbered slightly and donated less frequently but were clearly more likely to experience DAEs. VVRs accounted for ~50% of DAEs annually, of which over 95% were iVVRs that were significantly lower than dVVRs in deferral ($p = 0.01$), permanent deferral ($p = 4.2 \times 10^{-7}$), and female preponderance ($p = 7.1 \times 10^{-10}$). There was a clear cyclical pattern of monthly iVVR rates in whole blood donations that appeared to be driven by first-time student donors available mainly in school/college terms. We also observed unique patterns of interactions between gender and age group in iVVR rates differentiating the first-time from the repeat donations.

Multivariate logistic regression analyses for the first-time and the repeat donations respectively both identified commonly observable risk factors of gender and age, and novel risk factors of year and mobile collection sites and their interactions. Interactions with year were driven by the Covid-19 pandemic, where iVVR rates were roundly elevated in 2020 and 2021 probably because of Covid restrictions such as facemask wearing. Exclusion of the 2020 and 2021 data removed the interactions with year, but confirmed interactions of gender with mobile collection sites ($p = 6.2 \times 10^{-7}$) in first-time donations only and with age group in repeat donations only ($p < 2.2 \times 10^{-16}$), together indicating young female donors at the highest risk of iVVRs. Our results also revealed that donation policy changes contributed to the year effects; donors had a lower iVVR risk at mobile sites than well-medicalized donation centers probably because of under-reporting.

Summary/Conclusions: Modelling nonlinear interactions is highly valuable in identifying odds and revealing novel risk patterns of VVRs and new insights into blood donations.

P141 | Abstract withdrawn

Blood products

Blood processing, storage and release

P142 | Processing with a fully automated system, our experience

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Background: We have centralised processing of blood for our region and since 2016 we have implemented the automated processing of blood with Reveos System. Daily processing load is in average 85 units/day and maximal processing load is 150 units/day. Processing of blood has always been understaffed in our transfusion centre and during the last 2 years there have been many changings of staff within processing unit affecting of course quality of manual procedures that require skilled personnel.

Aims: To evaluate the quality of products produced with automated system Reveos compared to manual methods depending on personnel skills.

Methods: All donated blood in our region about 20,000 donations/year has been processed in our processing department of NBTC Tirana. Centrifuges used in our department are: Reveos and Hetich Roto silenta 630 PS. In one year we produce 25% of our Red Blood Cells (RBC) and 65% of Platelets Pools with Reveos. The benefits of automation have been evaluated with respect quality of products and staff satisfaction. For the quality of RBC three parameters have been checked: Volume, Hgb and Hct, whereas for the quality of PP volume and platelet yield have been checked. In total we compared the quality of 96 Reveos PP with with 85 T&B (top and bottom) PP mostly 5 and 6 pools from both sides with equal average units/pool and 120 Reveos Red Cells leukocyte depleted with 150 Red Cells T&B buffy coat removed.

Results: Our quality control data show significantly higher average platelet yield for Reveos PP compared to T&B PP respectively $4.35 \times 10^{11} \pm 0.7$ (Reveos filtered) and $3.19 \times 10^{11} \pm 0.8$ (T&B PP). RBC quality control data show similar quality of RBC (Hgb and Hct) between Reveos final filtered RBC and T&B RBC buffy-coat removed.

Summary/Conclusions: Processing with Reveos saves time, is user friendly and full automation leads to high quality final products (ready to be used for patients with special needs) and quality do not depend on skilled staff. Closed systems guarantee continuous maintenance leading to full efficiency of equipment very suitable for blood centres with difficulties in organizing maintenance. The high yield of platelets offers the possibility to divide product in order to better meet patient's needs, very efficient specially for countries with low donation rate. High platelet yield means also less transfusions/patient. Reveos Red Cells Leukocyte Depleted with pre-storage filtration, offer very good quality product ready to be used in patients with special needs like thalassemia patients or immunocompromised patients.

P143 | Comparison between platelet storage in butyryl trihexyl citrate and 2-diethylhexyl phthalate bags: The difference in quality and metabolic parameters

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Background: Phthalates are among the most used plasticizers in the world, with 2-diethylhexyl phthalate (DEHP) being the most common. It is widely used in many medical devices, including bags for blood components, making them more malleable and flexible. It also provides temperature tolerance, optical clarity, strength and resistance. DEHP bounds to Polyvinylchloride (PVC) in a non-covalently way resulting in the ability to leach from the polymer and affecting the content of the bags. While DEHP has a protective effect on red blood cell membranes, it has a lower permeability to oxygen and carbon dioxide, which restricts the shelf life of platelets and seems to reduce platelet aggregation. DEHP also seems to disrupt the reproductive and endocrine systems, kidneys, lungs, heart and liver.

Aims: The objectives of this study were to study the differences in quality and metabolic parameters between platelets pools stored in DEHP and Butyryl Trihexyl Citrate (BTHC) plastic containers.

Methods: Whole blood units were collected from voluntary, non-remunerated donors and processed into various components. Thirty-two platelet pools were obtained from Buffy-Coats and were randomly selected to be stored in either BTHC or DEHP plastic bags. All units were submitted to visual inspection for swirling, colour changes or clotting and evaluated for volume, platelet count and residual leucocytes. Once shelf life ended, units were evaluated for pH, oxygen, carbon dioxide partial pressures, bicarbonate, glucose and lactate concentrations and microbiological control.

Results: The decrease in pH was more accentuated in the BTHC bags ($p = 0.036$), while the variation in pCO_2 and pO_2 was significantly higher in the DEHP bags ($p = 0.026$ and $p < 0.001$, respectively). Platelet loss during storage was higher in DEHP bags ($p = 0.036$). There were no significant differences in the percent change of HCO_3- ($p = 0.950$), glucose ($p = 0.787$) and lactate levels ($p = 0.229$), nor in the mean platelet volume ($p = 0.885$).

Summary/Conclusions: When compared to DEHP bags, BTHC bags are non-inferior in platelet storage for a 5-day period. BTHC bags have a higher gas permeability and a lower platelet loss during storage. As such, we consider these bags appropriate for platelet collection and storage.

P144 | Reduced wastage of plasma after extending shelf life of thawed plasma from 24 hours to 5 days

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Background: The limited storage time of liquid blood products can lead to a high rate of wastage due to outdating. Recent data has documented both efficacy and safety of thawed refrigerated plasma for up to 5 days. Consequently, the blood establishment in the Health Region Skåne, Sweden implemented on January 1, 2021 an extension of the shelf life for all thawed plasma products stored between +2 and 6°C, from 24 h to 5 days, with the exception of components for paediatric patients.

Aims: To investigate if extending the shelf life of thawed plasma decreases the rate of wastage due to outdating.

Methods: This study was conducted at the Department of Clinical Immunology and Transfusion Medicine. Data regarding all thawed plasma over a 4-year period from January 1, 2019 to December 31, 2022 was extracted from the electronic blood bank system and analysed. The rate of wastage was defined as the percentage of issued plasma that was outdated during the specified year.

Results: In the two years preceding the intervention, 9929 units of plasma were issued, of which 3475 were outdated and discarded before they could be transfused. In the 2 years following the intervention, 9574 units of plasma were issued, of which 1435 units were outdated. The number of transfused units of plasma in 2019–2020 and 2021–2022 were 6363 and 5827 respectively, in line with global trends with decreasing numbers of transfused plasma units. No elevated transfusion reaction rate was detected with extended-thawed plasma. The plasma wastage rates were significantly reduced following the intervention: In 2019 and 2020, 34.8% and 35.2%, respectively, of issued plasma units were outdated, compared with 15.9% in 2021 and 14.0% in 2022 ($p < 0.00001$, Fisher exact test). After the intervention, a median of 29 (range 12–50) thawed plasma units per week were returned to stock available for immediate use. After thawing, 79.5% of transfused plasma were administered within 24 h. The percentage of transfusions on days 2–5 slowly decreased from 6.5% to 3.8%. The reduction of outdated plasma during the study period after the intervention represent a potential value of approximately 80.000 EUR per year, 26% of the yearly value for all thawed plasma in the region.

Summary/Conclusions: Extending the shelf life of thawed plasma resulted in a significant reduction in plasma wastage. A larger amount of thawed plasma appeared in stock, which led to a decreased time delay issuing plasma for urgent needs for example, in Massive Transfusion Protocol requests. In addition, the workload associated with thawing plasma was reduced for the laboratory technologists. A reduction in the quantity of outdated plasma also resulted in a sizeable cost reduction for the Regional Health Authority regarding plasma components.

P145 | Abstract withdrawn

P146 | Cold agglutinins: An uncommon cause of discarding donated whole blood units?

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Background: Follow-up on discarded whole blood (WB) units led to the discovery of a donor whose blood WB donations had been repeatedly discarded due to the inability manufacture components from the WB unit. Even though most cold agglutinins are clinically irrelevant, elevated titters of cold agglutinins in blood donors might negatively affect the ability to process their WB donations. We hypothesize that when donated WB is kept at 20–25°C overnight, cold agglutinins can cause red blood cell (RBC) agglutination and impact the ability to manufacture components from the WB unit.

Aims: To retrospectively investigate donors in which WB donations had been repeatedly discarded due to inability to manufacture the unit into components and to investigate whether cold agglutinins in the blood donor's plasma might be the aetiology of the agglutination.

Methods: The donor database at a regional transfusion service and blood bank was searched in order to identify donors with repeatedly discarded WB units due to component manufacturing issues. The database search spanned January 2013 to August 2022 with the search criterion: donors with ≥6 WB donations with >50% of their donations discarded due to issues manufacturing them into components. Using freshly collected samples from the donors identified by the search cold agglutinin-titrations at 4°C and direct antiglobulin tests were performed.

Results: Six out of 2480 donors with at least one discarded WB unit fulfilled the search criteria (6/2480; 0.24%). Cold agglutinin titters at 4°C in these six donors ranged 32 to 512 (see Table 1). For 5 out of the 6 donors the cold agglutinin titter exceeded the upper end of the reference range of the immunohematology laboratory (<64).

Summary/Conclusions: Blood donors with repeatedly discarded WB donations due to the inability to manufacture their WB donation into components were found to have 4°C cold agglutinin titters beyond the normal reference range, albeit one that was defined for patients not blood donors. However, a previously study found that the 95% reference range of cold agglutinin titters in blood donors were ≤ 4 (Bendix BJ, Transfusion, 2014). These findings indicate that cold agglutinins at even modestly elevated titters may cause significant RBC agglutinates in WB units following overnight hold between 20 and 25°C and interfere with the subsequent processing of the WB donation. Donor centers and hospital based blood banks should consider this aetiology when agglutinates are observed in WB units. Further investigation, including determination of a local reference range for cold agglutinins in blood donors, should be carried out.

P146 – Table 1

	Donations (N)	Discarded WB components N (%)	Cold agglutinin titter (4°)	Direct antiglobulin test
Donor 1	7	7 (100)	64	Negative
Donor 2	8	8 (100)	512	Negative
Donor 3	20	19 (95)	128	weakly positive IgG
Donor 4	30	27 (90)	128	Negative
Donor 5	16	12 (75)	32	Negative
Donor 6	23	15 (65)	64	Negative

P147 | Verification of the whole blood separation process

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Background: Separation of centrifuged whole blood is the staple process in blood component production. Automatization has brought many advantages and optimising the extraction process can help in getting the most out of each blood donation. WB separation also has a major influence on blood component quality.

Aims: To verify the performance of WB separation process on WB separator machines (WBS; Macopress Smarter®, Macopharma) and optimise the separation process on each machine to achieve the highest possible utilisation of donated blood and buffy coat (BC) stability.

Methods: Four units of WB were initially separated and all derived components from the initial separations were tested as follows. Red cell concentrate (RCC) units were tested for volume (VOL), residual leucocytes (LC), haemoglobin (HGB) content and haematocrit (HCT). Plasma (PL) units were tested for residual cells. BC units were tested for VOL, HCT, and total platelet content (PLT). If the tested parameters of RCCs and PL derived from the initial separations were meeting the EU standards, and the median parameters were within the set targets for BC (presented later), a further six separations were made. From these, only the derived BCs were tested with above mentioned tests. If the median parameters for the BC were within the target range, WB separation was deemed stable and the machine was put in routine use. If the median test results results for components from the initial 4 or the further 6 separations were not in the desired range, the parameters of WB separation were adjusted on the machine. Then, further WB separations were made and the derived components tested, in the same manner of initial 4 and subseqent 6 separations, until the right WBS configuration was found and set targets/standards for 10 BCs, 4 RCCs, and 4 PL units were reached. The described verification procedure was repeated on each individual WBS. The RCC and PL test results needed to comply with EU specifications. The set targets for BC parameters were median VOL 58–60 mL, median HCT 40%–44% and median PLT 10×10^{10}.

Results: A total of 14 machines were verified. In order to achieve the set goals for BC, 232 BCs were derived from WB and tested. In total, 82 RCCs and PL units were produced and tested. One RCC was nonconformant due to low HGB, with all other tested RCCs conforming to EU standards for the tested parameters. All PL units conformed to EU standards for residual cells. The median results for the last 10 derived BC for all WBS are VOL 58 mL (min 56 mL, max 61 mL), PLT 9.59×10^{10} (IQR 8.33–11.32), and HCT 43.4% (IQR 41.8–45.1). The median results from the last 10 derived BC for WBS 1-4 are summarised in Table 1.

Summary/Conclusions: The setup and verification of WBS is time and resource consuming. Separation performance must be verified on every individual machine to achieve optimal utilisation of donated blood and BC stability.

P147 Table 1: Median results for last 10 derived BC for WBS 1–4

Machine number	1	2	3	4
Median BC volume, ml	57	58	58	58
Median PLT content per BC unit, $\times 10^{10}$ (IQR)	8.47 (7.99–9.97)	9.35 (8.55–10.37)	9.06 (8.36–10.04)	10.11 (8.44–11.40)
Median HCT, % (IQR)	43.8 (42.5–45.7)	41.3 (40.1–42.4)	43.6 (43–44.9)	43.4 (42–45.4)

P148 | Validation data of a hypoxic red blood cell processing and storage system

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Background: During storage at 4°C, red blood cells (RBCs) undergo extensive changes in biochemical and biophysical properties collectively known as "storage lesions". One of the main contributors to the storage lesions is oxidative damage due to the presence of oxygen in the stored RBCs. This work presents the validation results of a system to produce O_2-reduced RBC concentrates (RCCs).

Aims: The purpose of this validation was to assess the quality of CPD/PAGGSM leukocytes-reduced (LR), O_2/CO_2-reduced RCCs stored for 42 days, and to determine if the produced RCCs were compliant with the current specifications.

Methods: Leuko-reduced RCCs were expressed after overnight room temperature hold of whole blood collected in a CPD/PAGGSM top-top bag system (Macopharma FQE641U, France). RCCs were then connected (within 20 h after whole blood collection) to the Hemanext ONE system (Hemanext Inc., Lexington, MA, USA) and agitated on a platelet shaker (72 rpm) for 3 h at room temperature for O_2/CO_2 adsorption. The resulting O_2-reduced RBC units were finally stored at 4°C within 24 h of whole blood collection for 42 days. Standard blood parameters, saturation of oxygen (sO_2), haemolysis, ATP, and extracellular K^+, lactate and glucose were recorded before and after the O_2 reduction step and at day 42 of storage. 26 procedures were fully analysed in a pilot unit and 5 more were handled in the production unit. Validation was carried out following the good manufacturing practice.

Results: The 31 tested units fulfil the European requirements for the preparation and storage of RCCs, except for one RCC with a haemolysis higher than 0.8% (0.81%) at the end of storage. While three RCCs had % sO_2 levels greater than 20% after O_2 reduction (21, 23 and 23%), the overall performance met the Hemanext specification for % sO2 (<20% with 80% confidence and 80% reliability). Consequently, all results were within the validation acceptance criteria. At day 1 ($n = 31$), haematocrit was of 0.595 ± 0.024, haemoglobin of 51 ± 5 g/unit, sO_2 of 11 ± 5% and end-of-storage haemolysis of 0.41 ± 0.19%. Additional parameters at days 1 and 42 ($n = 26$) showed a decrease of ATP and glucose levels, and an increase of lactate and of K^+. The O_2 reduction step induced an average haemolysis increase of 0.18 ± 0.15% and the storage itself an increase of 0.12 ± 0.11%. Compared to conventional PAGGSM-stored RCCs, haemolysis was higher, mainly due to the reduction step. Moreover, glucose consumption and the concomitant lactate production were higher because of anaerobic storage with a marked increase during the O_2 reduction step.

Summary/Conclusions: Despite differences in haemolysis and metabolic parameters, these validation data demonstrate that LR-RCCs in CPD/PAGGSM additive solution and O_2/CO_2 reduced and stored with the Hemanext ONE system are compliant with European regulations.

P148 – Table 1 RCC characteristics, $n = 31$

	Day 1 before reduction step	Day 1 after reduction step	Day 42
Haematocrit / 1	0.600 ± 0.022	0.595 ± 0.024	0.635 ± 0.034
Haemoglobin / g/unit	55 ± 5	51 ± 5	50 ± 5
sO_2 / %	n/a	11 ± 5	6 ± 4
Haemolysis / %	0.10 ± 0.02*	0.28 ± 0.16*	0.41 ± 0.19
ATP / µmol/g haemoglobin	4.9 ± 0.9*	5.5 ± 0.8*	3.5 ± 0.7*
Glucose / mM	28.9 ± 0.7*	26.9 ±0.8*	7.3 ± 2.0*
Lactate / mM	4.2 ± 0.6*	8.6 ± 1.0*	40.5 ± 2.8*
K+ / mM	< 1.5*	2.4 ± 0.3*	53.0 ± 4.2*

* $n = 26$

P149 | DEHT-PAGGSM blood bag devices do not affect the quality results on the processing: Multicentric and collaborative study

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Background: The plasticizer di(2-ethylhexyl)-phthalate (DEHP) is a common component in blood collection systems. However, exposure to DEHP is raising concern on about its potential carcinogenicity and reprotoxicity. Therefore, the DEHP will be banned following the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation in 2025. Macopharma has developed a non-DEHP whole blood collection system made of Dioctyl terephthalate (DEHT) plasticizer and phosphate-adenine-glucose-guanosin-saline-mannitol (PAGGSM) additive solution. This solution has been tested both by the "Établissement Français du Sang" (EFS) and by the "Service Francophone du Sang" (SFS) in a collaborative and multiparametric study.

Aims: The aim was to evaluate the solution proposed by Macopharma in DEHT/PAGGSM regarding the processing performances and the compliance of the red cell concentrates (RCC) and the plasma components regarding the European Directorate for the Quality of Medicines (EDQM), 20th version

Methods: 32 RCC and plasmas were obtained from Whole Blood (WB) non-therapeutic donations. WB were collected with DEHT devices in SFS Belgium. 16 WB were collected with Top and Top devices (TT) equipped with whole blood filter (LXT filter) and 16 with Top and Bottom (TAB) quadruple bag devices equipped with RCC leucodepletion (LCRD2 filter) and without plasma filter. Processing and analyses were carried out by SFS and EFS. The following parameters were compared to EDQM regulation: haemoglobin (Hb), haematocrit (Hct), residual White Blood Cells (rWBC) for RCC and residual platelets, residual red cells, rWBC, factor VIII (FVIII) for plasma.

The following parameters were measured and followed during the study: volume, glucose, lactate, pO_2, pCO_2, potassium (K^+), pH for the RCC and volume, fibrinogen for the plasma.

All the parameters have been measured at day 1

Results: Mean collected volume was 478 ± 5 mL with 53% of the donations from group O.

For all the RCC prepared, the results were above 40 g of Hb, between 50 and 70% of Hct and below 10^6 rWBC. The volume for TAB-RCC and TT-RCC were respectively 263 ± 20 and 303 ± 16 mL

For all the plasma prepared, the rWBC results were below 0.1×10^9 cells/L and below 1×10^6 cells/unit for TAB and TT, respectively, below $<50 \times 10^9$ residual platelets and below 6×10^9 residual red cells. 7 non-compliant results out of 32 were obtained with a FVIII below 70 IU/100 mL.

Summary/Conclusions: DEHT-PAGGSM blood bag devices do not affect the quality results on the processing. All the RCC units are compliant to the EDQM. All the plasma units are compliant to the EDQM except for FVIII with 80% of conformity. The FVIII is nevertheless compliant to the EFS local regulations. The non-conformities are likely attributed to the thermosensitivity of the FVIII and the late testing at 24 h for the plasma

P149 – Table 1

Results on RCC: Mean ± SD	EDQM criteria	TAB-RCC (day 1)	TT-RCC (day 1)
Hb (g/unit)*	>40	50 ± 6	58.4 ± 5.4
Hct (%)*	50 to 70	59 ± 3	60 ± 3
Haemolysis (%)*	<0.8	0.07 ± 0.01	0.08 ± 0.04
Median rWBC (cells/unit)*	$<1 \times 10^6$	0.05×10^6	0.13×10^6
Glucose (mmol/L)	/	31.1 ± 1.1	29.6 ± 1.1
Lactate (mmol/L)	/	3.7 ± 0.8	3.3 ± 0.7
K^+ (mmol/L)	/	1.8 ± 0.5	1.6 ± 0.8

P149 - Table 2: Results on plasma:

Mean ± SD	EDQM criteria	TAB-Plasma*	TT-Plasma
Volume (mL)	/	295 ± 18	301 ± 15
Median Residual WBC*	0.1×10^9 cells/L (not filtered) 1×10^6 cells/unit (filtered)	6.6×10^6/L	0.03×10^6
Residual platelets* (cells/L)	$<50 \times 10^9$	$6 \pm 3 \times 10^9$	$2 \pm 1 \times 10^9$
Residual red cells* (cells/L)	$<6 \times 10^9$	$0.25 \pm 0.24 \times 10^9$	$0.77 \pm 0.66 \times 10^9$
FVIII* (UI/100 mL)	≥70	94 ± 24	81 ± 16
Fibrinogen (g/L)	/	2.75 ± 0.51	2.62 ± 0.40

* Minimum of 90% of units tested should meet the required value.

P150 | Evaluation of the in vitro properties out to 7 days of buffy coat platelets stored in pas C or pas E and treated with amotosalen and a prototype LED UVA light source

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Background: Pathogen inactivation of platelets is implemented as a standard of care by EFS. The technique (INTERCEPT® Blood System, Cerus) combines amotosalen and a UVA light source (A-UVA). The UVA dose is delivered by an Illuminator equipped with fluorescent bulbs. Light emitting diodes (LED) in the target UVA wavelength are becoming available as alternate light sources. EFS has adopted a double dose platelet production method for whole blood derived platelet concentrates using pools of 8 buffy coats (BC-PCs). Pools are prepared in PAS C or PAS E platelet additive solution.

Aims: To evaluate the *in vitro* properties out to 7 days of BC-PCs stored in PAS C or PAS E and treated with A-UVA using a prototype LED Illuminator.

Methods: 16 BC-PCs were prepared from pools of 8 BCs with TACSI + (Terumo BCT) separators in 32%–47% plasma and 53%–68% PAS using 300 mL of either PAS C (InterSol, Fresenius Kabi) or PAS E (SSP +, Macopharma). The BC-PCs were treated with double dose processing sets (DS) using 17.5 mL of 3 mM amotosalen and UVA light (3.3 J/cm^2) delivered by a prototype LED Illuminator (Cerus) followed by 6–16 h storage in a compound adsorption device (CAD). BC-PCs were stored for 7 days and sampled at day (D) 1 (pre), D2 (post PI), D5, and D7 for the evaluation of platelet characteristics and quality markers: platelet content, pH, glucose, lactate, Mean platelet volume, pO_2, pCO_2, LDH, soluble p-selectin.

Results: Table 1 summarises the characteristics of BC-PC's after the A-UVA and CAD processing steps and split into two final storage containers. All units met the French guidelines for these characteristics.

Summary/Conclusions: The change of UV-A source to prototype LED had no substantial effect on 7- day storage properties of A-UVA BC-PCs. The use of PAS E in comparison with PAS C slightly improved platelet metabolism and generated less spontaneous activation or signs of apoptosis.

P150 - Table 1: Characteristics of A-UVA BC-PCs in PAS C and PAS E (combined results at D2)

N = 16	Volume (mL)*	Platelet concentration (10^9/L)*	Platelet content (10^{11})*
Mean ± standard deviation	**174 ± 6**	**1699 ± 194**	**3.0 ± 0.4**
French guidelines	**≥100**	**≥600**	**≥2.0 × 10^{11}**

* After split of the double dose BC-PCs in 2 final storage containers.

P150 - Table 2 presents the main *in-vitro* storage properties of the A-UVA BC-PCs over 7 days.

PAS C *N* = 8	Day 1 (pre-A-UVA)	Day 2	Day 5	Day 7
pH (22°C)	7.22 ± 0.11	7.10 ± 0.13	6.91 ± 0.05	6.90 ± 0.05
Glucose (mmol/L)	8.3 ± 0.4	7.5 ± 0.5	1.1 ± 0.7	0.0
LDH (U/L)	76 ± 7	94 ± 16	157 ± 17	211 ± 23
p-selectin (ng/mL)	31.2 ± 6.3	42.7 ± 10.3	116.0 ± 25.5	224.5 ± 35.1
PAS E *N* = 8	**Day 1 (pre-A-UVA)**	**Day 2**	**Day 5**	**Day 7**
pH (22°C)	7.17 ± 0.03	7.06 ± 0.03	7.2 ± 0.06	7.07 ± 0.08
Glucose (mmol/L)	8.1 ± 0.6	7.3 ± 0.6	4.0 ± 0.7	1.4 ± 0.9
LDH (U/L)	93 ± 23	102 ± 9	137 ± 23	168 ± 29
p-selectin (ng/mL)	30.4 ± 12.2	36.1 ± 8.0	70.2 ± 9.9	129.3 ± 25.4

Note: The *in vitro* biologic parameters reflect metabolism and an evolution of storage lesions comparable to the data (not shown) reported for previous A-UVA validations using a fluorescent UVA light source. All the units retained a relatively stable pH and retained swirling between D1 and D7. A significant difference was observed per an analysis of variance in favour of PAS E versus PAS C for the following parameters: pH, glucose, LDH, soluble p-selectin.

P151 | Abstract withdrawn

P152 | Assessment of Factor VIII levels in thawed fresh frozen plasma stored at 2 to 6°C for 5 days

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Background: Plasma is the cell free part of blood composed of water, proteins, electrolytes, lipids and carbohydrates. It can be prepared either from whole blood within 6 h of donation or by plasmapheresis. It must be stored at −18°C or colder and has a shelf life of 12 months. Fresh frozen plasma (FFP) contains all stable and labile coagulation factors. Upon request for transfusion, FFP is thawed for 30 min at 37°C. In routine practice, after thawing, FFP is stored for 24 h at 1–6°C if not transfused, and subsequently it is discarded, leading to wastage of valuable resources. Duration of storage and different storage temperatures might affect the coagulation factor activity in thawed FFP. This study measured the changes of coagulation factor VIII activities over 5 days in thawed FFP.

Aims: Fresh frozen plasma (FFP) contains all stable and labile coagulation factors. In routine practice, FFP is stored for 24 h at 2 to 6°C if not transfused after thawing, and subsequently it was discarded, leading to wastage of valuable resources. The objective of the study was to assess Factor–VIII levels in thawed FFP immediately after thawing and after storage at 2–6°C for 24 h, 72 and 120 h and thereby to assess whether thawed FFP stored for 5 days has the potential to ameliorate coagulation factor deficiencies in affected patients.

Methods: 100 blood donors, 25 each from A, B, AB and O blood groups were randomly selected. Coagulation test parameters like PT, APTT and factor VIII:C were measured at 0 h (Day0), 24 h (Day1), 72 h (Day3) and 120 h (Day5) using an ACL ELITE PRO coagulometer (Instrumentation Laboratory, USA).Measurements of coagulation factors were conducted using one stage clotting factor assays. Data were analysed statistically using ANOVA and paired *t* test.

Results: The average Factor VIII activity immediately after thawing (Day0) was 137.1%. Factor VIII activities after storage at 2–6°C for 5 days were reduced significantly ($p < 0.05$) up to 51% from Day 0 to Day 5. A maximum decrease of 34% was seen in first 24 h after thawing. The average factor VIII activity at Day 5 was 66.75% (Table 1).

Summary/Conclusions: Coagulation factor VIII activities in thawed FFP on Day 5 were still sufficient to treat bleeding patients as the activities were more than 30%. Therefore, thawed FFP stored for 5 days can be considered for use in needy patients in emergency cases as it can ensure immediate availability as well as reduction in wastage.

P153 | Characterization and validation of a new multiple sterile connection device: evaluation of mechanical resistance

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Background: Sterile connection is a critical and time-consuming step in the preparation of blood components. Macopharma has developed the Maconnect Sterile Connection device, an innovative and unique generation of closed-system device enabling multiple sterile connections in one-step. This new generation of sterile connection device has been designed to facilitate the process, increase productivity and reliability, and save time. To ensure a good integrity, a mechanical assessment was performed to analyse the sterile connections. Two parameters were studied: sterile connection leaks and tensile strength.

Aims: The goal of the study was to assess if the Maconnect is compliant with both criteria: <0.1% leak and resistance above 40N (ISO3826-1).

Methods: Samples of 3*4.1 mm DEHP PVC tube containing Red Cell Concentrate were prepared. Sterile connections were performed to connect two tubes together. For the leaks, 8663 sterile connections were performed by Maconnect and tested for 10 s at 0.500 bar under water to observe the presence of leaks.

For the tensile strength, a comparative study between the Maconnect device (Macopharma) and the TSCD®-II device (Terumo BCT) was performed. In the first condition, 30 sterile connections were made with the TSCD®-II device. In the second condition, 180 sterile connections were made with Maconnect. Each time, the sterile connection tensile strength was analysed through a dynamometer. 210 samples were analysed in total.

Results: Two leaks were obtained out of the 8663 results. The percentage of leak is 0.02%. A statistical-test (binomial capability analysis) was performed to ensure the Maconnect was statistically certain to be below 0.1% of leak. The Maconnect is significantly lower than 0.1% leak.

For the tensile strength, following results have been obtained

A statistical test (one-way analysis of variance) was conducted to compare the TSCD®-II device and the Maconnect. The Maconnect is significantly slightly better than the TSCD®-II device ($p = 0.046$).

Summary/Conclusions: Macopharma Maconnect has a significantly lower leak percentage than 0.1% and is compliant for the tensile strength (above 40N). The innovative design of Maconnect enabled to

P153 – Table 1

Tensile strength	Maconnect	Control (TSCD®-II)
N	180	20
Mean	89.7 N	85.2 N
Standard deviation	9.1 N	11.4 N

reduce the number of manipulations and connect 12 tubes with a connection cycle of 30 s.

P154 | Quality of methylene blue/light treated plasma using a DEHP-free bag system

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Background: Di-ethyl-hexyl-phthalate (DEHP) is currently one of the major plasticizer used in blood bags. Due to its endocrine disrupting properties European regulators decided to ban DEHP to be used in medical devices in the future. Although the final sunset date for DEHP in blood bags is not yet clear, blood bags used in Europe will have to be modified to DEHP-free versions within the next years.

P154 – Table 1

Parameter	Sample	Mean	SD	De- or increase [%]
aPTT [s]	a	34	2.9	
	b	37	2.3	7.4
	c	39	3.7	14.0
TT [s]	a	17.6	0.7	
	b	19.9	1.2	13.2
	c	19.7	1.1	12.0
Fibrinogen (Clauss) [g/l]	a	3.1	0.4	
	b	2.3	0.4	−24.9
	c	2.3	0.3	−23.7
Factor V [%]	a	105	20.3	
	b	94	19.3	−10.4
	c	83	17.1	−21.4
Factor VII [%]	a	96	23.5	
	b	94	22.3	−1.4
	c	106	24.5	10.5
Factor VIII [%]	a	107	30.7	
	b	84	28.9	−21.8
	c	72	20.5	−32.6
Factor X [%]	a	87	10.0	
	b	81	10.0	−6.9
	c	82	10.2	−5.3
Factor XI [%]	a	110	11.9	
	b	90	12.7	−18.4
	c	87	12.3	−20.7
free Protein S [%]	a	91	19.5	
	b	89	18.1	−2.5
	c	96	22.7	4.9

Aims: Aim of the current study was the investigation of the quality of Methylene blue/light treated plasma using the DEHP-free version of the THERAFLEX MB-Plasma system (Macopharma).

Methods: Whole blood units (500 mL) were collected into 70 mL CPD anticoagulant solution in regular, DEHP-containing bags (day 0) and kept at room temperature (+22 ± 2°C) overnight. After centrifugation plasma was separated and processed within 18 h after whole blood donation. Plasma units ($n = 8$) were connected to the DEHP-free THERAFLEX MB-Plasma disposable (REF PROSDV1, Macopharma) and MB/light treatment was done according to the instructions of the manufacturer using the Macotronic B2 illumination device with a light dose of 120 J/cm^2. Plasma was then transferred through the Blueflex filter into the storage bag and frozen. Samples were taken before treatment (sample a), after Blueflex filtration (sample b) and after storage for 1 month (sample c) for the measurement of plasma factors.

Results: MB/Light treatment had a significant effect on the plasma factors (Table 1). Coagulation times significantly increased (aPTT 7% and TT 13%) while the coagulation factors decreased significantly except for factors VII and protein S. Especially, fibrinogen (Clauss) (25%), factor V (10%), factor VIII (22%) and factor XI (18%) decreased. Specifications given by the European guidelines however were hold for all plasma units. Factor VIII was >70% in untreated samples (82%–170%) and >50% in MB/light treated samples (56%–146%). The decrease of fibrinogen activity was <40% (17.1%–28.6%) after MB/Light treatment.

Summary/Conclusions: The plasma quality of MB/light-treated plasma using the THERAFLEX MB-Plasma disposable PROSDV1 without DEHP showed the expected increase/decrease of plasma factors. Data was comparable to published data obtained for the DEHP-containing disposables

P155 | Aggregates in apheresis platelet concentrates: Can it be predicted from donors' history?

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Background: Visible aggregates made up of clumps of platelets and platelet fragments are occasionally observed in platelet concentrates, particularly in products collected by apheresis. Although the generation of platelet (PLT) aggregates seems multifactorial (i.e., collecting apparatus, aPC temperature during transportation and storage, seasonal effects, etc.), donor's characteristics could be one of the major elements underlying it.

Aims: Here we aimed at identifying factors which might be predictive of aggregate formation in apheresis platelets.

Methods: Apheresis donations ($n = 2830$) from 1117 donors were collected using a TRIMA Accel® Automated Blood Collection System (Terumo BCT, Tokyo, Japan) at one of our donation centres from early April to late August 2018. After a minimal resting time of 10 min, all

aPCs were sent to our processing laboratory to be received and thoroughly checked. In case of PLT aggregates, products were allowed to rest for an additional 1-h period before storage under agitation. The temperature at the donation centre was monitored throughout the study period. Donations containing visible aggregates were identified and counted, along with data related to donor (age, number of donations) and donation (ambient temperature, month of donation, apheresis apparatus, donation protocol). Ordinal, multilevel and simple logistic regressions were performed in order to identify trends in the data which might be predictive of the incidence of aggregates in apheresis platelet donations.

Results: PLT aggregates were observed in 15% (420 /2830) of aPC donations analysed. Temperature monitoring in the donation centre did not identify a single episode of temperature under 20°C. Spikes of temperatures above 24°C were brief and sparse. Statistical analysis of the incidence of aggregate-containing donations identified the donor as the main predictor of aggregate-containing donations. While 78% of donors (867 /1117) gave platelet aggregation-free aPCs, 14% of donors (158 /1117) that gave at least two aPCs had 50% or more of their donations containing aggregates and were responsible for 68% (285 /420) of all aggregate-containing aPCs observed during the study. It was estimated that an increase in the number of aPC donations from a given donor would likely increase the risk to detect aggregation in one of his subsequent donations (OR 15.2, 95% CI 7.9–29.4 for a seventh versus a first donation). Furthermore, it was shown that a first aPC donation with aggregates would increase the risk to detect aggregates in subsequent donations (OR 4.97, 95% CI 3.03–8.22).

Summary/Conclusions: Our results confirm previous reports suggesting that apheresis platelet donors with a history of aggregates in their donations are the main predictive factor of the incidence of aggregate-containing donations. To reduce product loss, donors could be redirected to other types of blood donations if aggregates are observed on their first donation.

P156 | Donor factors affecting platelet recovery from whole blood donations and best whole blood donors for platelets

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Background: The Reveos automated blood processing system is a device that separates 4 whole blood units simultaneously into RBCs (leukodepleted of-line), plasma (leukodepleted), Interim Platelet Units (IPUs) (subsequently pooled and leukodepleted) and residual leukocytes. The system provides with a platelet yield indicator (PYI) of every IPU which is used to make the pools. Every day it is decided how many whole blood units go for platelets and not in order to obtain more plasma (30 mL more plasma). In a previous study, we created a database of whole blood donors in order to have their historical PYI records and to analyse the correlation and stability of this parameter. The results showed that PYI was a very good estimator of the real count of platelets in the actual IPU, but it wasn't stable through different donations from the same donor and consequently does not have a predictive platelet recovery value for future donations.

P156 – Table 1

	Low haematocrit level	Medium haematocrit level	Height haematocrit level
Median male platelet recovery (%)	75.6	87.6	95.3

Aims: To study donor factors that could affect platelet recovery in order to select the best whole blood units for platelets concentrates prior to separation.

Methods: From June 2022 to January 2023, donations were randomly selected with the only criterion that the sample was well represented in terms of PYI database of whole blood donors. The following parameters were collected: whole blood weight, donor platelet count, donor red cells count, donor haematocrit, donor leucocyte count, IPU, leukocyte residue and plasma weight, IPU platelet count, donor's gender and age. For each donation, the percentage of platelet recovery was calculated. Since the normal range of haematocrit is different in men and women, we categorize this variable according to gender and sample tertiles. We analysed the platelet recovery according to donor age, gender, haematocrit and leukocytes count. Comparisons between groups were carried out using Kruskal-Wallis test.

Results: We had a representative sample of 106 donations (33% female and 67% male). There were statistically significant differences in donor platelet count between men and women (p-value < 0.05). The median platelet recovery was 86%. There was significantly higher platelet recovery in the group of male donors with a haematocrit greater than 46% versus those with a haematocrit less than 43% (p-value < 0.05). Trends in platelet recovery by haematocrit in women were similar. There were no statistically significant differences in platelet recovery according to donor leukocyte count.

Graphically platelet recovery in men decreases progressively with age and in women increases progressively with age, although the differences in practice are small and not statistically significant. Donor platelet count and donor haematocrit are statistically significant (p-value < 0.05) in jointly forecasting IPU platelet count through a linear regression model.

Summary/Conclusions: In addition to the donor's platelet count, other donor factors are involved in platelet recovery. Therefore, relying on platelet levels alone to select whole blood units is not the best way to proceed in order to obtain the highest platelet recovery. Donor haematocrit clearly impacts on platelet recovery too. Gender could also be taken into account for whole blood units selection.

Further studies would be needed to confirm whether pre-donation haemoglobin (digital prick) could be a good parameter for selecting platelet donations.

P157 | Abstract withdrawn

P158 | Abstract withdrawn

P159 | Comparative studies on two types of in-line filters used for whole blood filtration

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Background: Transfusion of leukocyte-contaminated blood components may induce a number of adverse reactions in the recipient. These include: non-haemolytic febrile post-transfusion reaction (FNHTRs) caused by cytokine release or alloimmunization with HLA antigens which leads to formation of anti-HLA antibodies that induce resistance to the transfused PCs. Residual leukocytes may also increase the risk of pathogen transmission (e.g., CMV, EBV or HTLV). Leukocyte-reduced blood components, that is, components with less than 1×10^6 leukocytes have been used for years in order to protect recipients and to reduce the risk of leukocyte-related serious adverse reactions. In-line or on-line leukofilters can be used for removal of leukocytes and microaggregates from blood components. Evaluation of any filtration system includes determination of the efficiency of this system (percentage of removed leukocytes and platelets) and its effectiveness (RBC and haemoglobin recovery). The filtration process depends on numerous factors such as: PC quality (no clots or microaggregates), the storage temperature prior to filtration and the type of filter used.

Aims: Comparative studies of two types of in-line leukofilters: Shandong Implements Co. Ltd (China) and Ashai Kasei Medical Co. Ltd. (Japan) were performed. Both the efficiency (percentage of removed leukocytes) and effectiveness (recovery of RBCs and haemoglobin) of the filtration process were evaluated as well as filtration time and reliability of the system.

Methods: 44 trials were performed using a total of 44 PC units. Whole blood was collected at the Regional Blood Transfusion Center in Warsaw into a quadruple blood bag system manufactured by Ravimed (CPD) with a built-in filter – either Shandong Implements filter (series number 22022601 - 22 pcs.) or Ashai Kasei Medical (GLT-H9, Sepacell - 22 pcs). WB units were filtered up to 4 h of collection. Each WB unit was weighed prior to filtration and after the procedure, then sampled for complete blood and residual leukocyte count. Testing was performed with the Sysmex XP-300 haematology analyser and residual leucocyte counts in the samples were determined microscopically using Nageotte chamber.

Results: Filtration was uneventful for all WB units. The results are presented in Table 1.

Summary/Conclusions: All WB units subjected to filtration with Ashai Kasei Medical filters were leukocyte depleted while only 15 out of 22 WB units (68.18%) met standard requirements following filtration with Shandong Implements.

P159 - Table 1: Quality control of PC subjected to filtration ($n = 22$)

Parameter	Value Shandong implements	Value Ashai Kasei medical	Range Shandong implements	Range Ashai Kasei medical
Filtration time [min]	12 ± 3	14 ± 1	8 – 15	11 – 16
Volume prior to filtration [mL]	507 ± 2	505 ± 3	502 – 511	496 - 510
Volume after filtration [mL]	463 ± 5	464 ± 3	457 – 479	455 – 468
Volume loss [mL]	44 ± 4	41 ± 2	27 -51	36 – 45
Volume loss [%]	8.6 ± 0.8	8.1 ± 0.4	5.3 – 10.0	7.2 – 8.9
RBC recovery (%)	91.31 ± 1.59	91.73 ± 0.84	86.27 – 94.44	90.32 – 93.33
Haemoglobin recovery (%)	91.6 ± 1.5	91.4 ± 0.6	89.3 – 96.2	90.0 – 92.6
Platelets removed (%)	98.9 ± 13.7	99.7 ± 1.5	46.9 – 93.8	93.2 – 100.0
WBCs removed (%)	99.98 ± 0.01 ($n = 15$, for leukoreduced components) and 99.79 ± 0.24 ($n = 7$, for non-leukoreduced components)	99.99 ± 0.01 ($n = 22$)	99.97 – 99.99 ($n = 15$, for leukoreduced components) and 99.31 – 99.96 ($n = 7$, for non-leukoreduced components)	99.99-100.00 ($n = 22$)

P160 | Implementation of cryopreserved platelets in routine production of blood components

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Background: Platelet transfusions is a critical part of treating trauma patients. Platelet shelf life is short, and for small hospitals having an adequate stock is challenging. Cryopreserved platelets have an extended shelf life, and can be stored at −80°C, beneficial for remote locations and in backup situations. During the last several years a protocol for cryopreservation of platelets has been validated and fine-tuned at Karolinska University Hospital. Cryopreserved platelets have been thoroughly characterised in vitro and a clinical feasibility study has been performed. Based on the results of these studies a decision to implement cryopreserved platelets in routine production was taken.

Aims: The aim of this study was to transfer the small-scale experimental production protocol to a larger scale routine production protocol, with a process reliability that ensures that ≥90% platelet content calculated from the original unit.

Methods: We adapted the experimental protocol to a routine protocol to be performed by production staff in the component lab, by standardizing several steps in the protocol. The reconstitution after freezing and thawing and was evaluated separately. In the experimental setup a 25% DMSO-freezing medium was prepared by adding DMSO and 9 mg/mL NaCl solution from open containers, using syringes, to a transfer bag (OMAVQE6004XB, Macopharma). Handling was done on ice in a laminar air flow (LAF) cabinet. One transfer bag was prepared for each platelet unit. To simplify handling, standardised volumes of DMSO and NaCl-solution required for cryopreservation of 4 transfusion units of platelets were calculated, making batch wise production of freezing medium possible. A closed system, which is possible to sterile dock, was used for DMSO delivery (Cryopur, SP-10 and SP-50, OriGen Biomedical). Unfortunately, 9 mg/mL NaCl is not available in packaging that is possible to sterile dock, but switching to delivery in a bag possible to spike (AFE1324, Baxter), simplified handling. Preparation no longer requires a LAF cabinet, although preparation on ice is still necessary. After preparation of the DMSO-NaCl-solution, the batch was divided into 4 separate units using pediatric bags (VQL0001XU, Macopharma). Platelet units (200 ± 3 mL) were transferred to freezer bags (VXX603B, Macopharma) and one paediatric bag containing 50 mL DMSO-NaCl-solution was added within 2 h of platelet production. After addition of freezing medium, the paediatric bag was removed, and a transfer bag (BB*T060CM, Terumo BCT) was added, and the platelets were centrifuged for 10 min at 1200 g. The supernatant was extracted to the transfer bag using a manual extractor, and the transfer bag containing the supernatant was removed. The freezer bag containing platelets in freezing medium was placed in a metal casing and immediately stored at −80°C. Volume and platelet count was determined using scales and a cell counter (SweLab alfa plus, Boule Medical) original platelet and for the removed supernatant for 20 units. Volume was similarly determined for the final platelet unit in freezing medium. Recovery (%) of cryopreserved platelets was calculated.

Results: Modification of the experimental protocol, allowing for standardised volumes, batch production and closed systems with sterile docking (where available) makes routine production of cryopreserved platelets possible. The remaining volume in the cryopreserved units was 12 ± 1.2 mL and recovery of platelet was 95 ± 2%, which meets set criteria.

Summary/Conclusions: The method for cryopreservation of platelets now consists of routine steps, that are well known to component production staff. Production is therefore possible in a routine setting, with a satisfactory platelet processing recovery.

P161 | Cooling kinetics of various freezing systems do not affect quality of plasma

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Background: To achieve the highest yield of Factor VIII (FVIII), the 20th edition of Blood Guide from European Council recommends that "freezing of plasma for direct transfusion has to be as rapid as possible and the core temperature of the plasma unit should be reduced to −25°C or lower within 60 min after commencing the freezing start". Thus, "freezing should take place in a system that allows complete freezing within one hour to a temperature below −25°C". EU guidelines specifies also that average of FVIII after freezing and thawing has to be >70% of freshly collected unit.

Aims: Objective of this study is to compare the quality of frozen plasma obtained by freezing systems which provide three different kinetics: high (H), moderate (M) and low (L) cooling rates. This study was performed in two French blood component facilities, EFS Bourgogne Franche-Comté (BFC) and EFS Grand Est (GEST).

Methods: 13 Apheresis ACD-A Plasmas (6 from O donors, 7 from non-O donors) of at least 640 mL were collected at EFS BFC ($N = 6$) and EFS GEST ($N = 7$) and were split in three sub-units of 200 mL (Unit #1, unit #2, unit #3). Each plasma unit was frozen 24 h after collection and stored at temperature below −25°C during 14 days. Plasma units #1 were frozen using high cooling rate (H) (kinetic of −25°C/60 min in both EFS) ($N = 13$). Plasma units #2 were frozen using moderate cooling rate (M) (kinetic of −25°C/90 min in both EFS) ($N = 13$). Plasma units #3 were frozen using low cooling rate (L) (EFS BFC: kinetic of −25°C/220 min; EFS GEST: kinetic of −25°C/>270 min) ($N = 13$). The plasma quality was evaluated before freezing and after 14 days storage period by measuring Factor VIII (FVIII), Fibrinogen and thrombin generation test (TGT).

Results: No statistical differences were observed between plasma units #1, plasma units #2 and plasma units #3 for FVIII, Fibrinogen and TGT (Table 1). All plasma units had a pre/post freezing yield > 80% for FVIII and for Fibrinogen and met the EU requirement. 33 plasma units collected from 11 apheresis plasma complied with FVIII Blood Guide requirement; > 0.7 UI/mL. The 6 plasma units with a FVIII < 0.7 UI/mL after freezing, came from 2 apheresis plasma where FVIII concentration were already below 0.7 UI/mL before freezing (apheresis plasma collected from O group donors).

Summary/Conclusions: Plasma units #1, #2, #3 meets the EU quality standards for therapeutic plasma, independently of cooling kinetics up to −25°C / 270 min. Other factors have to be considered in order to validate appropriate freezing system, that is, cost, airflow and ice front velocity, equipment capacity, efficiency and productivity, ease of use.

P161 - Table 1: Biological parameters of plasma units before and after freezing in function of the three cooling rates. (Mean ± SD from both EFS).

	Plasma units before freezing and after 24 h of collection ($N = 39$)	Frozen plasma units # 1 ($N = 13$) High (H) cooling rate	Frozen plasma units # 2 ($N = 13$) moderate (M) cooling rate	Frozen plasma units # 3 ($N = 13$) Low (L) cooling rate
Factor VIII (UI/mL)	1.05 ± 0.29	1.01 ± 0.29	0.99 ± 0.28	0.98 ± 0.30
Fibrinogen (g/L)	2.67 ± 0.93	2.72 ± 0.83	2.73 ± 0.87	2.66 ± 0.69
TGT using 5 pM Tissue Factor (TF) Lag Time (min)	—	3.8 ± 0.9	3.8 ± 0.7	3.9 ± 0.8
TGT using 5 pM Tissue Factor (TF) ETP (nM*min)	—	1351 ± 334	1347 ± 357	1334 ± 281
TGT using 5 pM Tissue Factor (TF) **Peak (nM)**	—	166 ± 60	192 ± 91	180 ± 81

P162 | The comparison of two methods of leukoreduction

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Background: The methods of collecting and processing blood and its components are constantly improved to guarantee the safety of their recipients. One of such methods is leukoreduction in the cell blood components.

In Regional Blood Center in Poznan, Poland we produce 100% of the leukoreduced platelet concentrates and only 50% leukoreduced red cell concentrates (the remaining 50% of red cell concentrates are without the buffy coat).

The leukoreduced red cell concentrates are produced using two methods: during the processing of the whole blood that was collected to a special kit of blood gabs with built-in in-line filter (primary processing) or during secondary processing when a special kit for filtering is attached using sealer to the already obtained red cell concentrates without the buffy coats.

Aims: The aim is to compare the results of the quality tests of the leukoreduced red cell concentrates that were obtained using the in-line filters (primary processing) and attached filtering kits (secondary processing).

Methods: We have tested 805 units of red cell concentrate in 2022: in 692 units of red cell concentrate the leukocytes were reduced during the primary processing and the blood was collected using the blood bag system by Fresenius (quadruple bags with in-line filter). In 113 units of red cell concentrates the leukocytes were reduced during the secondary processing—separate kits for leukocyte reduction by Fresenius were attached to the units of red cell concentrate without the buffy coat

After the leukoreduction following values were determined:

- the volume of the red cell concentrates
- the level of the haemoglobin and the haematocrit using the haematological analyser Pentra XL 80 by Horiba
- the level of the white blood cells (WBC) with the method of flow cytometry using the analyser BD FacsVia by Becton Dickinson
- the percentage of haemolysed erythrocytes on the last storage day.

The level of the haemoglobin in the supernatant was determined using the haemoglobin analyser for measuring low levels of haemoglobin Plasam/Low Hb by Haemocue

Results: Leukoreduced red cell concentrates obtained using the method of primary processing:

Volume (in mL): 249 ± 3 SD

Hb (g/unit): 47 ± 1 SD

Ht (%): 55 ± 1 SD

WBC ($\times 10^6$): 0.015 ± 0.006 SD

Haemolysis (%): 0.3

Leukoreduced red cell concentrates obtained using the method of secondary processing:

Volume (in mL): 248 ± 4 SD

Hb (g/unit): 48 ± 1.5 SD

Ht (%): 58 ± 1 SD

WBC ($\times 10^6$): 0.019 ± 0.01 SD

Haemolysis (%): 0.4

Summary/Conclusions: The results of the quality tests of the leukoreduced red cell concentrates obtained using the in-line filters (primary processing) and attached filtering kits (secondary processing) are comparable.

However, there are several advantages of leukoreduction during the primary processing (using in-line filters): the time between the collection and leukoreduction is shorter which allows for the leukocytes to be removed prior to their decay and release of intracellular pathogens for example, CMV and there is no need to use sealers for the tubes which eliminates the risk of contamination of the components due to the lack of sterility.

Taking into consideration all aspects mentioned above, the use of in-line filters seems highly recommended.

P163 | Characterization of residual cell populations in leukoreduction system disposable sets following apheresis platelet collections in plasma versus PAS

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Background: Frequent platelet pheresis has been associated with T-cell lymphopenia in platelet donors.
Aims: The residual cell content of Trima LRS chambers was characterised to determine the impact of plasma rinse back, a feature of the Trima Accel system, on the number of cells remaining in the disposable set. Collections in plasma were compared to collections in platelet additive solution (PAS).
Methods: Disposable tubing sets were obtained following apheresis platelet collections on Trima Accel V7 (Terumo BCT) in 100% plasma or with PAS (Intersol) using 'standard rinse back' or an added 'plasma rinse back' selected prior to collection. Residual cells were harvested separately from the LRS chamber and the remaining disposable set by gravity. Cells were analysed on a haematology analyser (Sysmex) and by flow cytometry (BD, Lyric) using antibodies as outlined in the Tables below. Flow cytometry counting beads were used to calculate absolute numbers of cells.
Results: Average platelet yields of the collections were 5.6×10^{11} for procedures with 'standard rinse back' (4.0 and 6.2×10^{11} with and without PAS respectively) and 5.1×10^{11} for procedures with added 'plasma rinse back' (4.0 and 5.5×10^{11} with and without PAS respectively). There was a difference in average volumes remaining in the kit after the donor was disconnected (56 mL Plasma compared to 124 mL with PAS). There was also a difference in observed chamber volumes comparing plasma (8.15 mL) to PAS (3.33 mL) platelet collections. After plasma rinse back, haematology analyser data show an average white blood cell (WBC) decrease after collections in plasma of 11% in the chamber and 71% in the kit compared to the standard process. For PAS collections, chamber WBCs are decreased by 6% and cells in the kit are decreased by 67% with an added rinse back. Similar trends were observed by flow cytometry.
Summary/Conclusions: The additional plasma rinse back step with Trima V7 may increase the number of WBCs returned to the donor and at the same time retain sufficient WBC numbers in the chamber for research purposes. A significant difference however was observed between platelet collections in plasma versus PAS. The total volume and cells remaining in the chamber after PAS collections was decreased compared to collections in plasma, with or without plasma rinse back, indicating more WBCs may be returned to donors after PAS collections.

P163 - TABLE 1: Residual WBC After 100 Plasma Collections

	Chamber		Kit	
Rinse back	−	+	−	+
Sample size	$N = 17$	$N = 14$	$N = 17$	$N = 14$
WBC CD45+ ($x10^7$)	352.9 ± 139.8	284.1 ± 150.3	729.6 ± 329.4*	186.3 ± 145.3
T-cells CD4+ ($x10^7$)	174.4 ± 74.2	160.0 ± 79.8	313.0 ± 148.8*	81.7 ± 56.5
T-cells CD8+ ($x10^7$)	12.3 ± 8.3	8.3 ± 4.6	17.6 ± 13.2*	5.5 ± 4.2
B-cells CD19+ ($x10^7$)	30.0 ± 15.8	27.8 ± 21.2	55.0 ± 33.2*	17.4 ± 21.5
Monocytes CD14+CD16- (10^7)	20.0 ± 23.2	13.4 ± 14.9	30.9 ± 48.8	16.7 ± 21.8
Platelets CD61+ (10^9)	6.0 ± 3.0	8.2 ± 5.6	13.7 ± 14.2	9.0 ± 4.2

Note: Mean ± standard deviation, *$p < 0.05$ for ± rinse back.

P163 - TABLE 2: Residual WBC after 65% Plasma / 35% PAS Collections

	Chamber		Kit	
Rinse back	−	+	−	+
Sample size	$N = 6$	$N = 6$	$N = 6$	$N = 6$
WBC CD45+ ($x10^7$)	20.4 ± 6.3	20.5 ± 7.0	615.3 ± 264.8	489.3 ± 83.4
T-cells CD4+ ($x10^7$)	12.9 ± 5.4	14.8 ± 5.2	352.5 ± 115.3	195.4 ± 47.9
T-cells CD8+ ($x10^7$)	0.7 ± 0.3	10.9 ± 0.4	23.8 ± 16.1	16.2 ± 3.8
B-cells CD19+ ($x10^7$)	1.7 ± 0.1	2.3 ± 0.6	59.4 ± 31.0	46.9 ± 86.4
Monocytes CD14+CD16- (10^7)	0.8 ± 0.5	0.5 ± 0.5	22.3 ± 17.4	19.3 ± 13.9
Platelets CD61+ (10^9)	5.0 ± 1.5	4.0 ± 2.3	80.6 ± 53.8	26.9 ± 21.1

P164 | Amotosalen/UVA light pathogen reduced plasma from previously quarantined units stored for 2 years

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Background: Two types of therapeutic plasmas are produced by the French Blood Service (EFS), plasma secured by quarantine (Q) and plasma pathogen reduced (PR) by amotosalen/UVA light treatment (A-UVA) (INTERCEPT™ Blood System, Cerus). A "previously frozen plasma" (PFP) process consisting after a frozen Q period in thawing the plasma, A-UVA treating and refreezing for storage was validated. This PFP process is approved in France since november 2022.

Aims: To evaluate 2-year stability of A-UVA PFP.

Methods: 18 apheresis ACD-A plasmas of at least 640 mL were collected, split in three sub-units, frozen with 24 hours and stored at <-25°C for 30 weeks. After thawing, pools of triplets were reconstituted and submitted to the A-UVA treatment split in 3 units and frozen within 6 hours for <-25°C storage. Similarly 18 groups of 5 CPD whole blood derived plasma units were frozen and stored at <-25°C for 30 weeks. After thawing, pools of 5 units were constituted, split in 2x 640 mL minimum. Each of the two sub-pools was A-UVA treated, split in 3 units and frozen within 6 hours. All plasma units were non-O. Plasma parameters were measured in the thawed plasma pools after the Q period (baseline) and in the plasma units after A-UVA treatment and up to 14-day frozen storage (post treatment), and at 1 year and 2 years from collection; total proteins, Albumin, Immunoglobulins, Coagulation factors and coagulation tests, coagulation inhibitors and fibrinolysis factors, thrombin generation test.

Results: The results of a selection of plasma parameters for the 36 combined replicates are reported in the Table below (post treatment not shown). The requirements from the French Official Journal (JO), at least 70 % of the units with FVIII ≥ 0.5 IU m/L and Fibrinogen ≥ 2.0 g/L were met at all periods.

Summary/Conclusions: Previously frozen plasma treated post-thawing with A-UVA retained sufficient levels of plasma proteins, coagulation factors and inhibitors and normal clotting time. A sufficient stability of these parameters was observed over a 2-year storage period at ≤-25°C. This process can also be applied in first intention to optimise production efficiency by batch processing of frozen plasma units.

P164 – Table 1

N = 36	Baseline (after 30-week ≤-25°C storage)	After thawing / A-UVA PR / & frozen storage up to 1-year from collection*	2 years from collection*
Total proteins (g/L)	62.9 ± 3.1	61.6 ± 3.4 s	60.5 ± 3.1 s
PTT (%)	95 ± 7	75 ± 5 s	75 ± 5 s
Fibrinogen (g/L)	2.81 ± 0.43	2.47 ± 0.36[s]	2.51 ± 0.39s
FVIII (IU/mL)	1.22 ± 0.29	0.83 ± 0.19[s]	0.77 ± 0.18s
VWF Ag (IU/mL)	1.11 ± 0.11	1.10 ± 0.10	1.07 ± 0.11s
AT-III (IU/mL)	0.97 ± 0.07	0.99 ± 0.06[s]	0.97 ± 0.06
Protein C (IU/mL)	0.97 ± 0.14	0.85 ± 0.13[s]	0.83 ± 0.12s
Protein-S (IU/mL)	0.84 ± 0.15	0.78 ± 0.13[s]	0.72 ± 0.12s
α2-antiplasmin (IU/mL)	0.99 ± 0.07	0.81 ± 0.05[s]	0.77 ± 0.05s

* two-tailed (alpha 0.05) t-test for paired values comparing each storage period to the "pre" data, "s" if significant difference $p < 0.05$.

P165 | How to minimise residual cells in specific blood components. Optimizing an automated processing system

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Background: Derived from our quality control follow-ups, an unexpected number of plasma units presented a higher amount of residual platelets according to regional and European quality guidelines. After consulting with our fractionation system provider, they suggested to perform modifications on the programs used for whole blood (WB) automated processing system (APS).

Aims: To analyse the variables influencing the concurrent presence of residual platelets in some plasmas obtained from APS, applying different modifications to the default separation program to choose the optimal one.

Methods: One of the APS used in our center separates whole blood into 4 products: plasma (PL), interim platelet units (IPUs), leukocyte residue (LR) and red blood cell concentrates (RBC). Modifications to the default program included adding a 30 seconds waiting pause before component expression (+30s), changes in the speed of expression (FAST/SLOW) and/or involving the detection of erythrocytes into the IPUs (Trigger -40). All the components obtained through APS modifications applied, along with their respective whole blood donations were analysed by flow cytometry and a cell coulter. Absolute platelet, leukocyte, erythrocytes and haemoglobin contents were calculated as well as volumes and haematocrit for each blood component. Results (mean ± standard deviation) were normalized and expressed as percentage of each cellular element into each fractionated component.

P165 – Table 2

		PLASMA		
	n	>50x10E9/L Platelet KO	>6x10E9/L RBC KO	>0,1x10E9/L Leukocyte KO
DEFAULT	16	6%	0%	0%
DEFAULT+30s	64	0%	0%	0%
DEFAULT+30s +TRIGGER40	32	0%	0%	0%
FAST EXPRESSION	63	8%	5%	0%
SLOW EXPRESSION	64	0%	0%	0%

Results: Default program and four different configurations involving the previously mentioned modifications were explored. Cellular elements distribution (expressed as percentage respect to whole blood ± standard deviation) in **Table 1.**

Summary/Conclusions: Our data suggested that from the various modifications applied to the default program, DEFAULT+30s better adjusted to our aims. This program resulted in no plasma bags out of specifications, while an acceptable amount of platelets were driven to the IPU and few got lost in plasma or the leukocyte residue. Our current APS has the capacity to include modifications to find an equilibrium between the optimal distribution of cellular elements and complying with quality standard specifications. This knowledge could provide us a tool to redirect future actions to solve compliance issues for blood components while reducing the presence of residual cellular elements.

P165 – Table 1

	% PLATELETS		
	Plasma	IPU	LR
DEFAULT	5,08 ± 2,41	75,9 ± 8,04	10,8 ± 5,99
DEFAULT+30s	4,31 ± 1,43	79,9 ± 11,1	8,21 ± 4,11
DEFAULT+30s+TRIGGER40	4,08 ± 1,27	76,4 ± 12,3	9,29 ± 4,70
FAST EXPRESSION	7,74 ± 4,42	82,3 ± 18,9	9,69 ± 6,61
SLOW EXPRESSION	3,67 ± 1,36	77,4 ± 11,6	9,38 ± 5,44

Note: Data regarding out of specification plasma counts are summarised in **Table 2.**

P166 | Elevated erythrocyte-derived microparticles (EMP) levels in storage red blood cells

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Background: Red blood cells product which is stored in the blood bank undergo changes in morphology, biochemistry, and metabolic which is called red blood cells storage lesion. Morphological changes of erythrocyte membrane phospholipids and damage due to oxidative damage and haemolysis can produce microparticles known as erythrocyte-derived microparticles (EMP). Increased EMP in the red blood cells product allegedly one of the causes bad outcomes in transfusions.

Aims: to know the difference levels of erythrocyte-derived microparticles (CD235a) in the packed red cells (PRC) product based on storage time on day 0, 7, 14, 21 and 28 at blood bank of Dr. M. Djamil Hospital, Padang, Indonesia

Methods: This is an analytical observational study with cross sectional study design of the 14 units of PRC stored in Blood Bank of Dr. M. Djamil Hospital, Padang, Indonesia. The tests were conducted during 28 days of storage at 1-week intervals. Measured of CD235a which is define as EMP levels was conducted by flowcytometry method. Statistical analysis by using T-test and Wilcoxon paired with p value <0.05 means significant. This research was approved by the Research Ethics Committee of Medical Faculty of Universitas Andalas.

Results: Fourteen units of PRC were obtained from almost male donors (85.7%) with the highest blood type is O (42.9%). The average of haemoglobin level of PRC product was 24.9 ± 1.4 g/dL and hematocrit was 77.1 ± 4.8%. In total 14 units we studied, EMP (CD235a) level was increased with time. The mean of EMP level was 228 ± 125/uL in 0 days of storage and increase to 956 ± 644/μL at the end of study. The EMP level increase significantly in linear with PRC storage duration ($p < 0.05$).

Summary/Conclusions: The erythrocyte-derived microparticles level increase significantly in linear with storage duration of packed red cells.

P167 | Abstract withdrawn

Blood products

Blood components

P168 | In-vitro quality of platelet concentrates X-ray-irradiated at the beginning or end of storage

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Background: X-ray-irradiation of blood product is used for inactivation of lymphocytes to prevent transfusion-associated graft versus host disease, similar to gamma-irradiation. Compared with gamma-irradiation, X-ray-irradiation is preferable from an operational point of view, as the radiation emission is easier to control and thus safer. However, only limited data is available from literature on the effect of x-ray-radiation on the quality of PC.

Aims: The aim of this study was to evaluate the in-vitro platelet quality of X-ray-irradiated platelet concentrates (PCs) compared with untreated controls, dependent on the storage time before treatment.

Methods: Plasma-reduced PC in SSP+ were derived from pools of 4 buffy coats after overnight storage of whole blood. PCs were used in two sets of experiments ($n = 6$ for each set). In a pool and split design, two PCs were merged and separated, with one bag remaining untreated, while the other was X-ray-irradiated at 25 to 50 Gy (average approx. 35 Gy) using an X-ray blood irradiator RADGIL2 (Gilardoni, Italy). Bags were irradiated either directly after PC production on day 2 (set 1) or on day 5 (set 2) at the end of storage. PCs were stored under agitation at 22 ± 2°C and sampling to determine platelet quality was done on day 6. The quality parameters observed for this study were PLT number, pH, presence of swirling, collagen-induced aggregation, CD62P exposition and Annexin V binding.

Results: PCs were in accordance with the German guidelines with respect to platelet content (>2 x 10^{11}/unit)) and pH (pH between 6.4 to 7.8). When comparing untreated controls with the corresponding X-ray-irradiated PCs no significant differences were detected for collagen-induced aggregation (table). Annexin V binding and CD62P exposition were slightly but significantly elevated in the x-irradiated bags. There was no significant difference in the quality of PCs x-ray-irradiated directly after production compared with those x-ray-irradiated at the end of storage (unpaired *t*-test, $p < 0.05$).

* p ≤ 0.05 compared to untreated control of the same set (paired *t*-test)

Summary/Conclusions: Platelet quality is well preserved after X-ray-radiation with only minor differences in platelet activation (CD62P and Annexin V) between X-radiated PCs and the corresponding untreated controls at the end of storage. The time point of irradiation, immediately after production or at the end of shelf life, had no effect on the platelet quality parameters we examined in this study.

P168 – Table 1: Summary of platelet quality parameters.

	Set 1: Irradiation day 2		Set 2: Irradiation day 5	
	Control [Mean ± SD]	X-Ray [Mean ± SD]	Control [Mean ± SD]	X-Ray [Mean ± SD]
Volume [ml]	269.62 ± 8.39	273.40 ± 8.73	278.82 ± 2.90	281.44 ± 3.54
PLT [×10^9 PLT/mL]	0.95 ± 0.07	0.97 ± 0.07	0.84 ± 0.03	0.85 ± 0.04
PLT/unit [×10^{11} PLT/unit]	2.57 ± 0.22	2.66 ± 0.19	2.35 ± 0.10	2.39 ± 0.09
CD62P (Sample) [1%] /	40.32 ± 4.45 /	43.38 ± 4.47* /	42.43 ± 4.18 /	44.18 ± 4.10* /
CD62P (TRAP activated control) [%]	97.47 ± 1.09	97.74 ± 0.28	97.43 ± 0.47	97.39 ± 1.02
AnnexIn V [%]	5.63 ± 1.08	7.2 ± 1.40*	5.68 ± 0.42	7.18 ± 0.48*
Collagen-Induced Aggregation [%] 10 µg/mL collagen	78.50 ± 6.17	78.64 ± 7.43	79.04 ± 2.49	80.63 ± 3.62
Collagen-Induced Aggregation [%] 2 µg/mL collagen	7.13 ± 5.08	4.67 ± 3.05	4.71 ± 1.49	5.50 ± 2.26
pH	7.34 ± 0.03	7.31 ± 0.03	7.35 ± 0.02	7.33 ± 0.02

* p ≤ 0.05 compared to untreated control of the same set (paired *t*-test)

P169 | Haemolysis in red cell concentrates is associated with buffy coats with 'white particulate matter'

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Background: Blood components with 'white particulate matter' (WPM) have been described in the literature since 2004 (Transfusion, Vol. 44). They are known as white, fatty particles and aggregates of platelets and leukocytes. WPM may pass leukoreduction filters but may disappear after warming to 37°C and has no impact on adverse reactions in patients after transfusion. In buffy coats (BCs) that immediately show WPM after separation of the whole blood it has been found that 40% of these BCs also immediately show haemolysis. Donations with WPM were obtained mainly from donors with obesity, a history of 'fatty' blood products or donors who take medication.

Aims: To investigate the quality of red cell concentrates (RCCs), corresponding to BCs containing WPM that also show haemolysis.

Methods: After centrifugation of a sample from a BC-with-WPM, the plasma supernatant was visually judged for haemolysis. Based on this observation, RCCs of the same donations were selected as the BC-with-WPM-and-haemolysis (group A, $n = 10$), or connected to a non-haemolytic BC-with-WPM as controls (group B, $n = 10$). The BCs were analysed on day 1 and the RCCs during storage on day 1, 35 and 42. The lipid profile was analysed from the BC supernatant plasma.

Results: Measurement of haemolysis in BCs confirmed visual judgement: 0.44 ± 0.34% (group A) vs 0.10 ± 0.06 (group B, $p < 0.01$). No significant differences were observed between quality parameters of both RCC groups, and haemolysis showed normal levels in 18/20 RCCs (<0.4%, day 42). However, in group A there were two outliers with 1) 1.1 % and 2) 0.8% haemolysis in the RCC (day 42). In 1) an abnormally high triglyceride concentration in the BC ('fat plasma', 3.6 mM; mean ±SD:1.8 ± 0.8) and a high metabolism of the red blood cells in the RCC seems to indicate a lower quality of the red blood cell (membranes). In 2) a very high haemolysis in the BC (1.3%) in combination with the maximum percentage of reticulocytes (6.8%; mean±SD:4.7 ± 1.1) and monocytes (16.2%; mean±SD: 9.2 ± 3.1) seems to indicate a high level of haemolysis in the donor (possibly caused by antibodies or complement activation). This may also explain why haemolysis in the RCC, that contained less plasma than the BC, was lower than in the BC.

Summary/Conclusions: In RCCs connected to BC-with-WPM in which no haemolysis was observed on day 1, haemolysis was normal during 42 days of storage. In RCCs of donations with haemolytic BC-with-WPM, 20% showed high haemolysis caused by two different mechanisms. So, red blood cells associated with haemolytic BC-with-WPM have increased risk for haemolysis. In further studies we aim to increase numbers to investigate and to gain a more mechanistic insight.

P169 - Table 1. Statistical results

		N	Mean	Standard deviation
Platelets (10^11/unit)	Trima	158	3.057	0.217
	Real	158	2.921	0.426
	Difference		0.136	
	Sig (p)		0.000	
Volume (mL)	Trima	158	291.854	22.036
	Real	158	280.258	21.939
	Difference		11.597	
	Sig (p)		0.000	

P170 | Analysis of the estimated volume and platelet count by an apheresis device

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Background: In order to fulfil GMP requirements in a blood bank it is necessary to trace the different procedures carried out on the products and reflect accurately the characteristics of these products.

Aims: To check that the estimated value of platelet count and volume of the apheresis platelets obtained by Trima apheresis device (Terumo BCT®) reflects the real value and, consequently, be able to confirm that the information systems we use allow us to confirm the correct traceability of data.

Methods: We have retrospectively analysed 158 apheresis platelets from a 16 months period. Information about those 158 units was transmitted from Trima device to our IT system through TOMEs software. Of the studied platelets, 128 units were single dose and 30 units double. From double dose platelets we have only analysed one of the two final products (Trima estimates the amount of volume and platelet count of the two of them at the same time). All the apheresis were donated in two different donation sites (site 1:153 units and site 2:5). Data analysed was registered and measured twice, one of them, from the value calculated by Trima device during apheresis processes, and the other, from the real weight of final product using an electronic balance and the count of platelets by using a cell counter (BC-3600; Mindray®). For calculations, we defined a density of 1,026 g/mL and a 47 g bag tare. We determine mean and standard deviation for descriptive purposes and Student's t test to establish significant differences among independent samples using the SPSS program.

Results: There are statistically significant differences between the value of volume and platelet count of apheresis platelets registered by Trima Equipment and real data of these products.

Summary/Conclusions: We obtained statistically significant differences in the studied parameters although we think the clinical significance of the difference is low due to low absolute values. Therefore, Trima device is able to estimate correctly the final volume and real platelet count in apheresis platelet products and offer good assistance to the

operator. In addition, the TOMEs system allows correct traceability of the data obtained and allows it to work under GMP conditions.

P171 | Abstract withdrawn

P172 | Abstract withdrawn

P173 | Abstract withdrawn

P174 | Abstract withdrawn

P175 | Abstract withdrawn

P176 | Assessment of blood bag plasticizer migrations in blood components

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Background: Polyvinyl chloride (PVC) plasticized with di(2-ethylhexyl) phthalate (DEHP) is a widely used material for medical transfusion devices. Not covalently bound to PVC, DEHP can migrate into blood products during storage. Recognised as endocrine disruptor and raising concerns about its potential carcinogenicity and reprotoxicity, DEHP is gradually being withdrawn from the medical device market. Therefore, the use of alternative plasticizers, such as diisononylcyclohexane-1,2-dicarboxylate (DINCH) and di(2-ethylhexyl) terephthalate (DEHT), have been investigated as potential candidates for the replacement of DEHP in medical transfusion devices.

Aims: The purpose of this study was to evaluate the quantification of PVC-plasticizers in the blood components according to their preparation, storage conditions and in function of the plasticizer.

Methods: Whole blood was collected, and labile blood products (LBPs) were prepared by the buffy-coat method with a PVC blood bag plasticized either with DEHP, DINCH or DEHT. DINCH and DEHT equivalent concentrations were quantified in LBPs by liquid chromatography-tandem mass spectrometry or coupled with UV and compared to DEHP equivalent concentrations.

Results: The plasticizer equivalent concentration to which a patient is exposed during a transfusion depends on the preparation of LBPs as well as their storage conditions, i.e., temperature, and storage time. At day 1, for all LBPs, the migration of DEHP is 5.0 and 8.5 times greater than DINCH and DEHT, respectively. At the end of the 49 days storage period, the DEHP equivalent concentration in red blood cells concentrate (RBC) is statistically higher when compared to DINCH and DEHT in the condition tested with maximal values of 30.19, 18.25 and 14.61 µg/mL, respectively.

Summary/Conclusions: In addition to lower toxicity, transfused patients using PVC-DEHT or PVC-DINCH blood bags are less exposed to plasticizers than using PVC-DEHP bags with ranging exposure reduction from 38.9% to 87.3%, due to lower leachability into blood components.

P177 | Evaluation of haematological analyser equipped with new blood bank mode for quality and safety of donor blood and transfusion products

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Background: XN-Series Blood Bank mode is a new analytical method for residual WBC (rWBC) and residual (rRBC) enumeration in blood products on haematology analyser. This enables to perform the quality as well as safety control of blood products on a single analyser.

Aims: Our objective was to compare the measurement of rWBC and rRBC in blood products with the XN Blood Bank mode and the laboratory standard operating procedures of manual counts. Moreover, to compare whole blood CBC values from blood donors and the quality of blood products from the Sysmex XN analyser versus XS-1000i analyser.

Methods: For blood donors, 190 samples from blood or apheresis donors were analysed in both the Sysmex XS-1000i and XN-1000 analysers and the mean values of six CBC parameters (WBC, RBC, HGB, HCT, MCV, PLT) were compared. For transfusion products, 164 samples were collected: 13 plasma products (whole blood), 9 plasma products (apheresis), 36 RBC concentrates (whole blood), 30 PLT concentrates (pooled buffy coats), 36 PLT concentrates (whole blood) and 55 PLT concentrates (apheresis). All blood product samples were analysed with manual counting methods and the XN Blood Bank mode (for rWBC and rRBC) or XN whole blood mode (for PLT) to measure the residual cells and compare the manual and automated method.

Results: All examined CBC parameters of blood donors showed equal performance, with excellent correlation coefficient (r) ranging from 0.821 to 0.995. A highest difference was found for platelets (15.29) although not statistically significant ($p = 0.075$). Most of the blood products did not have a quantifiable number of residual cells, meaning the number of rWBC and rRBC, if present, was below the LoQ of the different methods. rWBC were detected by Blood Bank mode in plasma products (whole blood) with mean rWBC of $0.012x10^9$/L and the PLT concentrates (pooled buffy coats) with mean rWBC of $0.19x10^9$/L. Correlation coefficient (r) in both analysers for all three parameters (HGB, HCT, RBC) in RBC concentrate (whole blood) was excellent, ranging from 0.95 to 0.99. For platelet count, r was in the

range of 0.98 to 0.99 with a high intercept for PLT concentrates (whole blood):23.48 and PLT concentrates (apheresis):12.96.

Summary/Conclusions: XN-Series analyser equipped with a Blood Bank mode can be reliably used for blood donor evaluation, enumeration of residual cells as well as measurement of quality of various blood transfusion products.

P178 | Evaluation of a new automatized methodology to perform blood components quality control

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Background: According to the main blood guidelines (CAT and EDQM), the 1% of blood components products have to be analysed to ensure the quality of the manufacturing process. The current quality control (QC) work-flow of blood products in the Blood and Tissue Bank of Barcelona include different techniques in order to evaluate all required parameters in each blood component. Nowadays there are no gold standard techniques for all the parameters, so, some techniques are analyst-dependent which could lead into variation in the sample manipulation or analysis. Moreover, blood components CQ is a large process time-and cost-consuming that in our lab is performed every weekday.

Aims: Validate a new methodology for blood components QC to reduce sample manipulation but also to reduce time and cost of the process, as well as to improve and automatize the analysis of the CQ samples of blood products.

Methods: Different techniques are being used for blood component QC. For residual (r) cell count, two different flow cytometry techniques are currently performed: Leukosure (Beckman coulter) for rWBC count, and an *in house* CD235a stain for rRBC., Platelets, RBC, haemoglobin (Hb) and haematocrit (Hct) are analysed with the XN-550 (Sysmex) analyser using impedance and Cyanide-free SLS haemoglobin modules. The aim of this study was to evaluate the use of Blood Bank (BB) mode of the XN-1000 (Sysmex) analyser which could allow us the automatization of the whole QC process. The BB mode is set up to analyse: rWBC, RBC, Hct and Hb in RBC concentrates; and rRBCs, rWBC, platelets and Hb in plasma and platelets products. The BB mode analyses platelets, (r)RBCs and (r)WBCs by a specific flow cytometry techniques whereas haemoglobin and haematocrit are measured using the same methodology as the XN-550 analyser. To perform this study, 950 samples from blood components were analysed in parallel using our current techniques and compared with the BB mode analysis.

Results: rWBC count perfectly correlates between methods with an $r^2 \geq 0.97$ in all leukoreduced blood components analysed (RBC concentrates, plasma and platelets). rWBC specification for leukoreduced products is 1E6 WBC/unit, and just a 1.9% ($n = 724$) of the product differs in conformity depending on the method used. Regarding rWBC count, we have observed that the count with BB mode is a very sensitive technique in which the rubber of vacutainers caps could interfere giving a false rWBC count. About rRBC count in plasma and platelet samples no differences in products conformity have been observed using both techniques ($n = 739$). Another method that changes is the platelet count, from impedance to an automated flow cytometry count. We have observed that platelet count in platelet and plasma products increases 17% ($n = 215$) and 50% ($n = 522$) respectively. Finally, no differences have been detected in hematocrit and haemoglobin levels in any blood component.

Summary/Conclusions: We have validated the new BB mode of the XN-1000 using more than 950 blood components samples and we have obtained similar results in the conformity of almost all the products due to the use of different techniques. We can conclude that, besides some differences observed, the BB mode of the XN-1000 is a good automated alternative for blood components CQ. With this new methodology we can significantly reduce the technical time, sample manipulation and the subjectivity in the flow cytometry analysis, as well as diminish the cost of blood components CQ.

P179 | More quality control–more confidence

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Background: Blood components are standardised. Their quality depends on both the source and processing. Most of produced blood components are issued for haematology and oncology patients, mostly multitransfused, and for acutely bleeding patients. Blood transfusions still rely on people who donate blood mostly regular blood donors. Modern transfusion therapy is guided by implementation of best available blood components and their quality.

Aims: Our goal was to determine the average blood test results of blood donors before donation process. After optimised processing of different blood components on automatic devices from blood donation through all steps of production, quality of all processed blood components was determined according to the Requirements and quality control from Guide to the preparation, use and quality assurance of blood components, EDQM 20th Edition 2020. More testing was done to observe the influence of intermediate steps in processing blood components (buffy coat BC, low level of haemoglobin in fresh frozen plasma FFP).

Methods: Blood tests were done for every blood donor before the donation (DxH 900 Beckman Coulter®). Totally 55 blood donations. Blood was collected in top&bottom quintuple blood bags with "in line" soft filters (Terumo Imuflex® CRC) on automatic blood mixers (Bagmatic NOVO ®). All units were separated on automatic blood separator (Luxomatic®) In RBC (red blood cells) BC (buffy coat) removed in AS (SAGM), and in RBC leucodepleted (by filtration) in AS, volume,

number of erythrocytes, level of haemoglobin, hematocrit was controlled. The volume, number of platelets and leucocytes was determined in BC. PC (platelet concentrates) single unit in plasma, were controlled for the platelet number and pH (Gem Premier IQM 3500®, blood gas analyser) at the end of stocking period. FFP (fresh frozen plasma) was controlled for the low level of haemoglobin (Haemocue low level®) immediately after processing.

Results: The average blood donor number of ER was 5,17X10^{12} /L; leucocytes 6,7 X 10^9/L; Htc 0,44; Hgb 156 g/L and Plt 217 $\times$ 10^9/L. In RBC BC removed in AS, the average volume was 285 ml, average number of ER was 7,66 $\times$ 10^{12} /L, Htc 0,65 and Hgb 231 g/L (65,83 g /unit). In RBC leucodepleted in AS, the average volume was 250 ml, the average number of ER was 5,58 $\times$ 10^{12} /L; Htc 0,52, Hgb 172 g/L (42,5 g/unit). BC units with average volume of 100ml and average level of Plt 297 $\times$ 10^9/L and 30,5 X 10^9/L leucocytes. In PC single unit in plasma the average number of Plt was 60,06 $\times$ 10^9/U, the average unit volume 70 ml and the average pH at the end of stocking period was 7,32. In FFP the average low level of Hgb was 0,04.

Summary/Conclusions: Everyday work is constant search for better blood component quality through continuous improvement in health education of our blood donors and implementation of best procedures in automatic blood separation. Quality is the responsibility and commitment of all doctors and technicians involved in all processes from blood donation to final component. More QC on every processing step, although not mandatory, enables better therapy for patients.

P180 | Evaluation of thawing Fresh Frozen Plasma (FFP) at 37°C and 45°C

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Background: Recently, a new thawing device (Plasmatherm V [PTV], Barkey GmbH & Co. KG) has been developed that uses both 37°C and 45°C temperatures and can thaw plasma even faster than its predecessor the Plasmatherm Classic (PTC) and Sarstedt Sahara-III Maxitherm and Thermogenesis Thermoline (TT) Water bath, which are the current methods in the UK. However, studies comparing the effect of plasma quality between Plasmatherm V and above devices have not previously described.

Aims: To assess the haemostatic quality of thawed plasma (LG-Octaplas) using the PTV method at 37°C and 45°C versus TT at 37°C and the PTC at 37°C and 45°C.

Methods: LG-Octaplas was thawed 8 times for TT, PTC @37°C and PTC @45°C and 6 times for PTV @37°C and PTV @45°C. Once thawed, plasma was stored at 2–4°C, and aliquots were taken for haemostatic testing at three different times: **1)** Baseline, 5 minutes after removal from the thawing device; **2)** 24 hours after thawing; and **3)** 120 hours after thawing. Samples were stored at −70°C until testing. Assays performed were prothrombin time (PT); activated partial thromboplastin time (APTT); Clause Fibrinogen (FibC); factor II, V, VII, VIII, and XI activity; free protein S antigen (Free PS) and protein C activity (PC). A one-way ANOVA and Tukey's post hoc analysis were conducted to determine if the haemostatic properties of plasma differed when thawed using different thawing methods. Data is presented as mean ± standard deviation.

Results: Baseline measurements for all assays with each thawing method were within the normal reference ranges for a healthy adult. There was no significant difference between PT, FibC, FII, FV, PC and Free PS between all methods tested. However, there was a significant difference in APTT, FVII, FVIII and FXI between PTV versus other methods, with PTV at 37°C and 45°C having a higher level of each of these factors and a shortened APTT at the baseline when compared with other methods. Over time clotting factors reduced with all methods with no difference between the methods.

Summary/Conclusions: The quality of plasma thawed by Plasmatherm V at 37°C and 45°C is no different to other thawing methods: if anything, at baseline certain clotting factors were more favourable in plasma thawed by PTV than other methods. The use of PTV at 37°C and 45°C does not impair plasma quality and should be considered in hospital laboratories to improve the rapid availability of plasma and reduce plasma wastage associated with extended thawed FFP.

P181 | Qualification of a residual white blood cell counter based on fluorescent microscopy

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Background: The gold standard for the residual white blood cell (rWBC) count in leukoreduced blood components is the cytometry. The apparition of this technology relieved the laboratory technicians and spent time compared to a manual count with chamber counter by microscopy. The ADAM rWBC HT is an automatic microscope with a high precision count and little human intervention.

Aims: This study has the objective to qualify the ADAM rWBC HT for precision, accuracy, linearity, limit of quantification and stability.

Methods: The ADAM rWBC HT is a digital microscope that counts rWBC stained by propidium iodide (PI) and detects its fluorescent expression. A mix of blood component and reagent containing PI and lysis solution (ratio 1:4 for platelet concentrates (PC) and red blood cell concentrates (RBCC), ratio 1:1 for plasma) is deposited on a disposable slide and placed on a rack to be automatically transported to the fluorescent microscope that counts rWBC in a minute. It automatically focuses on the slide and counts the cells from 63 images, then averages out the counting results to increase the accuracy and reliability.

The repeatability and reproducibility were tested on 4 control samples (PC with high and low WBC count and RBCC with high and low WBC

P181 - Table 1

Tests			CV Conformity (%)	CV (%)
Repeatability	High controls	PLT	< 10	6.64
		RBC	< 10	5.57
	Low controls	PLT	< 20	20.54
		RBC	< 20	21.07
	PC		< 10	8.20
	Plasma		< 10	3.46
	RBCC		< 10	9.50

P181 - Table 2

Tests			CV Conformity (%)	CV (%)
Reproducibility	High controls	PLT	< 10	8.96
		RBC	< 10	9.43
	Low controls	PLT	< 20	23.08
		RBC	< 20	25.35
	PC		< 10	8.49
	Plasma		< 10	3.76
	RBCC		< 10	9.70

count) and on blood components (PC, plasma and RBCC) on 10 replicates. The coefficient of variability (CV) was fixed at lower than 10% for repeatability and reproducibility for high controls and blood components and lower than 20% for repeatability and reproducibility for low controls. Linearity was tested on the 3 blood components with more than 98% as accepted limit. Stability tests were realized until 48h on plasma and PC products. Limit of quantification was determined by 10 x standard deviation after 24 tests of physiological water.

Results: All the results are included in tables 1 and 2. Repeatability and reproducibility for blood components and high controls respect the CV conformity with less than 10%. For the low controls, the CV is higher than the 20% of conformity with a maximum of 25.35%. The linearity has a R^2 higher than 98% for the 3 blood components. The stability of the sample is maintained until 24h after sampling with a coefficient of variation lower than 10% for PC and plasma at 24h. The quantification limit is 0.33 cell/µL for the ratio 1:4 and 0.45 cell/µL for the ratio 1:1.

Summary/Conclusions: The results respect the conformity, excepted with the low controls for repeatability and reproducibility. As expected, when the WBC results are low, the CV is high. Nevertheless, the repeatability and reproducibility are conform for blood components. The linearity for the 3 blood components is conform. The limit of quantification is close to 0 to allow counting weak contamination in the sample. The results confirm that the device is reliable for the counting of rWBC in plasma, PC and RBCC.

P182 | EBA working group, European quality control proficiency testing scheme

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Background: Biological parameters of blood products are very different compared to the physiology, making it challenging to find suitable quality controls. This working group is dedicated to improving the quality control methods in the European Blood Alliance (EBA) voluntary member countries.

Aims: The aim of the working group is to lead and co-ordinate a collaborative scheme of inter-laboratory testing to demonstrate the equivalence of the procedures between laboratories. The group also aims to compare new or established analytical procedures and to highlight difficulties or deficiencies in their performance.

Methods: In 2020, a four-cycle pilot phase was conducted by the French EFS to assess logistical organization and financial feasibility. Each cycle involved one laboratory preparing and shipping samples to other participants. A second phase was conducted in 2022 in order to improve the procedure. It included 4 news cycles, each of them being organized by different laboratories from SFS Belgium, CRL Luxembourg and EFS France.

Common parameters such as haemoglobin, haematocrit, haemolysis in Red Blood Cells and platelet count in Platelet Concentrates were studied.

A report was established for each cycle including a statistical exploitation of the results: estimation of the robust mean, calculation of Z-score and bias.

Results: Despite the variations in transport conditions across countries, (delivery times and temperature variations), the inter-laboratory was feasible. In 2022, 21 quality control laboratories from 11 different countries participated in four cycles, and three countries organized the exchanges. Four reports were published by the organizers of the assessment

Summary/Conclusions: The success of the inter-laboratory testing scheme demonstrates its potential for improving quality control methods for blood products. For 2023, we plan to study other parameters such as White Blood Cell contamination and consider long-term objectives such as benchmarking based on surveys on different topics like sampling methods and statistical process control monitoring of production processes.

P183 | Comparison between fluorescent microscopy and cytometry for residual white blood cell counting in blood components

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Background: White blood cells (WBC) in blood components are responsible for a number of well-known adverse effects in blood transfusion. The increasing use of WBC-reduced blood components has raised the need for a simple, stable and accurate routine method for counting low numbers of WBC.

Aims: The aim of the study is to compare the counting results from the cytometer FACSVia and the fluorescent microscope ADAM rWBC HT in different blood components.

Methods: The ADAM rWBC HT is a digital fluorescent microscope able to count stained residual WBC (rWBC) by propidium iodide (PI) in platelet concentrates (PC), plasma and red blood cell concentrates (RBCC). A mix of blood component and reagent containing PI and lysis solution (ratio 1:4 for PC and RBCC, ratio 1:1 for plasma) is deposited on a disposable slide and placed on a rack to be automatically transported to the fluorescent microscope that counts rWBC in a minute. It automatically focuses on the slide and counts the cells from 63 images, then averages out the counting results to increase the accuracy and reliability.

The study was based on the comparison of 50 samples of plasma, PC and RBCC measured with the cytometer and the fluorescent microscope. The plasmacount kit by Becton Dickinson was used to measure the rWBC in plasma units by cytometry and the leucocount kit was used for PC and RBCC. A Bland-Altman analysis was realized to detect a difference between the 2 devices.

Results: Results are presented in the table 1. For the plasma, the difference between the two counting methods is -0.45. The negative result means that the cytometer tends to count lower than the fluorescent microscope. 90% of the results are in the specification limits set at ± 2 standard deviations (SD). The difference between the two counting methods for the PC is -0.50 and 92% of the results are in ± 2 SD. Finally, the difference for the RBCC is -0.40 with 96% in the specifications.

Summary/Conclusions: The Bland Altman analysis shows that the ADAM rWBC HT gives same results in the 3 blood components in terms of difference (about -0.45) between the two counting methods and in terms of respect of specification limits (about 93% of results in the specification limits). The ADAM rWBC HT provides reliable results comparing the cytometer.

P183 - Table 1

Blood components	Difference FACSVia/ ADAM rWBC HT	Specification limits (± 2 SD) (%)
Plasma	−0.45	90
PC	−0.50	92
RBCC	−0.40	96

P184 | An in vitro comparison of red blood cells haemolysis in neocyte-enriched and leucocyte-poor blood

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Background: Red blood cell concentrates (RBCC) have a shelf life of 42 days when stored at 1-6°C. This may lead to shortages and wastage as biological changes due to storage lesions develop, causing these units to be discarded. Pooled neocytes could potentially extend the shelf-life of the RBCC unit. Patients with certain conditions, such as, sickle cell disease, depend on regular blood transfusions. These patients could potentially experience transfusion complications. This study was conducted in an attempt to improve the longevity of stored red blood cells, through isolating neocytes and examining the rate of haemolysis, biochemical changes and viability, by comparing these with pre-stored leucocyte reduced red cell concentrates.

Aims: The aim of this study was to determine whether neocyte-enriched blood could extend the survival of stored red blood cells compared to pre-stored leucocyte reduced red blood cells.

Methods: 30 pre-stored leucocyte reduced red blood cells units were processed. Two transfer bags were attached to the pre-stored leucocyte reduced red blood cells bag by using a sterile heat sealer. The neocytes and the additives were extracted from the pre-stored leucocyte reduced red blood cells. These were centrifuged and the two transfer bags were placed on separate scales. The additive was separated into one satellite bag while the concentrated neocytes were separated into the second satellite bag. The Saline Adenine Glucose Mannitol nutrients were added to the neocyte-enriched bag. The transfer tube between the two bags was sealed. The nutrient transfer bag was then discarded and the neocyte bag was the final required product. Samples were prepared every 14 days and tested for red blood cell count, mean cell volume, mean corpuscular haemoglobin concentration, haemoglobin, sodium and plasma haemolysis.

Results: The results demonstrated a decrease in the pre-stored leucocyte reduced red blood cells units and an increase in the neocyte-enriched unit for the red blood cell count. The difference is not seen as significant. The mean cell volume has increased, while the mean corpuscular haemoglobin concentration has decreased in both units. The sodium levels have decreased while the percentage plasma haemolysis has increased for both units ($p = 0.36$). The results therefore indicated that neocyte-enriched blood do not extend the survival of red blood cells significantly, compared to pre-stored leucocyte reduced red blood cells.

Summary/Conclusions: Biological changes have occurred in the pre-stored leucocyte reduced red blood cells as well as the neocyte-enriched unit. The outcome of this study demonstrates that neocyte-enriched blood does not have a survival advantage to pre-stored leucocyte reduced red blood cells by using the conventional manual collection method. This is aligned with the results from previous studies which utilised multiple methods to collect a high neocyte yield.

Feasibility was highlighted as the main challenge as many of these methods have proven to be too expensive and too laborious.

P185 | Validation of buffy coat pooled platelet concentrates in platelet additive solution versus in plasma; both prepared using an octopus pooling filter set

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Background: Preparation of Buffy coat pooled platelet concentrates is an established practice in Europe for more than two decades. Often considered as an alternative to apheresis platelets, these are prepared at low cost, contain the required dose of platelets and can be leukoreduced. Our center has been routinely preparing leukoreduced buffy coat pooled platelet concentrates in 100% plasma (PL-BCPPs) using the Train method of pooling. We are looking for the possibility to introduce Platelet Additive Solution (PAS) for platelet storage and prepare leukoreduced buffy coat pooled platelet concentrates in PAS (PAS-BCPPs).

Aims: Our study aimed to assess the quality of the PAS-BCPPs prepared and check if the results are comparable to the PL-BCPPs and meet the International guidelines for whole blood derived platelet concentrates. We also evaluated the performance of the Octopus Pooling set with Platelet filter.

Methods: Whole blood was collected in 450ml Top & Bottom Compoflex Blood Bags (Fresenius Kabi). Following hard spin centrifugation, the units were separated on the automated blood component extractor CompoMat G5 Plus (Fresenius Kabi) and buffy coats with a volume of 55-60 ml were produced.

Four ABO-matched buffy coat units was pooled together along with a platelet storage medium into the pooling bag of the Octopus pooling set BioP Flex Pool (Fresenius Kabi). The Octopus set involves rinsing of the buffy coat bags with the medium to avoid any buffy coat loss. 20 sets of pooled buffy coat units were prepared. Of these, 10 sets had the PAS Solution InterSol 280 ml (Fresenius Kabi) as the storage medium while the remaining 10 sets used same group plasma.

The pooled buffy coats were centrifuged using a soft spin and separated on the CompoMat G5 Plus, with simultaneous filtration for leukocyte reduction with the BioP filter of the pooling set.

The prepared PL-BCPPs and PAS-BCPPs (10 sets each) were analysed for quality parameters such as volume, platelet yield, pH and swirling (visual inspection). Filtration time and residual leukocyte count were also measured to assess the filter efficacy. Anti-A and Anti-B antibody titres were measured in both the groups of pooled platelets.

Results: The quality parameters of the both the types of pooled platelet concentrates were comparable. For PL-BCPPs and PAS-BCPPs, the mean platelet volume was 296 ± 22.99 mL and 281 ± 11.04 ml, platelet yield 385.18 ± 46.80 x 10^9 and 306.58 ± 67.90 x 10^9 cells while pH was 7.28 ± 0.56 and 7.17 ± 0.44 respectively. Swirling was observed in all the 20 sets of pooled platelet concentrates.

The residual Leukocyte count was 0.32 ± 0.24 x 10^6 and 0.24 ± 0.20 x 10^6 in PL-BCPPs and PAS-BCPPs respectively, while the filtration time was 2.32 ± 0.51 min and 1.84 ± 0.26 min respectively.

Both the PL-BCPPs and PAS-BCPPs groups had 8 units of Group O and one each of Group A and B. Among the PL-BCPPs, the Anti-A titres were in the range of 1:8 to 1:256 while the Anti-B titres were in the range of 1:8 to 1:1024. In the PAS-BCPPs group, the Anti-A titres were in the range of 1:4 to 1:16 while the Anti-B titres ranged from 1:4 to 1:32.

Summary/Conclusions: The PAS-BCPPs fulfilled the quality criteria of International and local guidelines on Blood components. Both the pooled platelet concentrates (PAS-BCPPs and PL-BCPPs) had similar quality. Leukoreduction and filtration time by the BioP filter also fulfilled the guidelines criteria. The ABO antibody titters were significantly reduced, in pooled platelets stored in PAS. Thus, use of PAS can be standardised and implemented in routine preparation of pooled platelets. The next step would be to assess the five days storage and clinical efficacy of these platelets.

P186 | A comparison between the strength of platelet lysate and Platelet Factor 4 to inhibit the bacterial growth in vitro

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Background: Recently the role of platelets in the tissue regeneration, wound healing, and prevention and control of infections have been reported. The treatment of bacterial infections has become a global challenge due to the spread of multidrug-resistant bacteria; therefore, the development of new treatment options for the mentioned infections is essential.

Aims: We assessed the antibacterial effect of platelet lysate (PL) derived from human platelet concentrates and recombinant platelet factor 4 (PF4) on bacteria commonly found in nosocomial infections.

Methods: In this study, the antibacterial activity of PL and PF4 was tested on *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Acinetobacter baumannii* by well diffusion and serial micro-dilution methods.

Results: *In vitro* results showed that PF4 inhibited the growth of *S. aureus*, *E. faecalis A. baumannii*, as a new antibacterial agent. While PL had no discernible impact on the growth parameters of any bacterium tested, statistical analysis showed that there is a significant difference between PL and PF4 by measuring the inhibition zone diameters.

Summary/Conclusions: PF4 that is a biocompatible and safe byproduct of platelets could be potentially useful to preclude the nosocomial infections. The use of PF4 is an attractive strategy for treating *A. baumannii* and *S. aureus*, *E. faecalis* nosocomial infections because it considerably inhibits the bacterial growth.

P187 | Abstract withdrawn

P188 | What usage of leucodepleted labile blood products in a middle-income country: A Tunisian report

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Background: High-income countries have implemented universal leukocyte depletion as part of their blood safety policy. As a middle-income country, the Tunisian health authorities can't afford universal leucodepletion. Thus, leukocyte depletion blood products are selectively prepared and issued based on specific indications

Aims: We aim to assess the indications of Leucodepleted-Packed Red Blood Cells units (L-PRBC) in Sfax, the second-largest city in Tunisia.

Methods: A prospective study was conducted over four consecutive years (2019 –2022). Fresh PRBC (<48 hours) are leucodeplated in our Blood Center both in closed and open systems. A minimal stock of closed-system L-PRBC is prepared on a weekly basis. Additional open-system L-PRBCs are prepared on demand during 2022. We also keep a traceability register for each prepared and issued L-PRBC with its indications. In the first two years of the study, indications were secondary prevention for patients with clinically significant anti-HLA antibodies. Then, indications were extended to primary prevention in haemoglobinopathies and chronic kidney failure when eligible for transplantation.

Results: A total of 3 742 L-PRBC were prepared and 3 696 issued or 3.5 % of the total issued PRBC. Haemoglobinopathies, other benign haemopathies, kidney transplants, chronic kidney failure, malignant haemopathies, and other diseases were respectively 50.2, 19.5, 14.2, 6.1, 3, and 7% of indications. Total issued L-PRBC varied from 664 to 1156. Over the years, the number (3 696) and % (3.5) of L-PRBC had a linear increase: 3% during 2019-2020 and 4% during 2020-2021 (linearity = 0.92; $p = 0.3$) and L-PRBC out-of-date decreased (overall 1.2%).

Summary/Conclusions: In our setting, L-PRBCs issuing was managed based on strict leucodepletion indications or secondary prevention of anti-HLA alloimmunization. Since 2021, the indications were extended to primary prevention of anti-HLA alloimmunization in two sets of patients, haemoglobinopathies, and chronic kidney failure to safeguard their transfusion and organ transplantation chances. This only led to a slight rise in our consumption and an acceptable out-of-date rate. Resources are needed for universal leucodepletion to insure better transfusion global security.

P189 | The effect of N-acethyl cysteine on lipid peroxidation and antioxidant status of packed red blood cell during storage

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Background: Red blood cell (RBC) storage lesion is a process can affect RBC viability and RBC quality during storage. In this regard, oxidative stress is one of the main causes of RBC storage lesion.

Aims: In this study, we evaluate the effect of N-acethyl cysteine (NAC) as an anti-oxidant on RBC oxidative damage during storage.

Methods: In this experimental study, 10 packed red blood cells (PRBCs) were randomly assigned to the Iranian Blood Transfusion Organization's innovation center. The effect of NAC was investigated on some biochemical variables, malondialdehyde (MDA) and total anti-oxidant capacity (TAC) of PRBCs up to 35 days of storage.

Results: The concentration of lactate and lactate dehydrogenase (LDH) enzyme activity in NAC-treated PRBC were less increased compared to the control group (without NAC) ($P<0.05$). Also, MDA concentration was less increased in NAC-treated group than the control during storage ($P<0.05$). Anti-oxidant capacity was so significantly higher in NAC-treated PRBC than the control, especially in the day of 28 of PRBC storage.

Summary/Conclusions: The results of this study showed that N-acethyl cysteine (NAC) can increase the survival and quality of red blood cell during storage by maintaining the anti-oxidant capacity of red blood cell and in the future it can be used as an additive to improvement of the PRBC quality during storage.

P190 | An innovative murine model for platelet transfusion

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Background: Platelets play a crucial role in haemostasis. With advances in medical care, there is an increasing demand for platelet transfusions. In the United States, platelet units can be stored for up to 7 days. Platelet units may undergo one of several modifications, including pathogen reduction techniques or storage in pathogen additive solution. Although platelet units are assessed by *in vitro* methods and post-transfusion recovery, it remains unknown how modifications and time in storage alter platelet function *in vivo*.

Aims: Despite the importance of platelet transfusion in medical care, there is no good animal model available to study platelet transfusions. We present a reproducible mouse platelet unit that meet the following standards: pH > 6.2; white blood cell (WBC) counts less than 2.08

x 10^3 per unit, and post-transfusion recovery (PTR) > 60% after 24 hours.

Methods: Using a commercially available transgenic mouse line with ubiquitously expressed green fluorescent protein (GFP), whole blood was collected by aseptic cardiac puncture and platelet-rich plasma (PRP) was diluted in phosphate buffered saline for better separation and isolated after two soft centrifugations. Platelets were pelleted from PRP with a hard centrifuge spin and resuspended in mouse fresh-frozen plasma. To measure PTR, we collected fresh whole blood by aseptic cardiac puncture from commercially available transgenic mice with platelets expressing red fluorescent protein (RFP). The transfusate was made from a mixture of fresh RFP whole blood and fresh or longer-stored GFP platelet unit and transfused into wild-type mice. Post-transfusion whole blood samples were collected at various time points following transfusion and were assessed by flow cytometry as the percent of transfused GFP platelets of the total number of transfused platelets (RFP + GFP platelets) still in circulation, normalized to the percent of GFP platelets transfused. Platelet counts, unit composition, and platelet activation were measured throughout unit storage. Platelets units samples were activated with 0.5U/mL high activity bovine thrombin for 2 minutes at room temperature. Platelets were labeled with anti-CD41 to label all platelets and anti-CD62P to label activated platelets.

Results: Using the Leucocount kit to measure WBC counts, units have ranged from 3.0 x 10^2 to 9.8 x 10^2 WBC/unit. The pH of fresh, 1-day stored, and 2-day stored platelet units were 7.25, 6.75, and 6.0, respectively. Although the trends are consistent between experiments, the activation of fresh platelets from one replicate was 26.77% at baseline and 70.72% following thrombin; activation of 1-day stored unit was 33.50% at baseline and 59.67% following thrombin; and activation of 2-day stored unit was 61.80% at baseline and 72.76% following thrombin. PTR of fresh, one-day stored, and 2-day stored platelets after 24 hours was 93.29%, 87.66%, and 0.00% respectively. Additionally, our platelet units were negative for bacterial contamination by culture in pediatric blood culture bottles.

Summary/Conclusions: These data demonstrate that we have developed a reproducible mouse platelet unit analogous to a transfusable human platelet unit, with a maximum storage time of 1 day storage for an "old" unit. Using this mouse platelet model, modifications and storage conditions can be applied to limitless transfusion scenarios. We anticipate that this powerful mouse platelet model will improve patient transfusion practices.

Blood products

Plasma derived products

P191 | Patient reported outcomes of serum eye drops manufactured from Australian blood donations and packaged using MEISE vials

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Background: Dryness and inflammation of the ocular surface can result in severe discomfort and reduced vision in one or both eyes. Dry eye predominantly affects females and can account for a quarter of referrals to an ophthalmologist. Serum from blood donation can be used as a substitute for tears as it contains similar growth factors and nutrients. In 2015, Australian Red Cross Lifeblood (Lifeblood) reported positive effects of autologous serum eyedrops (SED) (ASED) on self-reported ocular symptoms and vision-related quality of life (QOL). Although effective, collection of ASEDs can have barriers including poor venous access, low haemoglobin levels and other comorbidities. Patient-tailored (allogeneic) SEDs (PT-SED) made from healthy blood donors are an alternative. In May 2020, Lifeblood introduced the MEISE vial packaging process for SEDs.

Aims: This study aimed to conduct a prospective patient-reported outcome analysis to examine efficacy of SEDs and satisfaction with the MEISE vial packaging.

Methods: Patients that were provided SEDs between 1 November 2021 and 30 June 2022 were invited to participate. Patients were asked to complete standardised health questionnaires including the dry eye questionnaire (DEQ5), health-related QOL (SF-8^{TM}), Functional Assessment of Chronic Illness Therapy (FACIT) and general wellbeing. Existing patients were surveyed once, and new patients were surveyed at baseline then at 3 and 6 months post-treatment.

Results: Completed surveys were obtained for 24 existing and 40 new ASED patients and from 10 existing and 8 new PT-SED patients. The mean age was 57yrs (±13) for ASED and 70-74yrs (± 11) for PT-SED patients. Participants were predominantly female (76-100%) and had dry eye resulting from Sjögrens Syndrome (40-70%). Symptom duration ranged from 6-17yrs and nearly all patients (80-100%) had tried other treatments prior to SEDs. DEQ5 scores in new patients improved from 14.0 (±2.9) to 10.6 (±3.4) within 6 months. For ASED patients wellbeing scores improved from 7.0 (±1.9) to 7.8 (±1.7), however for PT-SED patients wellbeing decreased, but was not significant, from 7.1 (±2.0) to 6.7 (±3.0). QOL SF-8^{TM} measures improved in ASED patients from 19.6 (±6.7) to 18.7 (±6.0) but did not improve in PT-SEDs from 26.8 (±9.0) to 29.3 (±7.7). Patients used SEDs approximately 5 times per day and used 2 drops

each time. Most patients (78 – 100%) found it easy to open and close the vials.

Summary/Conclusions: SEDs improved dry eye symptoms in the majority of patients with positive feedback on DEQ5 measures. For patients receiving PT-SEDs some measures decreased during the survey period, however concurrent changes in other comorbidities were not assessed and these patients gave positive feedback for SEDs. Feedback on MEISE vial packing was positive, however some patients did report needing assistance to open and close the vials.

P192 | Treatment satisfaction and prescription refills among ocular surface disease patients treated with autologous serum: Data from the first Israeli study of blood bank-produced serum eye drops

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Background: In 2022, following the notification by the Israeli Ministry of Health assigning supervision and monitoring of autologous serum production to blood banks, the Rambam blood bank laboratory pioneered establishing and implementation of the first blood bank-produced autologous serum program in Israel.

Aims: To assess satisfaction and refill requests of patients with ocular surface disease (OSD) treated with autologous serum eye drops produced at the Blood Bank.

Methods: This retrospective study included the first consecutive OSD patients treated with autologous serum between 01/2022 and 01/2023 at the Blood Bank and Platelet Immunology Laboratories (Rambam Health Care Campus, Haifa, Israel). Patients received either 20% or 50% autologous serum at a dose of 1 drop 4-8 times a day for a period of 2-3 months (depending on usage). Patients were then asked regarding their satisfaction (rates) and refill requests (return rates).

Results: Overall, 135 patients (mean age - 55 years; 51.8% - females) were treated with autologous serum after referral from an eye care specialist. Main indications for treatment were: dry eye disease, ocular graft-versus-host disease and corneal neuralgia. The assessment of the first-year outcomes demonstrated that 5 patients (3.7%) passed away, 14 patients (10.4%) reported no improvement with the treatment, 12 patients were inaccessible or continued follow-up elsewhere (8.9%). To date, 45 (33.6%) have completed three months of therapy and elected to return for a second round of treatment (or more) and 58 (43.2%) are currently in their first round of treatment.

Summary/Conclusions: Hospital based blood bank supervised autologous serum increases availablitiy of this treatment for patients suffering from severe OSD. Providing this service in a tertiary care setting allows for efficient multidisciplinary treatment by hematologists, ophthalmologists and blood bank specialists

P193 | Use of fresh frozen plasma, fibrinogen concentrate and cryoprecipitate in Ireland

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Background: Based on the risk of transfusion transmitted vCJD, Irish plasma was removed as a therapeutic component over two decades ago. Following a review process in 2018, this decision was reversed. Considering the global shortage of Plasma Derived Medicinal Products (PDMPs), Ireland is now in a position to evaluate its contribution to the European supply of plasma, and move towards the use of Fresh Frozen Plasma (FFP) sourced from the Irish donor population.

The Irish Blood Transfusion Service (IBTS) has the sole responsibility for the distribution of fibrinogen concentrate and FFP in Ireland. The IBTS imports FFP (LG Octaplas™) and fibrinogen concentrate 1g (Riastap™) to supply Irish hospitals, which cater for a population of just over 5 million. Cryoprecipitate is prepared using Irish plasma and is used for paediatric surgery only. Ireland is currently collecting approximately 38,000L of whole blood derived recovered plasma, most of which is used for *in vitro* diagnostics.

Aims: This study is a retrospective, ten year review of the use of FFP, fibrinogen concentrate and cryoprecipitate in Ireland. This data can provide information on product usage in a country impacted by vCJD where alternative products were sourced to meet patients' needs.

Methods: IBTS figures for total units distributed to hospitals were collated from 2012 to 2022, for Uniplas™, Octaplas™, LG-Octaplas™, Riastap™ and Cryoprecipitate.

Results: Comparing 2012 and 2022, the FFP usage decreased from 21,122 units to 17,340 units, a 17.9% reduction. Fibrinogen concentrate (1g) usage increased from 4683 to 8955 units, a 91.2% increase. Cryoprecipitate usage decreased from 161 to 83 units, a 48.4% decrease.

Summary/Conclusions: The use of fibrinogen concentrate has widely replaced cryoprecipitate, with the exception of very limited usage in some paediatric surgery. Fibrinogen concentrate usage saw the most significant change with a 91% increase in usage since 2012. Since 2016 demand for FFP has been approximately 17,000 units per year. Demand for FFP and fibrinogen concentrate reduced in 2020 during the first year of the COVID-19 pandemic, however, demand has since increased. Demand for cryoprecipitate has decreased steadily since 2017. This product is used only in particular paediatric surgeries. The IBTS is working towards re-introducing Irish plasma for therapeutic use, both as FFP and as plasma for fractionation to make PDMPs. The figures analysed in this study will help inform the future demand for plasma in the Republic of Ireland.

P194 | Establishment of plasma fractionation project in Pakistan

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Background: Globally there is a shortage of blood and plasma donors for the Plasma Fractionation industry due to ageing population, increasing demand, Covid pandemic, Ukraine war etc. As a result the global production of Plasma Derived Medicinal Products (PDMPs) is seriously threatened. In order to address this grave situation, WHO has recommended that the Plasma Fractionation and Contract Fractionation initiatives be promoted in LMICs where there is excess of 'Recovered Plasma' which is mostly wasted due to non-utilisation. In addition, in most of the 'whole blood' donations, plasma is not separated due to lack of demand. WHO is therefore encouraging the developing countries to use the 'recovered plasma' and the potentially available plasma as well as promote the collection of 'source plasma' to generate PDMPs through Plasma Fractionation technology. The PDMPs thus produced will be cost effective and meet the unmet needs of the concerned country and also enable them to export the excess production. Presently, PDMPs are inaccessible to the patients in LMICs due to high cost, lack of availability, lack of awareness among physicians, inadequate diagnostic system etc.

Aims: To address the national insecurity of PDMPs and to make them accessible and affordable for the local population a Joint Venture (UPH Biopharmaceutical (Pvt.) Ltd.) has been formed between a large national private entity (JW-SEZ) and an international partner (Sinovac) to establish a Plasma Fractionation manufacturing unit in Pakistan.

Methods: UPH Pvt. Ltd. will in collaboration with the public and private sector blood banks in Punjab collect the excess 'recovered and source plasma' from the public and private blood banking system and provide the same to UPH for Plasma Fractionation. Necessary technical support to strengthen the system will be provided by UPH to improve the HR capacity, QA systems, screening systems, missing equipment etc. Special efforts will be made to conserve the 'recovered plasma', process all whole blood donations for plasma and in addition also collect 'source plasma'.

To ensure a consistent uninterrupted source of plasma a national campaign will be developed to promote voluntary blood and plasma donations as per the Blood Safety Acts, National Blood Policy and the WHO International Resolutions. Currently, the National Blood Policy for 2023-28 is being developed. The key areas being covered in the new Policy also include promotion of voluntary plasma collections and promotion of Plasma Fractionation in the country.

Results: 1. Provision of recommended effective treatment to the patients and less side effects leading to normal life style and life span in the affected patients.

2. Strengthening of the national blood sector.

3. Wastage of precious recovered plasma will be eliminated and utilised to prepare life-saving PDMPs.

4. Transfer of advanced technology to Pakistan

5. Employment opportunities especially for highly qualified scientists and professionals and in the Supply Chain and Logistics sector.

6. Substantial Foreign Direct Investment in the project and substantial annual exports.

7. Current expenditure on import of the PDMPs will be saved.

Summary/Conclusions: The national security of the life-saving PDMPs will be ensured and the mortality and morbidity of the affected haemophilia, immunodeficiency etc. patients will be controlled.

P195 | Primary immunodeficiency patients exposed to UK-sourced immunoglobulin: Surveillance for asymptomatic carriage of abnormal prion protein

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Background: Variant Creutzfeldt - Jakob disease (vCJD) is a very rare disease, associated with an abnormal form of prion protein. Most cases have been attributed to eating bovine spongiform encephalopathy (BSE) contaminated meat products. However, secondary transmission has occurred through blood transfusion and has been attributed, on one occasion, to treatment with a plasma product. Between 1996 and 2000, two intravenous immunoglobulin (Ig) products used to treat primary immunodeficiency diseases (PID) patients were manufactured from UK donor plasma-nine of these donors subsequently developed vCJD.

Aims: This study concerns the follow-up of patients exposed to these treatments to identify any case of vCJD, and, if found, to assess the likelihood of infection through this specific exposure.

Methods: Consenting patients had annual telephone and biennial face-to-face follow-up. Medical records were reviewed to identify any tissue and autopsy samples suitable for analysis. Blood samples were taken, anonymised and sent to the National Institute for Biological Standards and Control for storage pending possible future prion testing. *PRNP*-Codon 129 genotyping, histopathology, immunohistochemistry and PET blot analysis were undertaken. All cases are followed up to death or withdrawal.

Results: Of 79 cases included, none have yet shown symptoms or pathological evidence of vCJD. 58% were male, 70% born between 1960 and 1979, and 60% are still alive. The majority had a PID diagnosis of Common Variable Immunodeficiency (71%), and MM genotype (46%). The median time from first potential exposure to censor date was 20 years (range 8.9-28.2 years). Eight were known to have been treated with implicated batches (ones that included donations from pre-clinical vCJD donors), collectively contributing 155.1 person years of observation following first potential exposure. 46 cases donated 237 tissue specimens. Of these, 48 specimens from 22 cases were considered of sufficient quality (including from post-mortem). The average time from first exposure to specimen collection was

13.3 years (range 0-22.3 years). 7of the 21cases who have died underwent autopsy examination, with various causes of death, but not including vCJD: Aspiration pneumonia with disseminated carcinomatosis (1); Liver diseases (3); Alzheimer's disease/ cerebral amyloid angiopathy/progressive multifocal leucoencephalopthy (2); gastrointestinal haemorrhage of unknown cause (1).

Summary/Conclusions: We present the results of a follow-up study of patients with a potential exposure to vCJD infection via intravenous immunoglobulin products prepared from UK donor plasma, with some batches including donations from preclinical vCJD individuals. We found no evidence of transmission of vCJD, however, the incubation period from a potentially low dose exposure may be long. Uncertainties remain concerning the number of asymptomatic vCJD infections in the UK population, the risk of infection via blood components or plasma products derived from such individuals. The small number of vCJD cases, confirmed blood transmissions, and the absence of any vCJD transmission in this study is reassuring, and is evidence to support U.K. plasma being used in plasma product production. However continued surveillance for vCJD, and follow-up of these PID cases is important for public health confidence.

P196 | Allogeneic fresh frozen plasma preparation for the treatment of ocular GVHD

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Background: Graft Versus Host Disease (GVHD) develops in about 50% of patients undergoing allogeneic bone marrow transplantation. In many patients ocular GVHD)oGVHD) develops manifesting as severe dry eyes and chronic inflammation of the eye surface. Despite the use of topical therapies, anti-inflammatory drops and surgical procedures, oGVHD leads to a decrease in the quality of life and may even lead to visual loss. Autologous serum eye drops have been used for the treatment of dry eye syndrome. As plasma is routinely donated by healthy donors we sought to investigate the use of plasma drops for the treatment of oGVHD.

Aims: To describe the feasibility of preparing allogeneic fresh frozen plasma eye drops for the treatment of chronic oGVHD and to evaluate their safety and efficacy.

Methods: A Prospective, non blinded phase 2 open-label institutional study for adult patients with chronic oGVHD who underwent 2 previous lines of therapy. Patients were treated with 100% plasma drops QID or more for 3 months, on top of their usual treatment. The study was approved by Rabin Medical Center IRB. Plasma eye drops were prepared from fresh plasma donated by apheresis according to the Israeli ministry of health eligibility criteria. The plasma was connected using sterile connecting device to a closed and sterile tubing system (COL© system, Biomed Device Srl., Italy) containing 50 ampules of 1.45 mL. Validation and testing of the plasma eye drop sterility was performed by Hy Laboratories LTD. Following validation, release of the plasma drops was done after completion of the infectious disease markers and sterility testing. From each batch 30 eye drops ampules (individual month box) were packed and labeled according to ABO blood types. The patients received information and guidance on the use of the eye drops, transport conditions and expiry dates of the frozen and thawed plasma drops. Main outcome measures included OSDI (Ocular Surface Disease Index) questionnaire, Corneal Fluorescein staining NEI (National Eye Institute) grading and FACT-BMT quality of life questionnaire.

Results: Fourteen plasma units were donated from known apheresis plasma donors, the blood type of the plasma units was A in 6, 4 each were B and O. From each 600 ml plasma donation 9 individual month boxes were prepared. All the batches were sterile. The plasma eye drops were given according to the ABO blood type of the patients except for one AB patient who received A eye drops. Twenty five patients (49 eyes) completed the study. OSDI scores decreased from 53 ± 26 at baseline, to 32 ± 22 after 1 month and 30 ± 23 after 3 months (P<0.0001). In 47 eyes who had Fluorescein staining, NEI Fluorescein staining grade decreased from 7.6 ± 3.3 at baseline, to 5.9 ± 3.6 after 1 month and 5.5+3.1 after 3 months (P<0.0001). FACT-BMT scores did not change from baseline to 3 months (106.3 ± 22 vs. 112 ± 20, $P = 0.08$). One patient experienced irritation after 2 months of treatment, which resolved after providing a different batch of drops. No other adverse events were reported.

Summary/Conclusions: Production of allogeneic plasma eye drops is feasible and utilises established standards, infrastructure and quality control processes used by blood services. This prospective phase 2 study demonstrates that treatment of chronic oGVHD with allogeneic plasma drops is safe and effective, allowing rehabilitation of the ocular surface while avoiding the inconvenience of repeated autologous blood drawing.

In GVHD, this therapy has the advantage of not containing pro inflammatory cytokines and anti-self immune factors that may be present in autologous serum or plasma.

P197 | Establishment of plasma fractionation production in small countries as a key strategy for plasma derived product self-sufficiency and development of blood service

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Background: The demand for plasma-derived medicines is high in low and medium-income countries, largely due to their high cost and limited accessibility to a significant portion of the population. Having its own plasma fractionation site is important for small countries for several reasons. Firstly, it ensures a steady supply of essential plasma-derived therapeutics for the country's population, reducing

dependence on foreign imports and ensuring timely access to life-saving treatments. Secondly, it promotes self-sufficiency and reduces the financial burden of importing these products. It may also promote the growth of the country's blood service. Additionally, having control over the production process ensures quality and safety standards are met and reduces the risk of shortage or supply chain disruptions. In conclusion, owning a plasma fractionation site is a crucial step towards ensuring the health security of a small country's population.

Aims: The objective of this research project was to within the framework of public-private partnership develop production technologies for the manufacture of human blood plasma-derived albumin and normal immunoglobulin preparations within the Republic of Armenia through a collaborative effort between public and private sectors, with the aim of obtaining a trial sample for further scale up.

Methods: The Cohn 6 cold ethanol fractionation method has been widely used and validated for the production of high-quality albumin and normal immunoglobulin. This method is considered to be the gold standard for protein purification and is widely used in the biotechnology industry for the production of therapeutic proteins.

Production of 20% human serum albumin and 5% human normal intravenous immunoglobulin (IVIG) solutions were done with Cohn 6 cold ethanol fractionation method followed by additional purifications steps and viral removal steps including ultrafiltration, nanofiltration, *etc*. Production was wone at the pharmaceutical manufacturing facility of privet company which specialised in production of immunoglobulin products.

Results: Twelve-liter plasma pools were used for experimental production of albumin and IVIG preparations in three independent replicates. Based on obtained data, the yield of albumin was about 22 ± 0.5 g/L, which is comparable to 20-25 g/L produced by main plasma fractionators, while the yield of IVIG was 4.5 ± 0.2 g/L, which is excellent according to the largest plasma fractionation centers for both the Cohn and chromatography methods. The characteristics of the obtained albumin and IVIG preparations satisfied the requirements of the European Pharmacopoeia (the 11th Edition) respective monographs: Human albumin solution (0255) and Human normal immunoglobulin for intravenous administration (0918).

Summary/Conclusions: We suggest that development of plasma fractionation production in Armenia and other small countries is feasible and it can eliminate import issues and price fluctuations, while providing high-quality plasma proteins that are economically competitive and support the growth of the country's blood service.

P198 | Stability of vitamin A in allogeneic serum-based eye drops

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Background: Serum-based artificial tears mimic the composition of natural tears better than the pharmaceutical eye drops. Many components of the serum-based eye drops have similar properties to natural tears with the exception of values for vitamin A, fibronectin or the IgA and vitamin C content which is lower. Vitamin A deficit adversely affects the density of the conjunctiva goblet cells leading to dry eye syndrome. Because of the higher concentration of some elements, vitamin A among others, serum-based eye drops are very effective for Dry Eye Syndrome (DES) therapy and are therefore gaining popularity.

Aims: The study aim was to measure vitamin A concentrations in allogenic serum and to test the stability of the vitamin after 6-month storage at -18°C.

Methods: Serum was obtained in a standard procedure for the preparation of allogeneic serum-based eye drops. A total of 6 whole blood (WB) donations were collected from voluntary male AB group, (Rh irrelevant) blood donors with no history of transfusion. WB was clotted at 37°C for a maximum of 2 hours, and then centrifuged twice to remove cellular elements. The serum was subjected to pathogen inactivation in the MIRASOL system with riboflavin and UV light. 6 samples of riboflavin-inactivated serum were tested for vitamin A concentration using the HPLC-DAD method in fresh and in thawed material after 6 months of storage at -18°C.

Results: The initial vitamin A concentration in the samples was 0.35 mg/l average (0.29-0.49 mg/l). After storage, the average concentration was 0.58 mg/l (0.25-1.7 mg/l). For healthy individuals the reference range of serum vitamin A concentration is 0.2-0.43 mg/l. For sample with initial concentration above the normal range, a more than threefold increase in concentration was observed (standard deviation 0.856). The remaining 5 samples were within the reference range. In 4 samples, a slight increase was reported and in 1 sample, a decrease was noted (standard deviation of the remaining samples was between 0.007 to 0.028).

Summary/Conclusions: The high-range result were rejected and we concluded that the concentration of vitamin A in allogenic serum-based eye drops stored in standard conditions (-18°C) is stable for 6 months after collection and freezing of the material. The concentration of vitamin A is within the reference range. Research demonstrate that serum composition have a positive effect on tears' production and eye lubrication. Patients also report less inflammation and better vision.

P199 | High cytokine levels in donors who donate blood for allogeneic artificial tears

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Background: Since 2019, allogenic artificial tears have been prepared at the IHTM. By 2022, 114 patients had been included in the study. Allogenic serum eye drops have proved just as effective as autologous drops and have numerous advantages. The preparation procedure has high process efficiency and is time saving for patients. For elderly patients who struggle with veins problems there is also no need of venipuncture. Differences in the composition of serum collected from voluntary blood donors and that from patients were investigated. The patients who reported to the IHTM for artificial tears suffered from diseases such as Sjögren's Syndrome, Dry Eye Syndrome and GvHD.

Aims: The study design was developed to demonstrate the difference in cytokine concentrations in the serum of healthy blood donors and patients.

Methods: Serum was tested for the levels of interleukins IL-1β, IL-6, IL-2, IL-10 and vascular endothelial growth factor (VEGF). The BD FACSCanto™ II cytometer was used with appropriate BD™ Cytometric Bead Array (CBA) Flex Sets and Human Soluble Protein Master Buffer Kit (Beckton Dickinson). 38 serum samples from autologous whole blood donations and 10 serum samples from 7 allogeneic donors were analysed. Cytokine assays were compared with Ocular Surface Disease Index (OSDI) to determine the improvement in patients' well-being after application of allogeneic tears. Patients rate their responses on a 0-100 scale where highest scores represent severe dry eye symptoms.

Results: The cytokine concentrations in donor samples were much lower with the exception of IL-2 which demonstrated higher values in allogenic blood donors and lower values in the patients. During the study it turned out that the cytokine results of one of the donors was highly overestimated, with negative impact on the eye condition of the patients who applied the drops. Table 1 shows the mean results for 5 of 6 donors and those of the 'outstanding' donor. After application of artificial tears the mean OSDI in samples with appropriate cytokine concentration level dropped from 63,7 to 60. For patients who used tear drops prepared from the 'outstanding' donor's serum, the final OSDI was above 60; they reported improvement in eye's condition, but it was not as satisfactory as for eye drops prepared from the serum of the other donors.

Summary/Conclusions: Patients report satisfaction with allogeneic artificial tears. Not surprising, because Polish donors who volunteer to donate blood are subjected to comprehensive donor screening and only fully healthy donors are eligible for donation. The cytokine level of our 'outstanding' donor may be indicative of the onset of infection, an autoimmune disease or of a postoperative condition. Our study shows that donor serum with normal cytokine levels is more beneficial for DES therapy. It may therefore be advisable to expand the medical interview and to the range of tests to include a cytokine profile for donors of blood for the preparation of artificial allogeneic tears.

P199 - Table 1 Cytokine profile of blood donors.

Cytokines	Mean cytokine levels of 6 allogeneic donors * [pg/ml]	'Outstanding' donor's results [pg/ml]
IL-1B	0,65	47,26
IL-2	0,74	0,49
IL-6	1,9	55,6
IL-10	0,5	34,83
VEGF	31,42	63,86

* *One donor had much higher levels of inflammatory cytokines and growth factor, despite being classified as completely healthy prior to donation.*

P200 | Serum Eye drop experience in manufacturing: 11 years autologous and for 6 year allogeneic

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Background: Serum eye drops have been increasingly used world wide for years as an experimental therapy for severe forms of dry eyes. Until now only few prospective studies were published, several retrospective evaluations reported.

Aims: The aim of this study is to evaluate serum eye drops manufacturing in an allogeneous setting (1) with respect to donor health problems preventing autologous donation (2) with the goal of optimizing manufacturing.

Methods: Evaluated were data on donation, manufacturing and, where possible, clinical outcomes. Production in general has already been reported elsewhere.

Results: Since 2012 autologous serum eye drops (ASAT, ASED) have been produced in our blood center, and since 2017 allogeneic serum eye drops (FSAT, FSED) have been manufactured. In total, more than 2466 preparations were obtained. Approximately 2% of production was lost (bacterial contamination, IDM of the donors, technical issues).

In the period between 2017 and 2022, 556 allogeneic preparations were produced for 140 patients in individual healing attempts. Of these patients, there were children (15), previous autologous donors (49), and patients (76), who were already initially unable to donate due to underlying disease.

Even in the setting of a comparatively large University Blood center (24.000 whole blood donation, 60 -150 donors daily) the search for the suitable donor with respect to the eligibility criteria can be challenging, particularly as manufacturing is timed for each individual

patient. Due to quarantine until negative microbiological result, products can be issued up to 2 week after request only. This may be very long for urgent indications.

Summary/Conclusions: Due to eligibility criteria donor availability and quarantine manufacturing according to patients needs is difficult. Permission for general manufacturing and delivery from stock would be desirable. For this to achieve an international recommendation for standardization of donor eligibility and manufacturing in general would be very helpful.

P201 | Abstract withdrawn

Blood products

Pathogen inactivation

P202 | In vitro biochemical and functional characteristics of stored (double-dose) buffy-coat platelet concentrates treated with amotosalen and a prototype UVA light-emitting diode illuminator

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Background: Pathogen reduction of platelet concentrates (PCs) using amotosalen and broad-spectrum UVA illumination contributes to the safety of platelet transfusion by reducing the risk of transfusion-transmitted infections.

Aims: We evaluated the *in vitro* quality of stored buffy-coat (BC) PCs treated with amotosalen and a prototype narrow band width light emitting diode (LED) illuminator.

Methods: A pool-and-split strategy was used to obtain four study groups ($n = 5$ per group): i) double-dose BC-PCs prepared with PAS-C (InterSol)/plasma (55%/45%) treated with amotosalen and a conventional UVA lamp (INT100 illuminator 320-400 nm wavelengths, 3.9 J/cm^2), ii) double-dose BC-PCs prepared in PAS-C/plasma (55%/45%) and treated with amotosalen and a LED lamp (350 nm, 3.3 J/cm^2), iii) double-dose BC-PCs prepared in PAS-E (SSP$^+$)/plasma (55%/45%) and treated with amotosalen and a conventional UVA lamp, iv) double-dose BC-PCs prepared in PAS-E/plasma (55%/45%) and treated with amotosalen and a LED lamp. The *in vitro* quality and function of the platelets were evaluated by multiple biochemical and functional assays over 7 days of storage.

Results: Platelet counts were conserved during storage in all groups of PCs, as was platelet swirling without the appearance of macroscopic aggregates. Integrin αIIbβ3 and glycoprotein (GP) VI expression remained stable, whereas GPIbα and GPV declined similarly in all groups. UVA lamp- and LED-treated PCs displayed similar glucose consumption, lactate generation and pH levels. They also displayed comparable spontaneous P-selectin, phosphatidylserine and activated αIIbβ3 exposure, and similar maximal activation of these parameters upon strong agonist challenge, irrespective of the type of illuminator. Mitochondrial membrane potential, an indicator of mitochondria integrity, and lactate dehydrogenase (LDH) release, to evaluate premature platelet lysis, were similar for UVA lamp- and LED-treated PCs during storage. Finally, platelets prepared with both light sources and storage solutions retained adhesion to VWF and fibrinogen, and aggregate formation with similar thrombus volume on collagen under shear stress flow conditions over 7 day storage.

Summary/Conclusions: Replacing fluorescent UVA lamps with LED lamps for INTERCEPT treatment had no impact on platelet metabolism, spontaneous activation, apoptosis or viability, or on the *in vitro* haemostatic function of BC-PCs stored for 7 days in PAS-C or PAS-E/plasma.

P203 | Amotosalen and UVA treatment of enterobacter soli, leclercia adecarboxylata, and staphylococcus saprophyticus from a contaminated apheresis platelet unit

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Background: The INTERCEPT® Blood System for Platelets uses a combination of amotosalen and UVA light to inactivate pathogens and leukocytes in platelet concentrates (PC). The system is in routine use in US and EU blood centers to treat apheresis- and whole-blood derived platelets. In 2021, a report of an infection involving an INTERCEPT-treated apheresis PC at a clinic in the United States was published. Three bacteria were isolated from the bag and identified by sequencing as *Enterobacter soli*, *Leclercia adecarboxylata* and *Staphylococcus saprophyticus*. It could not be excluded that the PC was contaminated after the treatment as the bag was discarded before analysis of the unit could be performed. Prior TTIs associated with an INTERCEPT-treated PC have shown defects in the bag that lead to external contamination after treatment (Fadeyi *et al*., *Transfusion*, 2020). A previous study has also shown that *L. adecarboxylata* and *S. saprophyticus* can be inactivated by the INTERCEPT Blood System for Platelets (Fadeyi *et al*., *Transfusion*, 2020). This was the first report of an *E. soli* contaminated platelet unit and infection of a human. The only other report of an *E. soli* infection was in farmed fish in Malaysia. It is not known whether *E. soli* poses a risk for platelet transfusions. Prior to this study, there were no published data on pathogen reduction efficacy for *E. soli*.

Aims: The aim of this study was to assess the inactivation of *E. soli* alone and in combination with *L. adecarboxylata* and *S. saprophyticus* in apheresis platelets using the INTERCEPT Blood System for Platelets.

Methods: For the pathogen inactivation assessments, 335 mL of an apheresis platelet unit (35% plasma/65% platelets in PAS) was inoculated with 3.4 mL of an overnight culture of an individual bacterial species or a 1:1:1 (volume) mixture of *E. soli*, *L. adecarboxylata*, and *S. saprophyticus*. The contaminated platelet component was connected to an INTERCEPT Dual Storage Platelet Processing Set dosed with

amotosalen and illuminated with target dose of UVA light. The unit was then transferred to the compound adsorption device (CAD) container and incubated for 16 h at 22°C with agitation. After incubation, the unit was transferred into storage bags and held at 22°C with agitation. Samples were taken pre- and post-treatment, day 5, and day 7. Bacterial titre was measured by plating on LB agar. On day 7, potential residual bacteria were assessed by incubating the remaining unit with an equal volume of LB broth in a flask overnight at 37°C with agitation.

Results: For the individual assessment of *E. soli*, 7.5 ± 0.1 log CFU/mL was inactivated with no detectable bacteria observed at post-treatment, 5- and 7-days post-collection. For the combination assessment to assess whether INTERCEPT could inactivate the combination of pathogens found in the unit, inactivation of *E. soli*, *L. adecarboxylata*, and *S. saprophyticus* were observed at 7.2 ± 0.1 log CFU/mL with no detectable bacteria post-treatment, on days 5 and 7.

Summary/Conclusions: We confirm in this study that amotosalen/UVA treatment can effectively inactivate *E. soli*, *L. adecarboxylata*, and *S. saprophyticus* to levels below the limit of detection after treatment and throughout the 7-day storage period. This study highlights the efficacy of INTERCEPT at inactivating multiple bacteria in a single unit. Further studies are needed to understand the mechanism of contamination of units after treatment during the storage period.

P204 | Virus inactivation of plasma by methylene blue/light treatment using a DEHP-free bag system

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Background: Di-ethyl-hexyl-phthalate (DEHP) is currently one of the major plasticizers used in blood bags. Due to its endocrine disrupting properties European regulators decided to ban DEHP to be used in medical devices in the future. Although the final sunset date for DEHP in blood bags is not yet clear, blood bags used in Europe will have to be modified to DEHP-free versions within the next years.

Aims: Aim of the current study was the investigation of the virus inactivation capacity by Methylene blue (MB)/light treatment of plasma using the DEHP-free version of the THERAFLEX MB-Plasma system (Macopharma). In accordance with guidelines on virus validation studies, four different model viruses (Suid Herpes Virus (SHV-1), Bovine Viral Diarrhea Virus (BVDV), Feline Calici Virus (FCV) and Vesicular Stomatitis Virus (VSV)) were used that vary in their structural and biophysical characteristics.

Methods: Leuko-depleted plasma was prepared from whole blood in regular, DEHP-containing bags using standard blood banking technology. Plasma units ($n = 6$ for each virus) were spiked with virus suspension (10% v/v). MB/light treatment was done according to the manufacturer's instructions using the Macotronic B2 illumination device and the DEHP-free THERAFLEX MB-Plasma system PROSDV1. Samples were taken after spiking (load and hold sample) and after illumination with different light doses (30, 60, 90 and 120 (standard) J/cm^2)) for $n = 3$ replicates. For three additional replicates samples were taken only before and after illumination. The titre of SHV-1 (VR-135, ATCC) and VSV (VR-158, ATCC) was determined as tissue culture infective dose ($TCID_{50}$) by endpoint titration on Vero cells (ATCC, BE76-108B). FCV virus suspension (VR-2057, ATCC) on CRFK cells (ATCC CCL-94) and BVDV (VR-1422, ATCC) on MDBK cells (ATCC, CCL-22). In order to improve the detection limit additional large volume plating was done for some of the samples.

Results: Log_{10} reduction factors of ≥ 5.89 and ≥ 5.50 log steps were achieved for **BVDV** at a light dose of 120 J/cm^2 in the respective experiments with and without intermediate sampling. Log_{10} reduction factors of ≥ 4.29 and ≥ 4.17 log steps were achieved for **FCV,** ≥ 4.96 and ≥ 4.92 log steps for **SHV-1** and ≥ 5.35 and 4.77 log steps for **VSV** with and without intermediate sampling.

Summary/Conclusions: All of the viruses tested in this study were inactivated with reduction factors ≥ 4 log steps at the final illumination dose of 120 J/cm^2 using the DEHP-free bag disposable PROSDV1 and the illumination device Macotronic B2. Reduction factors are comparable to data achieved in the past for the DEHP-containing THERAFLEX MB-Plasma system.

P205 | Initial experience of switching pathogen inactivation system with extended platelet shelf life

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Background: After a first evaluation of the quality of platelets treated with two pathogen inactivation (PI) methods in 2021 combining either amotosalen and UVA light (A-UVA (INTERCEPT™ Blood System) or riboflavin and UV light (R-UV (MIRASOL™ PRT System)), the Luxemburgish Red Cross decided to use A-UVA to extend the storage time of platelets from 5 days (hours calculated) to the end of the 6th day after collection. The objective was to reduce the high percentage of outdated products and to ensure a higher level of self-sufficiency. The implementation of A-UVA had also required some process modifications, especially regarding apheresis platelet yield and concentration that were initially not in accordance with the process entry requirements.

Aims: To assess the impact of the switch of PI method on several aspects: platelet quality, percentage of expired products, process organization, level of non-conformities.

Methods: Several "routine QC parameters" such as platelet concentration, platelet yield, pH (22°C) and the percentage of outdated platelet products were analysed and compared to the period during which R-UV method had been used with a platelet storage time limited to 5 days. Moreover, the age of the platelet products issued was compared between both periods in order to assess the impact of storage time extension. Two types of platelet products were tracked: platelet from pools derived from Reveos whole blood separation (Terumo BCT®) and from Trima apheresis platelet collections (Terumo BCT®).

Results: QC results showed several statistical differences: in A-UVA platelet pools, the platelet yield and volume were lower while the platelet concentration was higher. Regarding the apheresis, all the A-UVA products (single and double donations) were statistically more concentrated for the same platelet yield. The storage time extension had an impact of the average age of the products issued, increasing from 2.73 to 4.03 days for apheresis platelets and from 3.31 to 4.26 for platelet pools.

The percentage of expired products has been reduced from 6.1 to 2.4% and from 29.6 to 20.4%, for apheresis and platelet pools, respectively. No increase of non-conforming products has been observed.

Summary/Conclusions: The main impact related to the change of PI method was a higher platelet loss for the platelet pools, mainly due to the processing, especially the step of compound adsorption. This was not observed for apheresis products: some parameters of the Trima collection program were initially modified (increase of the platelet collection concentration) in order to compensate this loss. The lower volume of photoactive compound added to the product to illuminate (15.0 or 17.5 for A-UVA vs 35.0 ml for R-UV) can explain the increase of platelet concentration

Even if the use of A-UVA implies a delay in the product release (additional steps) compared to the R-UV treatment, we didn't experience any shortage.

P205 – Table 1

Platelet Pools	R-UV 4 components ($n = 631$)	A-UVA 4 components ($n = 143$)	R-UV 5 components ($n = 2602$)	A-UVA 5 components ($n = 1192$)
Mean Platelet Yield 10e11/bag	3.15	2.82	3.51	3.23
Mean Platelet Concentration 10e9/l	978	1011	874	899
Mean Platelet Volume in ml	322	280	401	360

P205 – Table 2

Apheresis Platelets	R-UV single donation ($n = 607$)	A-UVA single donation ($n = 453$)	R-UV double donation* ($n = 384$)	A-UVA double donation ($n = 184$)
Mean Platelet Yield 10e11/bag	3.08	3.11	2.80	2.78
Mean Platelet Concentration 10e9/l	951	1144	945	1494
Mean Platelet Volume in ml	323	272	296	186

* After splitting, product characteristics are considered as "equivalent"

P206 | Improvement of platelet quality after pathogen inactivation

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Background: Pathogen inactivation increases the microbial safety of platelet concentrates, but has a negative impact on the *in vitro* quality. Clinically, this results in lower increments after transfusion, resulting in an increased transfusion requirement in certain patient populations. In order to improve the *in vitro* quality of pathogen inactivated platelets, we investigated the potential beneficial effect of various ingredients added to the platelet additive solution E (PAS-E).

Aims: To determine the effect of pyruvate as alternative nutrient, and the effect of antioxidants glutathione and vitamin C on the *in vitro* quality of pathogen inactivated platelet concentrates in PAS-E.

Methods: Ten buffy coats were pooled and split in two equal parts. To one, unmodified PAS-E (T-PAS+, Terumo BCT) was added, to the other, PAS-E with ingredients as described in Table 1 were added. The ingredients, their concentration and pH were determined in a series of pilot experiments. After pathogen inactivation (Mirasol, Terumo BCT), the platelet concentrates were stored for 8 days with regular sampling for determination of in vitro quality on day 2, 6 and 8.

P206 - Table 1b.

Day 8				
Glucose, mM	0	0	0	0
pH	6.63	6.67	6.73	6.68
CD62P, % pos. cells	14	26	21	27
Annexin A5, % pos. cells	29	66	36	78
TEG, max. amplitude	66	46	59	39

Results: The addition of pyruvate leads to a lower glucose consumption, consequently resulting in less lactate formation, and thus better maintenance of pH. Both glutathione and vitamin C result in lower activation (CD62P expression) and lower levels of apoptosis (annexin A5) than controls. *In vitro* functionality (TEG maximum amplitude) is better maintained than in controls.

Summary/Conclusions: The inclusion of pyruvate and antioxidants are promising ingredients for a PAS to mitigate some of the in vitro measured effects of pathogen inactivation. Further confirmatory studies are needed. How these changes affect the efficacy of pathogen reduction needs to be established, as much as how these changes affect clinical performance.

P206 - Table 1a.

	Experiment 1		Experiement 2	
Day 6	PAS-E +10 mM glutathione +10 mM pyruvate pH 7.0	PAS-E	PAS-E +10 mM glutathione +10 mM pyruvate +50 µM Vitamin C pH 7.5	PAS-E
Glucose, mM	2.2	0	0.9	0
pH	6.82	6.65	6.75	6.62
CD62P, % pos. cells	14	28	18	39
Annexin A5, % pos. cells	25	42	20	27
TEG, max. amplitude	71	48	63	61

P207 | Pathogen reduced plasmas from maxi-pools combined with fast thawing for use in emergency situations

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Background: Early and timely use of transfusions has effect on survival in trauma setting. Transfusion packs containing 4 units of erythrocytes, 4 units of plasma and 1 platelet unit have been adopted in our institution for trauma patient care. This practice had a detrimental effect on the outdating of plasma. 25% of plasmas for transfusion were outdated in 2018. The plasma needed for the transfusion packs is thawed as soon as the order comes in, and the ward can return the unused units back to the blood bank. If there is no need for thawed plasma within 7 days after thawing, the plasmas are discarded. Clinicians also expressed a wish to have more standardised products with regards not only to volume but also content. We developed a 10-unit plasma pooling technique allowing to optimise the use of pathogen reduction (PR) processing sets and delivering 12 units of 200 mL end products subsequently fast-thawed before transfusion.

Aims: To assess, based on plasma quality parameters tested *in-vitro*, a preparation procedure based on pools of 10 previously frozen plasma units subsequently split into volumes compatible with the process for PR treatment and thawed post-frozen storage with a fast thawer.

Methods: 100 WB-derived leukocyte depleted plasma units were frozen within 24 hours at ≤25°C and stored for 7 days. After thawing, 10 maxi-pools of 10 A, B or AB plasma units were constituted. After splitting each into 4 sub-pools of 650 mL, they were PR treated using amotosalen and UVA (A-UVA, INTERCEPT® Blood System, Cerus). Further splitting into units of 3 results in a total of 120 PR plasma units at 200 mL. The units were frozen at ≤25°C for 1 week, then thawed either in a CS201 (Conroy) fast plasma thawer for 5 min or in other control devices (Barkey Plasmatherm and Sahara, 17 to 23 min). Factor VIII, Fibrinogen, Albumin, IgG, Protein S, and vWF were measured in plasma units, maxi-pools, and plasmas after PR treatment and thawing.

Results: Results of the assays in the Table, given at the different phases of the process. Mean ±SD

Factor VIII and fibrinogen levels were not significantly reduced after freezing and thawing procedures. However, after PR, there was a statistically significant ($p < 0.05$) but still clinically acceptable reduction of these levels with 69% and 87% recovery for Factor VIII and fibrinogen, respectively. These concentrations are still over the recommended levels of ≥0.5 IU/mL and ≥2 g/L. Only Factor VIII was lower ($p < 0.05$) using control devices versus the CS 201 fast thawer. Other studied proteins were not significantly affected in the processes.

Summary/Conclusions: Pooling 10 plasma units before the PR treatment standardises volume and protein content of plasma units. Besides the economic value of generating 12 products for transfusion, this procedure combined with a thawing time of about 5 minutes is of value in emergency situations and allowed to decrease outdating of plasmas to 12%.

P207 – Table 1

Parameter	Plasma units (N =100) Frozen, thawed	Maxi-pools of 10 units (N = 10) Frozen, thawed	Plasma units after A-UVA PR, 7-day ≤25°C storage and thawing (N = 20) CS 201	Control thawers
Factor VIII:C (IU/mL)	1.07 ± 0.3	1.08 ± 0.1	0.72 ± 0.07	0.69 ± 0.08
Fibrinogen (g/L)	2.7 ± 0.4	2.6 ± 0.2	2.3 ± 0.1	2.3 ± 0.1
Albumin (g/L)	32.7 ± 2.1	33.1 ± 1.6	32.6 ± 0.5	32.5 ± 0.5
IgG (g/L)	8.66 ± 1.57	8.63 ± 0.52	8.42 ± 0.52	8.44 ± 0.50
Protein S (IU/mL)	0.94 ± 0.17	0.94 ± 0.05	0.84 ± 0.08	0.84 ± 0.05
vWF (IU /mL)	1.20 ± 0.36	1.19 ± 0.14	1.13 ± 0.13	1.13 ± 0.13

P208 | Bacteria elimination from plasma by methylene blue/light treatment using a DEHP-free bag system

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Background:
Di-ethyl-hexyl-phthalate (DEHP) is currently one of the major plasticizers used in blood bags. Due to its endocrine disrupting properties European regulators decided to ban DEHP to be used in medical devices in the future. Although the final sunset date for DEHP in blood bags is not yet clear, blood bags used in Europe will have to be modified to DEHP-free versions within the next years.

Aims: Aim of the current study was the investigation of the bacteria elimination capacity by Methylene blue (MB)/light treatment of plasma using the DEHP-free version of the THERAFLEX MB-Plasma system (Macopharma). The study was designed to investigate the relevant process steps of the THERAFLEX MB-Plasma procedure including the filtration steps for leukocyte depletion (Plasmaflex filtration) and removal of MB and photoproducts (Blueflex filtration) for their impact on elimination of two different bacteria species (*Klebsiella pneumoniae* and *Brevundimonas diminuta*).

Methods: Leukodepleted plasma was prepared from whole blood in regular, DEHP-containing bags using standard blood banking technology. Plasma units ($n = 3$ for each bacteria strain) were spiked with bacteria suspension to reach a titre of approx. 10^6 CFU/mL. MB/light treatment was done according to the manufacturer's instructions with a light dose of 120 J/cm^2 using the Macotronic B2 illumination device and the DEHP-free THERAFLEX MB-Plasma system PROSDV1. Samples were taken after spiking, Plasmaflex filtration, illumination and Blueflex filtration. The bacteria titre was determined by plating on agar plates and the $\log_{10}$ reduction was calculated.

Results: *K. pneumoniae* was reduced by ≥ 5.7 log steps by filtration through the Plasmaflex filter. A reduction factor of at least 5.9 log steps was achieved by the entire process. *B. diminuta* was reduced by[3] 2.2 log steps by Plasmaflex filtration. A further reduction below the limit of detection was achieved by subsequent irradiation and Blueflex filtration so that an overall reduction factor of at least 4.7 log steps was achieved for the entire process.

Summary/Conclusions: In the current study it could be demonstrated that bacteria species *Klebsiella pneumoniae* and *Brevundimonas diminuta* were efficiently removed from plasma by using the THERAFLEX MB-Plasma System PROSDV1 (non-DEHP). Due to a higher spiking concentration log reduction factors achieved in this study are even higher than formerly published for DEHP-containing THERAFLEX MB-Plasma systems.

P209 | Inactivation of WHO reference bacterial strains in platelet and plasma components using Amotosalen/UVA treatment

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Background: The INTERCEPT® Blood System for platelets and plasma utilises amotosalen and UVA light to efficiently inactivate a wide range of pathogens and leukocytes in platelet concentrates (PC) and plasma. The INTERCEPT Blood System for platelets routinely used for the treatment of apheresis and whole blood (WB) derived platelets in Europe, and in the US for the treatment of apheresis platelets (TRIMA® in 100% plasma or AMICUS® for 65% PAS-3/35% plasma). The INTERCEPT Blood System for Plasma is available both in Europe and US. The World Health Organisation (WHO) Expert Committee on Biological Standardisation (ECBS) in association with the Paul-Ehrlich-Institut (PEI) approved an extended panel of bacterial strains to evaluate methods for improving the microbial safety of blood components (Spindler-Raffel et al, Vox Sanguinis, 2015).

Aims: The aim of this study was to evaluate the inactivation of WHO reference (PEI) bacterial strains in platelet and plasma components using the INTERCEPT Blood Systems.

Methods: Apheresis PC collected in 100% plasma or 65% PAS-3/35% plasma were pooled into individual units of 420 mL with platelet doses of 4.0 to 5.0 $\times 10^{11}$ and 4.0 to 7.9 $\times 10^{11}$ respectively (INTERCEPT Blood System for Platelets – Dual Storage (DS) Processing Set). Human plasma donations were collected and pooled to yield individual units of ~650 mL (INTERCEPT Plasma Processing Set). Four replicates per platelet matrix were performed for each PEI strain of transfusion-relevant bacteria, including *K. pneumoniae* and *S. aureus* in plasma, with each replicate consisting of one unit spiked with a single PEI strain. The contaminated PC and plasma units were then treated with amotosalen and UVA light in the INTERCEPT Blood System for platelets and plasma, respectively. ~5 mL and ~50 mL samples were taken pre- and post- UVA treatment, respectively and were analysed for bacterial titre by plating on appropriate media (100µL – 10 mL/plate).

Results: Platelet and plasma units contaminated with PEI bacterial strains (Table 1) were treated with amotosalen and UVA in the INTERCEPT Blood System for platelets and plasma, respectively. Robust bacterial inactivation was observed post-treatment (Table 1).

Summary/Conclusions: The INTERCEPT Blood System for Plasma consistently inactivated high titres of *K. pneumoniae* and *S. aureus*. The INTERCEPT Blood System for Platelets efficiently inactivated *K. pneumoniae*, *S. aureus*, *E. coli* and *S. epidermidis*. These data demonstrate that the INTERCEPT Blood System for platelets and plasma robustly inactivates WHO standardised bacteria strains associated with TTBI.

P209 - Table 1: Bacterial inactivation using amotosalen/UVA treatment for human plasma and platelet concentrates in 100% plasma and 65% PAS-3/35% plasma

Bacteria (Strain)	Matrix	Log Reduction* (Log cfu/mL)
K. pneumoniae PEI-B-P-08	PC In 100% plasma	4.7 ± 0.4
K. pneumoniae PEI-B-P-08	PC In 65% PAS-3/35% plasma	5.6 ± 0.2
K. pneumoniae PEI-B-P-08	Human plasma	4.5 ± 0.5
S. aureus PEI-B-P-63	PC In 100% Plasma	6.7 ± 0.0*
S. aureus PEI-B-P-63	PC In 65% PAS-3/35% plasma	7.6 ± 0.1*
S. aureus PEI-B-P-63	Human plasma	6.5 ± 0.1*
E. coli PEI-B-P-19	PC In 100% Plasma	7.4 ± 0.2*
E. coli PEI-B-P-19	PC In 65% PAS-3/35% plasma	7.2 ± 0.1*
S. epidermidis PEI-B-P-06	PC In 100% Plasma	7.7 ± 0.1*
S. epidermidis PEI-B-P-06	PC In 65% PAS-3/35% plasma	7.8 ± 0.0*

* No residual bacteria were detected at post- UVA treatment.

P210 | Implementation of a UVC-based pathogen reduction treatment for apheresis platelets at a regional blood service in Germany

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Background: Until now, pathogen reduction treatment for platelet concentrates (PCs) is not mandatory in Germany. Nevertheless, the Bavarian Red Cross Blood Service started to implement the THERAFLEX UV-Platelets System (Macopharma) as effective and preventive measure to increase blood safety.

Aims: The UVC-based system for PCs was validated as required for manufacturing license and marketing authorization approval.

Methods: Six double apheresis PCs were collected and split into 12 single units. Six PCs were treated with the THERAFLEX UV-Platelets System, while the other six corresponding PCs were left untreated and served as control. Pathogen reduction of PCs using the THERAFLEX UV-Platelets System was performed under routine-like conditions. PCs were stored under agitation at 22 ± 2°C and samples were collected on day 2 (day after collection and UVC treatment), day 6 and day 9. Several platelet in vitro quality parameters were tested according to the local standard operation procedures. Manufacturing steps of the THERAFLEX UV-Platelets procedure were validated, and UVC treatment was monitored in the platelet units using a mitochondrial DNA multiplex real-time polymerase chain reaction inhibition assay.

Results: THERAFLEX UV-Platelets treatment was finished about three and a half hours after end of apheresis. The processing time including all handling steps and UVC illumination was about 20 minutes for a single PC. Platelet content per unit was between 3,12 x 10^{11} and 3,75 x 10^{11} (mean 3,43 x 10^{11}) in UVC-treated PCs and between 3,25 x 10^{11} and 3,91 x 10^{11} (mean 3,56 x 10^{11}) in control units. Residual red blood cells and white blood cells were less than 3 x 10^{9} and 1 x 10^{6} per unit, respectively, in all units. Pathogen-reduced and untreated PCs showed similar result in swirling and hypotonic shock reaction until day 9 and were tested negative for anaerobic and aerobic bacterial growth. During time of storage glucose concentration decreased and lactate concentration increased in all units. Glucose consumption was higher in UVC-treated units than in untreated units; however, glucose was still present on day 9. The mitochondrial polymerase chain reaction analysis correctly discriminated between UVC-treated and untreated PCs.

Summary/Conclusions: UVC treatment of apheresis PCs is a fast and easy procedure and was successfully integrated into the local manufacturing processes. All UVC-treated PCs fulfilled the specifications of the THERAFLEX UV-Platelets technology and met the requirements of the German guidelines for pathogen-reduced PCs.

P211 | A pilot study tracking pathogen reduced RBCs in vivo using surface acridine and biotin flow cytometric markers

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Background: Pathogen reduction of red blood cells (PR-RBCs) with amustaline/glutathione is an investigational process designed to reduce transfusion-transmitted infection risk of RBC transfusions. Low titre antibodies specific for acridine (a breakdown product of the PR process) bound to RBC surfaces have been detected in a small number of naïve and exposed patients.

Aims: We designed an *in vivo* RBC survival study to track PR-RBCs by flow cytometry using a monoclonal antibody specific for acridine-RBC adducts to measure RBC survival in a patient with sickle cell disease.

Methods: The patient received three ~7 mL aliquots of different biotin dose labeled RBCs: Two aliquots drawn from one RBC unit before and after PR treatment (Pre-PR RBCs, 2 µg/mL biotin; PR-RBCs, 6 µg/mL biotin), and one from a conventional RBC unit (Control, 18 µg/mL biotin). The remaining unlabeled PR-RBC and Control RBC units were also transfused. Semi-quantitative flow cytometry for acridine and biotin was performed in triplicate on samples drawn at designated time points post-transfusion.

Results: Flow cytometry for biotin labelling within 1 hour of transfusion detected 0.72% of PR-RBCs, 0.76% of Pre-PR RBCs and 0.92% of Control RBCs. The acridine assay detected 12.3% of circulating PR-RBCs representing the entire transfused unit. RBC surface biotin density declined slightly over 98 days: ~7,000-5,000 molecules/RBC at 18 µg/mL (Control); 2,500-2,000 molecules/RBC at 6 µg/mL (PR-

P211 - Table: Detection of acridine surface antigen

Day	Acridine molecules/RBC	% Acridine positive RBCs
0 (≤ 1-hr)	8,414	12.30
1	3,764	12.60
7	1,360	11.20
14	1,417	11.00
25	1,039	7.10
54	946	3.40
67	760	1.80
82	649	0.50
98	643	0.10

RBCs); and 800-600 molecules/RBC at 2 μg/mL (Pre-PR RBCs). Acridine antigen density on RBC surfaces (Table) was approximately equivalent to the 18 μg/mL biotin label within 1 hour of transfusion. Acridine density declined ~55% at 24 hours post-transfusion and by ~87% on day 7 with elution of acridine in a uniformly monotonic fashion suggesting that no RBCs completely lost the acridine label. Acridine labeling remained detectable throughout the 98-day observation period. The shapes of RBC survival curves for both biotin and acridine labeled cells were similar over 98 days. Screening for antibodies to acridine and to biotin-RBCs were negative throughout the survival study.

Summary/Conclusions: PR-RBC survival can be tracked *in vivo* by flow cytometry for RBC surface acridine with similar sensitivity as biotin labeling. Acridine labels allow PR-RBC tracking *in vivo* without extra processing. Acridine antigen density on PR-RBCs declines rapidly and uniformly and stabilizes at ~8-13% of the transfused level within 7 days. Low residual acridine levels on RBC surfaces may account for the lack of laboratory evidence of haemolysis with low titre treatment-emergent antibodies to PR-RBCs seen in clinical studies. Additional research is needed to confirm this hypothesis.

P212 | Evaluation of CD 44, CD47 and annexin V expression in leuko-reduced packed RBCs stored in additive solution obtained from WB subjected to pathogen inactivation and separation

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Background: Implementation of pathogen inactivation methods in plasma and PC has significantly strengthened the safety of blood component transfusion. Pathogen inactivation of packed RBCs (the most frequently transfused blood component), has not so far been implemented into routine practice. The Mirasol PRT system for whole blood has been developed for the treatment of whole blood for transfusion and has acquired CE mark in 2015. It is based in the same principle as the technology used for the treatment of platelets and plasma, namely: using riboflavin (vitamin B2) plus UV light to induce damage in nucleic acid-containing agents. The method was also found effective in the inactivation of T lymphocytes and can therefore be an alternative to irradiation of PC and packed RBCs indicated to high-risk patients. In our institute we fractionated the inactivated WB to obtain inactivated packed RBCs, plasma and PC for research purpose only, as this is an off-label application of the technology. In Poland, WB is rarely transfused but we believe that the protocol for whole blood inactivation has great potential as it allows to obtain three pathogen inactivated blood components

Aims: The aim was to evaluate the quality of leuko-reduced packed RBCs stored in additive solution (PAGGSM), obtained with automated separation (REVEOS system) from WB units subjected to pathogen inactivation (Mirasol PRT system for whole blood, Terumo Blood and Cell Technologies), as measured by CD 44 and CD 47 expression. These are considered important markers of intercellular interaction and phagocytosis respectively

Methods: The study material involved 40 units of WB (mean volume 445 ± 7 ml), collected into TERUMO BCT Reveos® LR sets from healthy blood donors at the Regional Blood Transfusion Center in Łódź. The WB units were equally distributed into study (S) and control (C) groups (20 units for each group). WB units from the study group were pathogen inactivated (Mirasol system), while the control WB units were not. The leukocyte-depleted packed RBCs with PAGGSM (separation in the Reveos system) – from both the control and study groups - were stored at 2°C - 6°C. Test samples were collected on the 1st, 7th, 14th, 21st, 28th, 35th and 42nd day of storage.CD44 and CD47 antigen expression and the percentage of apoptotic cells during storage of leuko-reduced packed RBCs in additive solution were determined, as follows: murine monoclonal antibodies which bind to CD47-FITC and CD44-APC antigens were used. Annexin V was used to determine the percentage of apoptotic cells. 10,000 red blood cells were analysed using a FACSCalibur flow cytometer (Beckton Dickinson, USA). The population of RBCs was isolated on a dot-plot FSC vs SSC (on a logarithmic scale). CD44, CD47 and Annexin expression were analysed using the CellQuest PRO program (Beckton Dickinson, USA). The percentage of positive cells (cut-off value for control test) and the mean fluorescence intensity of the study population, MFI (mean fluorescence intensity) were determined.

Results:

P212 – Table 1

	Day 1		Day 42	
Parameter	**C**	**S**	**C**	**S**
CD44 (%)	119,4 ± 40,5	114,1 ± 42,1	116,3 ± 34,3	115,0 ± 30,3
CD47 (%)	142,3 ± 40,2	140,3 ± 24,2	172,6 ± 34,2	168,9 ± 38,0
Annexin V	1,04 ± 0,62	1,15 ± 0,63	1,83 ± 1,47	1,58 ± 1,02

C- control
S- study

Summary/Conclusions: No significant differences were observed between the two groups of leuko-reduced packed RBCs stored in additive solution (study and control) as regards the mean fluorescence intensity of CD44, CD47 and Annexin V.

Blood products

Novel blood products/ components

P213 | 1,2-α-fucosidase treatment effectively converts O group blood components into phenotypical Oh units compatible with Bombay patient plasma

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Background: Blood transfusion is a lifesaving intervention at the center of many routine medical procedures, including acute haemorrhages and cancer treatments. Yet transfusion is often out of reach for patients with the rare Bombay (O_h) and para-Bombay blood groups who have naturally-occurring haemolytic antibodies against carbohydrate determinants on common blood types.

Aims: The aim of this study was to investigate the ability of *1,2-α-fucosidase* from the probiotic bacteria *Bifidobacterium bifidum* to hydrolyze the H antigen on O type blood components and thus convert them to phenotypical Oh units.

Methods: The enzymatic reaction was performed at 4 degrees, directly in blood bags containing SAGM additive solution. The resultant H-antigen expression was evaluated using Ulex lectin and monoclonal anti-H antibodies in gel column based micro typing system cards, microscopy and flow cytometry. Furthermore, 20 sera from Bombay patients were crossmatched against ECOh RBCs and three of those sera were used for the Monocyte Monolayer Assay. We also cross-matched 1000 healthy blood donor plasmas against ECOh RBCs to screen for cryptic antigens. The physical properties of ECOh RBCs were evaluated using Ektacytometry.

Results: The enzymatic reaction effectively generated blood units phenotyped as Oh. Enzyme converted Oh (ECOh) red blood cells were crossmatch negative when tested against 20 unique Bombay patient sera. This compatibility was further confirmed through functional *ex vivo* testing using the Monocyte Monolayer Assay (MMA). *Fucosidase* treatment did not affect the physical membrane properties of the RBCs and it did not expose cryptic antigens.

Summary/Conclusions: Clinical availability of this easy-to-use *1,2-α-fucosidase* at Blood Banks could increase the chance to survive acute haemorrhage situations for the 100,000+ Bombay and para-Bombay individuals around the world as well as enable them to undergo modern medical treatments, e.g. chaemotherapy against cancer, with reliable access to transfusion support.

P214 | Abstract withdrawn

P215 | Differential expression of microRNAs in room temperature and cryopreserved platelets

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Background: Platelets contain an abundance of microRNAs (miRNAs) which are involved in the regulation of platelet function. The expression of miRNAs change during conventional platelet storage at room temperature, which is likely related to the platelet storage lesion and apoptosis. However, changes in expression of miRNAs in platelets stored under alternative conditions, such as cryopreservation, have not been examined. Analysis of apoptosis-related miRNAs in platelet components may provide new insight into the regulation of platelet function during component storage.

Aims: To characterise differences in the relative abundance of apoptosis-related miRNAs in fresh and cryopreserved platelets using a commercially available panel.

Methods: Apheresis platelets (day 1 post-collection) were cryopreserved with 5-6 % dimethyl sulfoxide (DMSO) before freezing at -80°C. Cryopreserved platelets were thawed and resuspended in a unit of plasma before sampling. Samples were taken before (fresh) and after cryopreservation ($n = 3$), and RNA was extracted using TRIzol reagent. RNA was reverse transcribed and PCR was performed with 25 ng cDNA using a miRCURY LNA miRNA Human Apoptosis Focus

P215 - Table 1: miRNAs with a relative increase in abundance in cryopreserved platelets compared to fresh platelets.

miRNA	p-value	Hypothesised role in platelets
miR-181c-5p	0.022	Inhibition of Bcl-2, inhibition of PI3K/Akt signalling pathway
miR-29a-3p	0.005	Inhibition of Bcl-2
miR-29c-3p	0.004	Inhibition of Bcl-2, inhibition of Bcl-w
miR-32-5p	0.032	Regulation of BIK
miR-365a-3p	0.047	Regulation of Bak and Bax
miR-497-5p	0.018	Inhibition of Bcl-2, inhibition of Bcl-w

V2 panel (QIAGEN). miRNA abundance was normalised to 5 reference miRNA and a spike-in control, and the transcript abundance was analysed using the $2^{-\Delta\Delta Ct}$ method. This analysis enables relative quantitation of the miRNAs between treatment groups. The abundance of miRNA was compared between fresh and cryopreserved groups using a paired t-test. A p-value of less than 0.05 and a fold-change of >1.5 were set as cut-offs for up-regulated targets.

Results: A total of 84 apoptosis-related miRNA targets were screened. Approximately 14 % of these targets were either absent or present at low abundance (Ct >35). Of the targets identified, 12/72 were statistically different in fresh and cryopreserved platelets. However, the abundance of only six targets was at least 1.5-fold higher in the cryopreserved platelets (Table 1). The role of these miRNAs has been documented across various cell types and disease states, providing an indication of their potential role in platelets, which likely includes the regulation of pro-survival proteins, such as Bcl-2, and pro-apoptotic proteins, such as Bak and Bax.

Summary/Conclusions: This screening identified six miRNAs that were differentially expressed between fresh and cryopreserved platelets. Broadly, these miRNAs are thought to play a role in platelet regulatory pathways, including platelet activation, the platelet storage lesion, and apoptosis and further research to fully elucidate their role in cryopreserved platelets is warranted.

P216 | Abstract withdrawn

P217 | Granulocyte pooling: An alternative to the classical collection method from apheresis?

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Background: Granulocyte transfusions are used to provide functional neutrophils to patients with severe neutropenia, who are at high risk of bacterial and fungal infections. These transfusions involve collecting white blood cells, including neutrophils, from a compatible donor through an apheresis and sedimentation method, with hydroxyethylstarch (HES). Since the PRAC (Pharmacovigilance Risk Assessment Committee) recommended the suspension of the authorization of commercialization of medicines with HES in the European Union (May 2022), novel methods for collecting granulocytes need to be developed.

Aims: To describe a new method of obtaining granulocytes for transfusion from the pool of a concentrated leukocyte by-product (leukocyte residue, LR) derived from automated whole blood (WB) fractionation. This product should have a granulocyte yield equivalent to the granulocyte concentrate collected by apheresis.

Methods: We retrospectively evaluated 49 granulocyte pools (GP) transfused between 2021-2023 in patients who were candidates for GP infusions. The patients evaluated ($n = 11$) aged between 5 months and 11 years were diagnosed with acute myeloblastic or lymphoblastic leukemia ($n = 7$), non-Hodgkin lymphoma ($n = 1$), congenital leukemia cutis ($n = 1$), CBL syndrome ($n = 1$) and APLS-like syndrome ($n = 1$). All of them were screened to rule out the presence of anti-HLA/HNA prior to transfusion. Once the transfusion order was received with patient's weight and ABO group, the transfusion dose/Kg was calculated. Based on the average viable granulocyte count in each LR (0.97 ± 0.20 × 10E8), fresh WB donations drawn from non-transfused male donors that afternoon were selected according to ABO/Rh compatibility and time since extraction. These WB, with confirmed negative screening tests, were processed according to internal protocols. Then, LRs were taken and suspended in platelet additive solution to obtain a GP. To comply with EDQM standards, quality control samples were analysed (cell counter and flow cytometry). Once said compliance was verified, GP was irradiated to 30 Gy (Cs137) and immediately delivered for transfusion. Granulocytes expire 24h after WB donation, according to stability studies.

Results: Patients (weight from 7 to 54Kg) received an average of 2.94 ± 1.07x10E8 viable granulocytes/Kg. This was accomplished by using a mean of 0.30 ± 0.09 LR/Kg, and with a production yield target of 2-4x10E8 viable granulocytes/Kg. According to clinical situation, one GP was infused every 2-3 days, with a range of 1 to 12 GP per patient. Nevertheless, a mean of 2.70 ± 0.83 mL of residual erythrocytes/Kg were also transfused with the GP. Just one patient didn't show neither peripheral blood count recovery nor signs of clinical improvement. No patients showed adverse reactions to transfusion.

Summary/Conclusions: Granulocytes collected from WB were effective in clinical resolution of all patients. In fact, one is under chaemotherapy treatment and GP transfusions are still required. The process described above enabled us to ensure granulocyte transfusions despite of HES discontinuation without the need to pharmacologically mobilize granulocytes from donors. While granulocyte doses were achieved, a limitation of this product was the presence of a high amount of residual red blood cells, so we must develop purification methods to maximize the quality of the component, reducing possible immunohematological sensitization. Additional functional studies have to be performed.

P218 | Abstract withdrawn

P219 | Pathogen-reduced, cryopreserved platelets to maintain individual platelet support

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Background: Providing adequate platelet (PLT) support for donors requiring cross-matched or HLA-typed platelets could be challenging, especially during holiday seasons. Cryopreservation of platelet concentrates (PCs) is a potential solution to overcome shortages and

maintain platelet support. In our institution, we prepare cryopreserved PLTs from pathogen-reduced platelets (CPRPs) to increase blood safety. It is known that cryopreservation of platelets induces a higher level of basal activation, lower level of activatability, increased clot formation time (CFT) and reduced clot firmness (MCF). That may lead to reduced post-transfusion recovery, but was reported not to impact haemostasis. It also was reported elsewhere that pathogen reduction treatment did not significantly impact these parameters additionally in cryopreservation procedures. Polish guidelines require a platelet count of $\geq 3x10^{11}$ per unit and a white blood cell (WBC) count of $<1x10^{6}$ per unit pre-freezing, and a recovery of >40% of platelets and a volume of 50-200 mL post thawing.

Aims: Aim of the study was the assessment CPRPs manufacturing data and quality compliance with Polish guidelines, based on 2020-2022 data.

Methods: Leukoreduced apheresis platelet concentrates (APCs) were collected with an Amicus® device (Fresenius) in 65% SSP+ (Macopharma) and 35% plasma followed by pathogen inactivation treatment with amotosalen/UVA (INTERCEPT® Blood System, Cerus). The APCs were pelleted in a centrifuge, reconstituted in 100 mL autologous plasma with 5% DMSO and subsequently frozen at -80°C (1 year shelf life) within 24 h post collection. The CPRPs were thawed at 37°C in a water bath, washed gently with 100 mL washing solution (SSP+, pH 6.5 with 2.5 mL ascorbic acid), pelleted in a centrifuge and reconstituted in 100 mL autologous plasma (post-thawing shelf-life 2 h). PLT and WBC counts were determined with an XN-550 hematology analyser (Sysmex). The procedure was developed and validated solely by the Warsaw Regional Blood Donation Center independently.

Results: 13.587 PCs were collected in 2020, 298 of them (2.2%) were cryopreserved. 14.884 PCs were collected in 2021, 282 of them (1.9%) were cryopreserved. 15.426 PCs were collected in 2022, 260 of them (1.7%) were cryopreserved. 290 CPRPs were thawed and distributed in 2020, 284 in 2021 and 249 in 2022. Data from 156 CPRPs were included in this study to obtain representative results (approx. 20% of annual production). The WBC count of all APCs was $<1x10^{6}$/unit pre-cryopreservation, the average PLT count was $3.4x10^{11} \pm 0.6$, post-cryopreservation $2.3x10^{11} \pm 0.5$ (average recovery rate 68.5% ± 10.5 (40.4-97.8)). The average volume post-thawing was 102.2 mL ± 4.5 (90-117). No CPRP transfusion-related adverse events were reported.

Summary/Conclusions: We recognised a trend for a decreased demand for CPRPs from2020 to 2022, which may be explained by the stepwise cessation of pandemic measures, releasing restrictions for blood donors. CPRPs are used only when fresh PCs are not available as safety pool to ensure platelet availability for donors requiring HLA-typed/crossmatched platelets in holiday/vacation seasons with limited availability of donors. CPRP fulfilled Polish quality requirements, the recovery rate was in line with previous published data (70.6% in Sandgren, Blood Transfus, 2022).

P220 | Trehalose-treated freeze-dried platelets–Effect on ex-vivo haemostasis in post-cardiopulmonary by-pass

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Background: Platelet transfusion is a cornerstone in the treatment of acute bleeding after cardiopulmonary by-pass; however, platelet supply is often compromised by the short lifespan of platelets in storage, and platelets stored in blood banks often show decreased activation which may be critical in acute bleeding. Freeze-dried platelets may be a solution to these challenges as they can be stored for extended time and has been hypothesised to have increased haemostatic potential compared to standard platelets.

Thrombosomes® (Cellphire Therapeutics Inc., USA) are lyophilized, freeze-dried platelets derived from blood type O donor platelet-pools. Platelets are pathogen-reduced and treated with trehalose prior to freeze-drying to attenuate the damage usually associated with the procedure.

Aims: We sought to characterise the effects of Thrombosomes® on haemostasis in patients after cardiopulmonary by-pass (CPB) measured by thrombelastography (TEG®).

Methods: We did ex-vivo spiking of post-protamine blood samples from 12 patients undergoing open heart surgery on CPB. Four groups: Two different concentrations of Thrombosomes® (**LOW**: $35x10^9$ particles/L; **HIGH**: $59x10^9$ particles/L.), standard pooled platelet concentrate (**PLT**: $33x10^9$ platelets/L.), and a saline placebo (**Saline**).

Functional haemostasis was assessed using TEG® 6s Citrated Kaolin channel with heparinase (CKH) to eliminate residual heparinization from CPB immediately after spiking.

Results: 12 patients: 2 females and 10 males, median age 67 years, range 49 – 77 years. Mean(±SD) pre-spiking platelet count 207(±64) $x10^9$/L.

Summary/Conclusions: In this small, ex-vivo study, functional haemostasis assessed by thrombelastography with heparinase was unaffected by the addition of Thrombosomes®. Further research on monitorering the ex-vivo effect of Trombosomes is needed.

P220 – Table 1

TEG® CKH, Median (IQR)	R-time (min.)	Angle (°)	K-time (min.)	Maximum amplitude (mm.)
Saline	7.9 (6.9 – 9.1)	71.4 (68.6 – 72.5)	1.4 (1.3 – 1.7)	61.1 (58.1 – 63.5)
LOW	6.9 (6.0 – 8.1)	73.4 (71.0 – 74.4)	1.3 (1.1 – 1.7)	61.1 (59.1 – 64.0)
HIGH	7.1 6.3 – 7.7)	72.6 (69.4 – 73.6)	1.6 (1.3 – 1.8)	58.7 (57.6 – 62.1)
PLT	7.1 (6.1 – 8.0)	72.7 (68.4 – 74.6)	1.3 (1.2 – 1.6)	62.1 (61.2 – 64.5)

No significant differences among means (Tukey's HSD, $\alpha = 0.05$)

P221 | Freeze-dried platelets provides faster onset of coagulation ex-vivo

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Background: Platelet transfusion plays an important role in treatment of thrombocytopenia, including acute and massive haemorrhage. Shortage of platelet and limited shelf-life, combined with possibly decreased activation of standard pooled platelet concentrates, calls for innovative thinking. Alternative platelet products have been investigated for several years; however, all are facing difficulties with platelet recovery.

Thrombosomes® (Cellphire Therapeutics Inc., USA) are lyophilized, freeze-dried platelets derived from platelet-pools from type O donors. Thrombosomes® are pathogen-reduced and treated with trehalose before freeze-drying. Freeze-dried platelets have been hypothesised being procoagulant, providing a higher thrombin-'burst', compared to room-temperature stored platelets.

Aims: To explore the haemostatic effect of freeze-dried platelets post CPB ex-vivo.

Methods: We performed ex-vivo spiking of post-protamine blood samples from 12 thoracic surgical patients undergoing open heart surgery on cardiopulmonary by-pass (CPB). Addition of two different concentrations of Thrombosomes® (Low: $35x10^9$ particles/L; High: $59x10^9$ particles/L), standard pooled platelet concentrate (PLT: $33x10^9$ platelets/L) and a saline placebo (Saline).

Immediately after spiking we measured R-time, Clot Formation Time (K), Angle and Maximal Amplitude (MA) using Thrombelastography TEG® 6s citrated kaolin (CK). Differences among medians calculated using Tukey's-HSD test and α=0.05.

Results: 12 patients were included in this study, 10 males and 2 females, median age 67 years, ranging from 49-77 years. Data (medians, IQR) are shown in the table below.

Summary/Conclusions: In this ex-vivo study, Thrombosomes® reduced clotting time compared to saline placebo, as lower R-values were observed. MA-values were slightly decreased after addition of Thrombosomes® compared to after addition of standard pooled platelet concentrate.

Our data suggests a faster onset of coagulation with the addition of Thrombosomes®, and therefore potentially a promising role for early treatment of patients with acute or massive haemorrhage. However, these results need to be investigated in further studies.

NB! Lise Høj and Camilla Sterll contributed equally to this work.

P221 – Table 1

TEG® CK	R-TIME (min.)	K-TIME (min.)	ANGLE (°)	MA (mm.)
SALINE	8.8 (7.7-10.6)	1.5 (1.3-1.7)	70.7 (67.6-72.0)	60.1 (57.0-62.7)
LOW	7.5 (6.6-8.0)*	1.5 (1.1-1.8)	70.0 (68.5-74.0)	58.6 (56.1-54.7)
HIGH	7.7 (6.8-8.6)	1.8 (1.7-2.1)	68.8 (66.8-69.8)	57.1 (54.8-60.6)#
PLT	7.7 (7.1-8.7)	1.5 (1.1-1.8)	68.7 (67.8-75.0)	61.9 (61.1-64.8)

* Different from saline ($p < 0.05$); #Different from PLT ($p < 0.05$) using Tukey's-HSD test.

Transfusion transmitted infections

Screening strategies for TTI

P222 | Abstract withdrawn

P223 | Multivariate linear regression analysis of four mandatory blood screening serology markers in national blood centre & 12 regional blood centre of Thai Red Cross Society, Thailand.

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Background: The Four Transfusion transmissible infections (TTIs) namely, Human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Treponema pallidum (syphilis) are mandatorily screened for all the blood donations at all blood centres in Thailand for the provision of a safe blood supply. The National Blood centre (NBC) and 12 regional blood centres (RBC) of Thai Red Cross Society (TRCS), Thailand utilise Alinity i immunoanalyser (Abbott Diagnostics, IL) to perform the HIV Ag/Ab combo, HBsAg, anti-HCV and syphilis serology screening since Sep 2019. We recently conducted method verification and correlation of these four Alinity i screening assays between NBC and RBCs using multivariate linear regression (MLR) before the implementation of new Alinity i analysers in Nov 2022 for the routine blood screening in the blood centres.

Aims: To perform MLR analysis of four TTIs screening markers - HIV Ag/Ab combo, HBsAg, anti-HCV and syphilis on 32 Alinity i immunoanalysers in NBC and 12 RBCs of Thai Red Cross Society, Thailand for the result correlation.

Methods: MLR analysis was performed using 50 donor plasma samples (25 negative and 25 positive) for each TTI screening marker that is, HIV Ag/Ab combo, HBsAg, anti-HCV and syphilis provided by NBC to all the RBCs. Total of 32 Alinity i analyser at NBC and 12 RBC were included in the study.

Results: MLR analysis of all the 4 TTIs markers on 32 Alinity i analyser at NBC and 12 RBCs demonstrated excellent correlation between the expected and the observed results with HIV R^2 ≥ 0.99, HBV R^2 ≥ 0.89, HCV R^2 ≥0.99 and syphilis R^2 ≥0.96.

Summary/Conclusions: The study showed excellent correlation for all the 4 evaluated TTIs markers on 32 Alinity i analyser at NBC and 12 RBCs of Thai Red Cross Society. Robust MLR results not only provided objective evidence towards high performing blood screening serology in the TRC labs before the implementation but also allowed us to set the baseline standard for future monitoring of the labs performance.

P224 | Prevalence of acute hepatitis E virus infections in Swiss blood donations 2018–2020

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Background: Hepatitis E virus (HEV) genotype 3 is the major cause of acute viral hepatitis in several European countries. It is acquired mainly by ingesting contaminated pork, but has also been reported to be transmitted through blood transfusion. Although most HEV infections, including those via blood products, are usually self-limiting, it may become chronic in immunocompromised persons. It is thus essential to identify HEV infected blood donations to prevent transmission to vulnerable recipients.

Aims: Prior to the decision whether to introduce HEV RNA screening for all Swiss blood donations a 2-year nationwide prevalence study was conducted. The findings of the study is presented.

Methods: All blood donations were screened in pools of 12-24 samples at five regional blood donation test centres using the commercial HEV RNA assays from Roche Molecular Systems, Inc. and from Grifols Diagnostic Solutions. The HEV RNA positive pools were subsequently resolved to the individual donation (X donation). On the X-donations the viral load, HEV IgG and IgM serology and the HEV genotype were determined. Follow-up investigations where conducted on future control donations (X+1) and previous archived donations of the donor (X-1) where available.

Results: Between Oct 2018 and Sept 2020, 541,349 blood donations were screened and 125 confirmed positive donations were identified (prevalence 1:4,331 donations; Table 1). At the time of blood donation, the HEV RNA positive individuals were symptom-free. The median viral load was 554 IU/mL (range: 2.01 - 2,500,000 IU/ml). Men (88; 70%) were more frequently infected than women (37; 30%) as compared with the sex distribution in the Swiss donor population (57% male / 43% female, $p < 0.01$). Of the 106 genotyped cases (85%), all belonged to genotype 3 (Table 2). Two HEV sub-genotypes predominated; 3h-s (formerly 3s), a variant endemic

P224 - Table 1: Characteristics HEV RNA-positive blood donations

Number donations screened	541,349	
Number HEV RNA positive	125	
HEV prevalence	1:4,331	
HEV median viral load	554 IU/ml	
HEV viral load range	2.01 - 2,500,000 IU/ml	
Number males/females (%); median age; viral load	males: 88 (70); 47.5 y; 734 IU/ml	females: 37 (30); 38.4 y; 266 IU/ml

P224 - Table 2: Distribution of HEV sub-genotype 3 identified

HEV sub-genotypes	Number (%)
HEV 3h-s	42 (40)
HEV 3c	40 (38)
HEV 3f	10 (9)
HEV 3ra	5 (5)
HEV 3e	2 (2)
HEV 3a	1 (1)
various HEV 3 (3/3o/3t)	6 (6)
Total	**106**

in the Swiss pig population and which is practically confined to Switzerland and 3c, a variant often encountered in northern Europe. The remaining sub-genotypes are all known to circulate in Europe. Five 3ra genotypes were identified, a variant associated with rabbits. 85 (68%) X donations were negative for HEV IgM and IgG. The remaining 40 (32%) were positive for HEV IgG and/or IgM and are consistent with an active infection. We found no markers of previous HEV in the 91 analysed archive samples (X-1) (Table 3). Three donors were HEV IgG positive in the X-1 donation suggesting insufficient immunity to prevent HEV reinfection. Time of collection of the 91 (72.8%) analysed X+1 donations varied between 2.9-101.9 weeks (median of 35 weeks) after X donation. As expected, none of those tested were positive for HEV RNA. Most donors (90; 98.9%) were positive for anti-HEV IgG / IgM (i.e. seroconversion). HEV IgM positive (24; 26%) indicates an often long persistence of IgM antibodies post HEV infection.

Summary/Conclusions: The data collected during the first year of the study provided the basis for the decision to establish mandatory HEV RNA universal screening of all Swiss blood donations in minipools.

P225 | 21 years experience in NAT screening of blood donors: analysis of data recorded at ASST-Spedali Civili, Brescia (Italy)

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Background: Since October 31, 2001 all the donations collected in Brescia (Italy) and in its province are tested using a molecular qualitative screening for HIV1-2, HCV, HBV (HBV-DNA since March 30, 2006) at the NAT Laboratory of the ASST Spedali Civili Brescia. In 21 years, until October 31, 2022, 1.344.637 donations have been screened.

Aims: Evaluating the role of NAT screening in the detection of transfusion trasmitted viruses

Methods: Data about donations from the last 21 years were investigated retrospectively. Blood samples were collected in EDTA tubes.

Until February 2014, the units were tested by ID-NAT (Individual donor nucleic acid test) using TMA (transcription-mediated amplification) technology: from October 2001 to March 29, 2006 by a semiautomated system (e-sas), the Procleix HIV/HCV Assay, then by Procleix Ultrio Assay (HIV/HCV/HBV) and, from August 2008, using Tigris automated system. In February 2014, minipool Multiplex PCR was introduced: maximum 6 samples were analysed using Cobas s201, then Cobas 6800; since October 5, 2016 the units were tested individually by MPX using Cobas 6800.

According to the Italian law, all initially reactive samples (IR) were retested in duplicate on the pilot screening tube and in triplicate from the fresh frozen plasma (FFP) collected from respective donations.

The serological status of IR donors was also investigated. Anti-HBc test was performed on HBV-DNA IR samples to identify occult HBV infections (OBI).

All the IR donations were discarded

Results: From the 781.113 donations tested by TMA, 1121 were IR (352/256.999 tested only for HIV and HCVRNA); a discriminatory test was performed on IR samples: 5 were confirmed HCV positive (1 anti-HCV negative), 15 HIV positive (2 anti-HIV negative) and 22 HBV positive (19 HbsAg negative).

174.999 samples were screened in minipool using MPX; 204 were IR: 11 were HBV repeat reactive (RR), 1 also HBsAg+ while 17 were IR and HBcAb+.

Out of 388.525 individual donations tested by MPX, 597 were IR: 1 was HIV RR and Anti-HIV +, 43 were HBV RR while 89 were HBV-DNA IR and HBcAb+

Summary/Conclusions: According to our experience, the introduction of NAT screening for three viruses on blood donations has improved blood safety in Italy by reducing the transfusion transmitted risk and the window period: 16 HIV (2 seroconversions), 5 HCV (1 seroconversion), 4 acute hepatitis B and 178 OBI were detected.

Our analysis support the effectiveness of using NAT for detection of OBI.

P226 | ID-NAT screening of donated blood: A one year experience

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Background: Since the introduction of nucleic acid testing (NAT) of donated blood, there is a growing evidence concerning the impact on blood safety associated with the shortening of the window period and detection of occult infections.

P226 - Table 1. Prevalence of TTI in blood donors

Technique	HBV No (%)	HCV No (%)	HIV No (%)	Total No (%)
Serology	76 (0.34)	7 (0.32)	7 (0.032)	90 (0.40)
NAT	83 (0.37)	4 (0.02)	7 (0.032)	94 (0.42)
Serology+NAT	85 (0.38)	7 (0.32)	7 (0.032)	99 (0.45)

Aims: To correlate the prevalence of serological and molecular markers of transfusion transmissible infections (TTI) in blood donors and to analyse the discrepant results.

Methods: In 2022, multiplex ID-NAT was introduced in the Institute for blood transfusion of North Macedonia and 53630 blood donations from 22084 blood donors were screened for the presence of HBV DNA, HCV RNA and HIV RNA using Procleix Panther System, as well to the presence of HBsAg, anti-HCV and anti-HIV/p24 using Architect 2000 platform. Repeatedly seroreactive samples were subjected to confirmatory testing using HBsAg neutralization test and immunoblot for HCV and for HIV. Repeatedly reactive samples by NAT were subjected to individual testing using Procleix Ultrio Elite HIV, HCV and HBV discriminatory assay.

Results: Out of 53630 blood samples, 309 (0.57%) were initially seroreactive, 160 (51.7%) of them were repeatedly reactive and 90 (56.2%) of them were confirmed positive. Concerning NAT, 156 (0.30%) blood samples were initially reactive, 115 (73.0%) of them were repeatedly reactive and 94 (81.7%) of them had positive discriminatory test. The prevalence of HBV DNA and anti-HCV is bigger than the prevalence of HBsAg and HCV RNA respectively.

The NAT yield of 22008 HBV seronegative blood donors was 9 (0.040%) from which 7 (77.7%) were repeat donors. One case was suspected to be occult hepatitis B infection and the rest of the NAT only positive donors were window period infections. In 3 out of 7 serologically HCV positive donors, NAT was negative. There were no discrepant HIV results between serologic and NAT testing.

Summary/Conclusions: Optimal TTI screening strategy according to the epidemiologic situation which includes complementary NAT technology is essential towards maximal reduction of the residual risk of infection via blood transfusion.

P227 | Seroprevalence of TTI markers: A comparative study

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Background: According to the literature data, the prevalence of HBV, HCV and HIV is much lower in blood donors, especially in regular and no remunerated donors in comparison to the general population. In spite of that, besides blood safety, blood donor transfusion transmissible infections (TTI) markers testing is among the largest screening programs concerning public health in terms of prevention of infectious disease spread among the population.

P227 - Table 1. Seroprevalence of HBV and HCV in first time and repeat blood donors

Period	HBsAg		Anti-HCV	
	Fist time No (%)	Repeat No (%)	Fist time No (%)	Repeat No (%)
2017-2019	147 (0.30)	4 (0.015)	15 (0.030)	3 (0.011)
2020-2022	235 (0.68)	11 (0.033)	15 (0.043)	1 (0.0030)

P227 - Table 2. Seroprevalence of HIV in first time and repeat blood donors

Period	Anti-HIV/p24	
	Fist time No (%)	Repeat No (%)
2017-2019	2 (0.004)	3 (0.011)
2020-2022	5 (0.014)	9 (0.027)

Aims: To compare the seroprevalence of TTI markers in blood donors in two consecutive periods in order to evaluate the impact of donor characteristics.

Methods: The seroprevalence of TTI markers (HbsAg, anti-HCV and anti-HIV/p24) in 34450 first time and 33142 repeat donors who donated blood in the period 2020-2022 was compared to the seroprevalence in 49711 first time and 26052 repeat donors who donated blood in the period 2017-2019. Blood screening was performed using Architect 2000 platform. Confirmatory testing of the repeatedly reactive blood samples was performed using neutralization test for HBsAg and immunoblot test for HCV and HIV.

Results: The overall, the first time and the repeat donor TTI marker prevalence was 0.23%, 0.32% and 0.038% respectively in the period 2017-2019. In comparison, the overall, the first time and the repeat donor TTI marker prevalence was 0.41%, 0.74% and 0.063% respectively in the period of 2020-2022. The prevalence of HBV (0.36%) and HIV (0.020%) was significantly higher in the period 2020-2022 in comparison to the period 2017-2019 when the prevalence of HBV and HIV was 0.20% and 0.006% respectively. The prevalence of HCV (0.023%) was the same in the two analysed periods. The prevalence of HBV and HCV is higher in first time donors which is not the case for HIV which prevalence is higher in repeat donors as shown in Table 1 and 2.

Summary/Conclusions: The overall prevalence of TTI markers is increased by double in the past three years. One reason may be the fact that proportion of family donation reached almost 40% in 2020 due to SARS-CoV-2 pandemic. In 2021 and 2022 family donation dropped to less ten 1%, like before the pandemic, but the TTI prevalence remained increased. Further analysis, as well as donor education improvement, revision of the donor selection criteria and strengthening collaboration with the public health centers is necessary.

P228 | Global nucleic acid amplification testing use and confirmatory testing approaches in blood donor screening

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Background: Nucleic acid-amplification testing (NAT) is used for the detection of specific sequences of nucleic acids. Most blood operators in high- and middle-income countries use NAT for the detection of viral nucleic acid to reduce the occurrence of transfusion-transmitted infections (TTIs). HIV combined with HCV NAT was initially implemented in 1999, and soon after for HBV. NAT is now also routinely used for other TTIs including HEV in selected regions, West Nile virus (WNV) during times of increased viral circulation, and during outbreaks of other viral infections including Zika virus (ZIKV), dengue, and chikungunya.

Aims: To review NAT usage for screening blood donations for TTIs, including confirmatory testing procedures, globally.

Methods: A survey was developed by ISBT WP-TTID, based on previous work (Roth, Vox Sanguinis, 2012). The survey asked questions about NAT usage and confirmatory testing approaches applied within the year 2019. The survey was distributed to ISBT WP-TTID members as well as the customer base of the two main suppliers of NAT for blood screening. Results were analysed using descriptive statistics, while reported variables were expressed as frequencies and percentages.

Results: Responses from 43 blood operators in 30 countries were included in this analysis. Survey responders primarily performed NAT for HIV, HBV, and HCV; NAT screening for HEV, WNV, and ZIKV was employed less often (performed by 11, 11 and 3 responders, respectively). No respondents indicated use of NAT for all six viruses. Individual-donation NAT (as opposed to testing of mini-pools, MPs) was used for HIV, HCV and HBV by 51% of responders, while HEV was screened for in MPs by 91% of responders. MP sample sizes ranged from 4 to 96 samples, with 6 being the most frequently reported for HIV, HBV, HCV and WNV. MPs of 16 samples were most frequently reported for HEV. Survey responders generally performed confirmatory testing for NAT yield samples (NAT reactive but non-reactive by serology, if used) by NAT on a sample from the same donation (either the same sample or an alternative sample); using both NAT and serological based assays or using follow-up samples were used less frequently.

Summary/Conclusions: Overall, there has been increased NAT usage since 2008, when data for the last international review of NAT were collected. NAT use for HIV, HBV and HCV has remained relatively stable since the previous NAT survey, with only a small expansion of use for those pathogens. The last decade has, however, seen a rise in blood donation NAT for other viruses, particularly WNV in donors travelling to WNV endemic regions, and the introduction of HEV NAT. ZIKV NAT was implemented in some regions, but has now been discontinued due to no NAT yield and an absence of transmission since 2019. Compared to the previous survey, there has been a trend towards use of smaller pool sizes or individual donation NAT. This survey captures the current usage of NAT globally and provides insights into confirmatory testing approaches used for NAT yields, which may be useful for blood operators looking to undertake NAT in the future.

P229 | A validated model for predicting the status of Hepatitis B infection in non-discriminated reactive blood donors from a single blood center in China

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Background: Analysis using the transcription-mediated amplification (TMA) method revealed that there were ELISA-negative and non-discriminated reactive blood donors who were positive in the screening tests but negative in the discriminatory NAT assay. This raised the question whether such donors were false positive.

Aims: This study aimed to develop and validate a model for predicting the individualized status of Hepatitis B infection in these donors.

Methods: A prospective cohort study was performed in the Blood Center of Zhejiang Province. In addition, 436 initial non-discriminated reactive blood donations were obtained from two successive periods. All samples with initial non-discriminated reactive were analysed two times using the same screening and discriminatory assays, and then sub-categorized into three groups as follows: (1) non-repeated positive group, (2) non-discriminated positive group, and (3) non-repeated HBV-DNA positive group. Further serological and viral load assays were conducted to identify determine the HBV infection status of the donors and the results were encoded as binary variables indicating infection (1) or non-infection (0). The sample was randomly divided into the training and testing datasets. Several variables of donors including age, gender, BMI, marital status, education level, the donation type, and total donation number were recorded. Stepwise

P229 - Table 1 Predictors for the HBV infection in non-discriminated reactive blood donors in the final regression model for the training dataset

Intercept and Variable	β Coefficient	OR(95% CI)	P Value
Intercept	−5.784	/	/
Donations group	2.884	15.26 (6.07-38.34)	0.00
Age	0.055	1.06(1.02-1.10)	0.01
Numbers of donation	0.037	1.03(1.01-1.06)	0.00

forward algorithms were applied to identify informative predictors based on Akaike's information criteria. Multivari ablelogistic regression analysis was used to construct the model which was validated using an independent testing dataset. The Hosmer-Lemeshow test and receiver operating characteristic curves were used to assess the performance of the model. A P<0.05 was selected as the threshold for statistical significance.

Results: There were 285 HBV-infected donors, 204 in the training dataset ($n = 311$), and 81 in the testing dataset ($n = 124$). The S/Co values in first screening assay, donation group in repeat tests, donors age and the numbers of donation were predisposing factors to HBV infection with ORs(95% CI) 1.07(1.01-1.13, $p = 0.03$), 15.32 (7.09-33.07, $p < 0.01$), 1.04(1.01-1.08,$p = 0.01$), and 1.04(1.01-1.06, $p < 0.01$), respectively. Donation groups, donors age and the numbers of donation were identified as predictors and used for the construction of the model (Table 1). The area under curve for the training tests was 0.877(95%CI, 0.839-0.914), which indicated an adequate discrimination in the test dataset(0.859; 95%CI, 0.796-0.921). In the testing dataset, the model was well calibrated, with a Hosmer-Lemeshow χ^2 statistic of 4.51($P = 0.92$). The confusion matrix was also summarised, and the accuracy was 79.4 and 76.6% in the training and testing dataset, respectively.

Summary/Conclusions: This study has proposed an effective model for predicting the status of HBV infection in non-discriminated reactive blood donors. The results demonstrated that repeat tests in screening and discriminatory assay can identify more donors with HBV infection, especially for those with low level of viral loads OBIs. Also, the older repeat non-discriminated reactive blood donors may have higher risk of HBV infection. In summary, the model has important implications for the formulation of NAT screening strategies and the management for the reentry of blood donors.

P230 | Seroprevalence of transfusion-transmissible infections among blood donors in Nigeria: A retrospective analysis of blood donor data in the six geo-political regions

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Background: Millions of lives at risk of loss from critical disease conditions and massive blood loss are saved using therapeutic blood and blood products. However, the life-saving potential of blood is often marred by the risks of transfusion-transmissible infections (TTIs) from blood donors for which sub-Saharan African countries record some of the highest burdens.

Aims: We aimed to assess the seroprevalence of HBV, HCV, HIV, and syphilis among blood donors in Nigeria, and determine the association of seropositivity with specific blood donor characteristics.

Methods: A retrospective cross-sectional study was conducted to determine the seroprevalence of HBV, HCV, HIV, and syphilis amongst blood donors in thirteen blood bank/service establishments in Nigeria's six geopolitical zones from January 2018 to December 2019 following laboratory screening with highly sensitive Enzyme-Linked Immunosorbent Assays (ELISA). Data was collected from the country's web-based software District Health Information System, Version 2 (DHIS2) and analysed using R Studio v2022.07.2+576. pro12.

Results: Out of a total of 98,610 blood samples donated and screened in the 13 blood bank/service establishments, the seroprevalence of TTIs was 10.15%. This was higher in 2018 (11.46%) compared to 2019 (8.08%). Male donors were eight times more likely than female donors to be TTI seropositive (AOR = 8.3, 95% CI: 7.70–9.01, $p < 0.00$), while donors aged 46–55 years were more likely to be

infected with TTIs compared to those aged 18–25 years (AOR = 1.11, 95% CI, 1.02–1.20, $p = 0.014$). Additionally, donors at mobile blood drives were more likely than those from fixed donation locations to be seropositive (AOR = 4.2 95% CI, 3.6–4.4, $p < 0.00$); and replacement blood donors were five times more likely than voluntary donors to be infected with TTIs (AOR = 5.4, 95% CI, 5.05-5.88, $p < 0.00$). Furthermore, first-time donors were found to be nearly nine times more likely to be seropositive for TTIs than donors who had donated more than two times (AOR = 8.8, 95% CI, 8.0–9.7, $p < 0.00$). Finally, the hospital centre in the South-South geopolitical region and the stand-alone blood centres were found to be less likely to have TTI seropositive donors (AOR = 0.62, 95% CI, 0.55–0.68, $p < 0.00$; AOR = 0.21, 95% CI, 0.19–0.24, $p < 0.00$; AOR = 0.13, 95% CI, 0.11–0.15, $p < 0.00$; AOR = 0.16, 95% CI, 0.13–0.19, $p < 0.00$; AOR = 0.54, 95% CI, 0.44–0.65, $p < 0.00$; AOR = 0.22, 95% CI, 0.16–0.30, $p < 0.00$; AOR = 0.51, 95% CI, 0.41–0.63, $p < 0.00$; and AOR = 0.22, 95% CI, 0.19–0.25, $p < 0.00$).

Summary/Conclusions: The seroprevalence of TTIs in Nigerian blood donors is high. This study revealed significant findings regarding the association between the seroprevalence of TTIs and blood donor characteristics such as age, gender, donor type, number of donations, type of donation site, and particular regional blood bank/service establishments in Nigeria. It is therefore critical that tailored health education, as well as donor recruitment and retention strategies, are deployed to enhance population health promotion, and positive behavioural change for blood donor safety, thus reducing the risk of TTIs.

P231 | Comparison of two automated NAT systems for HIV, HBV, and HCV

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Background: Advances in technology have led to automated equipment that can perform many of the steps that previously required manual manipulation. Automation of equipment has helped laboratory testing with the increased ability to test larger volumes of specimens, reducing personnel hands-on time and the risk of potential errors due to human error, and decreasing turnaround times for release of test results.

One such instrument is the **cobas**® 5800 System, an automated platform for nucleic acid testing (NAT) that is used for low volume donor screening of transfusion-transmitted pathogens.

Aims: The aim of the study was to compare the sample process flow and operator interactions for NAT pooling and testing on the **cobas**® 5800 System to the **cobas s** 201 system.

Methods: The **cobas**® 5800 System provides sample preparation followed by PCR amplification and detection in a single integrated instrument. The **cobas s** 201 system consists of the COBAS® AmpliPrep Instrument for sample processing and COBAS® Taqman® Analyser for amplification and detection. The two instruments may be stand-alone or linked with a docking station. A Hamilton Microlab® STAR (STAR) is used for pooling of individual samples.

De-identified human plasma previously screened and found negative by serology and NAT for HIV, HCV and HBV (NHP) was aliquoted into 119 test tubes labeled with unique barcodes. A HCV external run control (EC) (HCV 1E3 IU/mL Precision (HCVL201), Exact Diagnostics) was included as a positive sample.

The 119 aliquots of NHP and 1 HCV EC were pooled on the STAR to create minipools consisting of six individual samples and each minipool was tested by **cobas**® MPX on the **cobas**® 5800 System. The pooling process was repeated with each minipool tested by the **cobas**® TaqScreen MPX Test, v2.0 on the **cobas s** 201 system.

Resolution of reactive pools with the positive EC was performed on each system to identify the reactive EC sample.

The sample process flow and operator interactions were documented for the STAR, **cobas**® 5800 System and **cobas s** 201 system.

Results: Total processing time, which includes pooling, testing and operator time, was 3:26:06 (hrs:min:sec) on the **cobas**® 5800 System compared to 5:32:50 on the **cobas s** 201 system. Pooling time was comparable for the two process flows of 0:38:15 and 0:37:26, respectively. The machine time of the **cobas**® 5800 System was 2:37:00 compared to 4:43:05 on the **cobas s** 201 system, which included processing on the COBAS® Ampliprep Instrument, COBAS® Taqman® Analyser, and the automatic transfer of samples with a docking station between the two instruments. The number of operator touchpoints for the total process was 9 for the **cobas**® 5800 System and 14 for the **cobas s** 201 system.

The total processing time for resolution of a positive pool was 2:40:32 on the **cobas**® 5800 System and 4:21:22 on the **cobas s** 201 system. Individual samples did not need to be pipetted on the STAR prior to testing on the **cobas**® 5800 System compared to the **cobas s** 201 system.

Summary/Conclusions: The **cobas**® 5800 System streamlines and shortens the turnaround time from sample processing to test result compared to the **cobas s** 201 system. The difference in machine time and the increased number of operator touchpoints was due to the manual steps required for sample setup on the STAR and COBAS AmpliPrep Instrument for the **cobas s** 201 system.

The combination of automation and workflow with the **cobas**® 5800 System minimises the opportunity for manual errors. The **cobas**® 5800 System is an ideal instrument for low volume laboratories processing donor samples in pools or by individual donation compared to the **cobas s** 201 system. It can also be used as a backup to the **cobas**® 6800/8800 Systems.

P232 | Incidence of syphilis in blood donors and comparison of various methods for screening

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Background: Blood transfusion is a life saving modality in medical practice and is associated with complications which are seen in 1% of all transfusion. These also include the most dreaded risk of acquiring transfusion transmitted infection (TTI). The Serological tests most commonly need to screen for the disease are Non Treponemal test like RPR (Rapid plasma reagin) or VDRL (Venereal Disease Research Laboratory) measure the hosts response to Non Treponemal antigens such as cardiolipid and lecithin and Treponemal tests like TPHA (Treponema pallidum Hemagglutination Assay) and Micro hemagglutination assay for Treponema Pallidum have high sensitivity for all the stages of disease other than very early primary syphilis, It is known that syphilis can be transmitted through blood, so it is significant to choose a method with high sensitivity and specificity to test syphilis when do the blood screening.

Aims: - To Find out Incidence of Syphilis in Voluntary and Replacement donors.

- Comparison of three methods (RPR, TPHA, ELISA) for syphilis detection.

- To evaluate the suitability of commercially available Non Treponemal and Treponemal test like RPR, TPHA in comparison with ELISA for either screening or confirmation of infection or disease.

Methods: This was a prospective cross sectional study conducted on 5000 blood samples from blood donors over 12 months from November 2020 to October 2021 in the Department of Transfusion Medicine of a tertiary care teaching hospital, in western India. Blood sample was collected and centrifuged until the serum was separated. All 3 tests, TPHA (Kit:-Meril-Diagnostics, Gujarat) RPR (Kit:-Pathozyme diagnostics, Kolhapur) and ELISA (Kit:-ERBALISA, Mfg:-Transasiabiomedicals, Ltd, Daman) were performed and results were achieved according to the kit's manufacturer's instruction.

Results: A total of 5000 donors (2500 voluntary donors & 2500 replacement donors) were tested by ELISA, TPHA & RPR in our study. Out of 5000 donors 61(1.22%) were reactive for syphilis using ELISA as a diagnostic test in this study. Among them 22 were voluntary donors and 39 were replacement donors. we also found 27 were false positive and 8 were false negative by RPR test. we reported 2 false negative with TPHA test. so the overall sensitivity and specificity of RPR was 86% and 99.4% respectively and sensitivity and specificity of TPHA was 96% and 100% respectively as compared to ELISA.

Summary/Conclusions: As is apparent from results of present study, higher incidence of transfusion transmitted syphilis have been observed among replacement donors compared to voluntary donors, It can be concluded that given the high sensitivity and specificity of ELISA, it can be considered as a suitable screening test as ELISAs are ideally suitable for the blood centre where in large number of samples are being processed with an added advantage of an objective reading in the form of printout.

P233 | The role of information technology in blood safety, survey of 18 years TTV frequency in blood donors at Fars province, Iran

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Background: Blood transfusion safety is the essential necessary in blood transfusion. Transfusion Transmitted Virus threatened the blood safety. Information technology systems facilitate the workflow and save the time and budget. When Transfusion Transmitted Infection screening tests results and information technology attached and combined together can be helpful for us to transfused safe blood.

Aims: The effectiveness of 18 years of comprehensive information technology implementation and its role on blood safety was assessed.

Methods: This retrospective cross sectional study included 2014981 blood donors that admitted to Fars Blood Transfusion Service during from 16 01 2005 to 22 09 2022, 6459 days. A total of 2622729 donations were tested by HBS Ag, HCV Ab, HIV Ag-Ab. Initial reactive samples were repeated duplicate. Compatible repeat reactive samples confirmed by confirmatory assays. Data were analysed by SPSS.

Results: Donation frequency was 1.3 donation/donor. Total HBS Ag, HCV Ab, HIV Ag_ Ab initial reactive test: 0.81% (21308/2622729) and repeatedly reactive test: 0.63% (16644/2622729). Confirmed donation test: 0.2% (5328/2622729) and confirmed donor test: 0.26% (5249/2014981). Total positive donor test frequency 0.2% during 18 years. The incidence of positive rate in 2005 was highest (0.35%) and the lowest range was (0.06%) in 2022. Significand decreasing trend showed at first year past of used IT at 2006.

Summary/Conclusions: Our findings showed the IT have essential guaranteed role an improve blood safety. Preparation of a comprehensive database will eliminate donation of people who had positive history of TTV, which will greatly helpful to public health. Offline donation method causes increased risk of reentry of positive recognised donors. National online communication and unique database between blood transfusion centers recommended.

P234 | Abstract withdrawn

P235 | Improved efficiency using sequential screening assays for Treponema pallidum (syphilis) in blood donations

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Background: Australian Red Cross Lifeblood performs serological testing for *Treponema pallidum* for blood donations used to manufacture transfusible components. Prior to December 2020, syphilis screening was performed using an automated microhaemagglutination assay (TPHA). Reactive samples were further tested using a rapid plasma reagin (RPR) assay and also referred to an external reference laboratory for confirmatory testing (*Treponema pallidum* particle agglutination assay – TPPA, venereal disease research laboratory test – VDRL and fluorescent treponemal antibody absorption test – FTA-Abs), with all results used to determine the donor's syphilis status. Donors who were RPR non-reactive and negative for all confirmatory tests were assigned as biological false reactives (BFR), with transfusible components discarded and plasma accepted for fractionation. While Lifeblood has successfully used a second immunoassay (IA2) to clarify the antibody status of donors giving repeatedly reactive results on primary screening immunoassays for human immunodeficiency virus (HIV), hepatitis C virus (HCV) and human T-lymphotropic virus (HTLV) for over two decades (Seed, Transfusion, 2003), this approach had not been used for syphilis screening. In December 2018, the Abbott ARCHITECT Syphilis TP CMIA assay was implemented as the sequential second-line assay (IA2) to the TPHA.

Aims: To analyse the impact of implementing sequential screening assays for syphilis by reviewing the screening and confirmatory testing data, in the six months following implementation of the IA2.

Methods: Syphilis testing data from donations collected from 1 January to 30 June 2019 was analysed. The IA2 results and any confirmatory testing, with the final syphilis status, were reviewed.

Results: 641 samples were reactive for TPHA and subsequently 585 were non-reactive on the IA2 (91.3%) and categorised as BFR. While the transfusible components from these donations were discarded, the plasma was accepted for fractionation as *Treponema pallidum* testing is not a mandatory test. The plasma component from each whole blood donation is approximately 250 mL which resulted in 145 kg of plasma being issued to the plasma fractionator in this six-month period. With the baseline screening strategy, this plasma would have been discarded.

The 56 samples which were TPHA reactive and IA2 reactive had confirmatory testing completed. Following donor counselling, 37 (5.8%) were found to represent past treated cases, 14 (2.2%) reported no known history of syphilis and five (0.8%) were deferred for a suspected current infection. Donors from the latter two categories were referred to a medical practitioner for follow-up.

Summary/Conclusions: The application of the sequential screening assay method to resolve nonspecific reactivity from the primary screening assay was successfully applied to donor syphilis screening in Australian donations. Introducing a highly sensitive and highly specific IA2 enabled the prompt determination of screen reactive BFRs, significantly reduced the number of samples being referred for external confirmatory testing and increased the yield of plasma available for fractionation.

P236 | Abstract withdrawn

P237 | Detection of bacterial contamination in platelet components using 16S ribosomal RNA real-time PCR

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Background: Platelets are small, enucleated cells that derive from megakaryocytes in the bone marrow. Upon activation at sites of vascular injury, they undergo changes and play a critical role in repairing the vasculature and defending against microbial infections, thereby contributing significantly to haemostasis. Currently, platelet concentrates can be stored at room temperature for up to five days from the collection date. However, because of this storage condition, platelets are more prone to contamination than other blood components stored in refrigerators. As a result, many countries have implemented various screening systems, such as visual observation and blood culture, to detect bacterial contamination in platelet components.

Aims: The aim of this study was to develop and evaluate a screening test for bacterial contamination in platelet components using 16S ribosomal RNA real-time PCR.

Methods: Twenty kinds of bacteria were selected for the development and evaluation of primers for 16S ribosomal real-time PCR using Fast Plus Evagreen Master Mix (Biotium, Hayward, CA, USA). These bacteria were distributed from the regional branch of the National culture collection of pathogens in Korea. We conducted a prospective study on the accuracy of real-time PCR using in-house-designed primer sets targeting 16S ribosomal RNA sequence. DNA extracted from each colony of 20 kinds of bacteria was measured with a spectrophotometer (Nanodrop 2000®, Wilmington, USA) and serially diluted to check the limit of detection. We spiked 20 kinds of bacteria at a concentration of 50 CFU/ml into human platelets stored in blood transfusion bags, incubated for 48 hours at room temperature, and then extracted nucleic acids and performed PCR.

Results: Two primer sets showed distinct positive peaks from all kinds of bacterial DNA and no significant peak from negative control such as distilled water during real-time PCR. The limit of detection measured was different between species (0.006 ~ 0.7 ng/uL of bacterial DNA; 10^2 ~ 10^4 CFU/mL, if a bacterial cell contains approximately 20fg DNA). And also distinct positive results were detected in all platelet components spiked with 20 kinds of bacteria, incubated 48 hrs at room temperature. But, no positive results were observed in negative control such as platelet components without any bacteria.

Summary/Conclusions: We recommend 16S ribosomal real-time PCR as a good tool for screening bacterial contamination in platelet components. We can screen bacteria growing in platelets with our primer sets successfully because most bacteria contaminated could be reached over 10^5 CFU/mL after 48 hours at room temperature.

P238 | Blood donor screening for hepatitis E virus in the Kazakhstan

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Background: About 20 million cases of hepatitis E virus (HEV) infection are recorded annually, 3.3 million of these patients registered with clinical manifestations (WHO, 2020).

HEV causes serious morbidity and mortality worldwide, especially in developing countries, where it is endemic among both animals and humans. At the same time, the number of reported cases of HEV transmitted by blood transfusion in patients in need of donated blood is steadily increasing. Due to the trend towards an increase in the serological prevalence of infection among the population and the serious clinical consequences that HEV can cause, the need for screening is becoming increasingly important as an important public health problem in the world.

Aims: To determine the prevalence of HEV among blood donors in Kazakhstan.

Methods: The study was conducted in November 2022 on the basis of the Scientific and Production Center of Transfusiology of the Ministry of Healthcare of the Republic of Kazakhstan (Republican Reference Laboratory of the Blood Service). Blood samples of donors were examined for HEV RNA by PCR on the Cobas 6800 automatic analyser (Roche Diagnostics Ltd.) by combining 6 samples.

Inclusion criteria: donor blood samples that have passed laboratory testing for all laboratory parameters and are ready for delivery to medical organizations.

Exclusion criteria. Samples of donated blood with laboratory deviations from the references and unsuitable for use in medical organizations.

Results: Blood samples of 16147 (6.8% of the number of donations per year) blood donors were examined. None of the donors from the blood service institutions of the South, North, West and East regions of the Kazakhstan were found to have hepatitis E RNA.

Summary/Conclusions: For the first time in Kazakhstan, screening of blood donors for HEV was conducted among healthy individuals. The results obtained show the lack of relevance of the problem of high blood pressure among blood donors in Kazakhstan at this time.

P239 | Diagnostic performance of the serology screening of blood donors in Northern Greece

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Background: The use of screening tests in blood donor population, a population with a low prevalence of infectious markers, is a complex process. All assays should have a high level both of sensitivity and specificity. Increased sensitivity is of outmost importance to detect and remove potentially infectious blood products from the blood supply and eliminate false negative results. At the same time, specificity is also important in reducing the number of false positive results.

Aims: The aim of our study was to analyse the seroprevalence of transfusion-transmissible infectious agents of voluntary blood donors within the period 2018–2022 and verify the accuracy of screening tests followed by confirmatory assays.

Methods: The study conducted at the Serology Laboratory of AHEPA Blood Center, which is responsible for the serology screening of blood donations collected from 36 blood services, located in northern and central Greece. Blood samples were tested on the LIASON (DIASORIN) platform for syphilis (CLIA technique) and on the PRISM (2018-2019) / Alinity s (2020-2022) platforms for HBsAg, anti-HCV, anti-HTLV I/II and HIV Ag/Ab, using the respective ChLIA and CMIA assays (Abbott Diagnostics). The screening strategy included repeated testing of reactive samples, followed by confirmatory testing. The applied confirmatory tests were the fluorescent (FTA) and haemoagglutination (TPHA) assays for syphilis, the HBsAg qualitative neutralization assay for HBsAg, the immunoblot assay (INNO-LIA) for HCV and HTLV, and the Western Blot assay for HIV.

Results: Results of screening and confirmatory testing for HBsAg, HCV, HIV, HTLV and Syphilis. (RR: repeatedly reactive, TP: true-positive, FP: false-positive, SP: Specificity (0%), PPV: positive predictive value).

HBV: RR:576, TP:*448 (0,05%)*, FP: *128*, SP: *99.98%*, PPV: *77,77%*
HCV: RR:858, TP:*134 (0,01%)*, FP: *724*, SP: *99.92%*, PPV: *15,62%*
HIV: RR:507, TP:*40 (0,004%)*, FP: *467*, SP: *99.95%*, PPV: *7,89%*
HTLV: RR:*298*, TP:*7 (0,0008%)*, FP: *291*, SP: *99.97%*, PPV: *2,35%*
Syphilis: RR:*1879*, TP:*397 (0,04%)*, FP: *1482*, SP: *99.83%*, PPV: *21,13%*

Summary/Conclusions: Our results confirmed the low prevalence of transfusion transmitted infections in Greek blood donors. As a consequence, the PPV of the screening assays was poor and a relevant number of uninfected donations were excluded. Remarkable exception was the high PPV of the HBsAg screening assay, indicating a

possible intermediate prevalence of the disease among blood donors. Specificity for all assays was within our expectations and definitely equal or better than the value demonstrated on the reagent inserts. The use of the confirmatory algorithm offered valuable help in the management of blood donors.

P240 | Abstract withdrawn

P241 | Evaluation of NAT screening and anti-core testing of blood donors in a Greek blood center: Its contribution to blood safety

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Background: NAT screening of blood donations contributes to transfusion safety, by narrowing the window period and detecting occult hepatitis B. Additionally, anti-HBcore screening contributes also to blood safety, since it reveals donors with occult hepatitis B.
AHEPA Molecular Center of blood donors was the first established in Greece in June 2006. The last few years is screening blood donations from the 37 Blood Banks of Macedonia, Thrace, Thessaly and Epirus.
Molecular screening for HBV, HCV, HIV, is obligatory in Greece but anti-core testing is applied only in specific cases.
Aims: To show the variability of NAT HBV-DNA reactivity in cases of occult hepatitis B and determine whether the anti-core screening provides additional blood safety
Methods: Individual blood donation screening was performed using the multiplex agent (MPX- HIV, HCV, HBV) in Roche Cobas 8800 system.
All donations were also tested using screening serological methods for HBV, HCV, HIV(I,II), HTLV and syphilis.
In case, of HBV-DNA reactive result, the algorithm includes retesting the initial sample, testing a plasma sample (from the blood unit) and additional testing the all the HBV serological markers (anti-HBcore, anti-HBs, HBeAg anti-HBe). Furthermore, a follow up donor sample is requested, to confirm reactivity.
Samples that are HBV-DNA reactive, HBsAg negative and anti-HBcore positive, are characterised as "occult hepatitis B"
We recorded all HBV-DNA reactive samples, since January 2021 to December 2022.
Results: We examined 373212 samples and confirmed 214 HBV-DNA reactive.
119/214 (55,6%), were reactive only in the initial testing and were also anti-HBcore positive.
91/214 (42.5%) showed reactivity in the initial testing and also in the second testing of the same sample
73/214 (34.11%) showed reactivity in all examinations: initial testing, retesting and testing of sample of blood unit.
We examined 42/214 (19.63%) follow up samples and 13/42 (30.9%) were HBV-DNA reactive.
We also found 20 (0.005%) samples initial reactive (without repeatedly reactivity and all HBV serological markers negative)
Only 9/214 (4.2%) were anti-HBcore negative.
Summary/Conclusions: Molecular blood testing for HIV, HCV, HBV in our Center, increased the safety of transfusions. It prevented transfusion of 214 infectious blood units (donors with OBI) which would have been administered, if we relied only on serological testing. 55.6% of these units were positive only in the initial sample and 95.8% were also anti-core positive.
We found also that only 42% gave a positive result on the retest of initial sample and only 34% gave a positive result on the blood unit. Blood donors were retested in a very small percentage of only 19.6% and 30.9% of them were positive due to very low viral load
This represents a small gap in transfusion safety, that may be filled by use of even more sensitive NAT reagents and/or either systematic anti-HBcore screening of all blood donations or selective screening of first-time donors or donors from regions with high HBV prevalence.

P242 | Abstract withdrawn

P243 | Screening for antibodies against treponema pallidum with enhanced chemiluminescent immunoassay as a replacement for already rapid plasma regain test

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Background: Syphilis is a transfusion transmissible infections and it is mandatory to do serological test for syphilis on all donor blood samples. It is usually based on detection of antibodies against the cardiolipin-lecithin antigen or against the Treponema-specific antigen. Syphilis testing with good sensitivity and specificity helps enhance blood safety and consolidation along with other transfusion transmittable infections such as human immunodeficiency virus, hepatitis-C virus, and hepatitis-B virus helps in reducing the errors and enhances efficiency. Recent availability of a chemiluminescent microparticle immunoassay for detecting antibodies against Treponema pallidum has led to adopt chemiluminescent based immunoassay for screening of syphilis
Aims: This study was designed to evaluate evaluated the utility of chemiluminescent immunoassay for routine screening of syphilis. We assessed the performance of VITROS® syphilis Treponema pallidum agglutination (TPA) assay based on enhanced chemiluminescence principle for its use as a replacement for already existing rapid plasma regain (RPR) testing method on donor blood samples at a newly operation tertiary care health center.
Methods: Antibodies against Treponema pallidum were screened in 1000 serum samples using enhance chemiluminescent immunoassay (VITROS® syphilis TPA assay) . Enhanced chemiluminescent immunoassay-positive samples were reflexively tested with rapid

plasma reagin tests and Treponema pallidum particle agglutination assays. Dot-immunoblot assays were used to confirm results of chemiluminescent microparticle immunoassay-positive and Treponema pallidum particle agglutination-negative serum samples. The ethical permission was taken from hospital ethical board.

Results: Overall, 13 samples (1.3%) were enhanced chemiluminescent immunoassay-positive, and 11 (84.6%) of those chemiluminescent microparticle immunoassay-positive serum samples were also Treponema pallidum particle agglutination-positive. Samples signal to cut-off ratio for enhanced chemiluminescent immunoassay correlated with diagnostic reliability, as hihger samples signal to cut-off ratio corresponded with increased concordance between chemiluminescent immunoassay and Treponema pallidum particle agglutination results. Dot-immunoblot testing of immunoassay-positive and Treponema pallidum particle agglutination-negative serum samples showed that 3 samples (23.2%) were Dot-immunoblot-positive, 4 (30.7%) were indeterminate and 6 (46.1%) were negative.

Summary/Conclusions: Performance of the VITROS® syphilis TPA assay meets the requirements for its use as syphilis testing method in our blood centre thus allowing consolidation with other transfusion transmittable infections screening assay on chemiluminescence platform. While in screening populations discrepancies between chemiluminescent microparticle immunoassay and Treponema pallidum particle agglutination results are quite prevalent further analysis by Treponema pallidum particle agglutination is recommended to confirm diagnostic results

P244 | Target-plexing“ in PCR design increases safety in blood donation screening

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Background: In 2009 the first transmission of HIV-1 by a cellular blood product after mandatory NAT screening in Germany was reported. Between 2007-2010 in total 17 HIV transmission cases were reported and submitted to root cause analyses. The analyses showed that low viral loads and mismatches in the primer/probe region led to the detection failures of the NAT tests. As a reaction to the transmission cases the Paul Ehrlich Institute mandated the use of dual target HIV-1 NAT tests for donor screening in Germany. As a result, the next generation of HIV NAT tests cover at least two genome regions.

Aims: We investigated whether ‘Target-Plexing’ may also be used to increase the sensitivity of an assay and not only compensate for target mutations.

Therefore, a new PCR assay to amplify and detect three genomic regions (‘amplicons’) of the DNA of CMV was designed. The probes of the multiple amplicons use the same fluorescent dye. Parallel amplification of the individual amplicons shall lead to increased net fluorescence signals compared to single-plex PCRs and lower Ct values in real-time PCRs. Combined these two effects can translate into a more sensitive test.

To support these hypotheses, the new triplex CMV assay was tested in comparison with single-plex and duplex PCRs. The new assay was then subjected to a system comparison test with two CE-marked IVD test systems for CMV DNA.

Methods: After multiple sequence alignments, a number of primers and probes were designed with the aid of bioinformatics software. The best performing triplex assay was developed to the standards of a commercial IVD product. Precursor single-plex and duplex assays were used as comparators.

In its validation phase the triplex assay was submitted to a system comparison test with two different CE-marked CMV tests. The new triplex CMV assay (‘PoET CMV’) was run on GFE’s PoET Instrument and the two other tests on the respective accompanying automated systems. The performance comparison was conducted with a panel of 96 samples incl. 86 potentially CMV reactive and 10 CMV negative samples. The samples were split into three aliquots, one for each system. The sample panel consisted of ring trial material, seroconversion panels and the CMV WHO IS. The panel encompassed low CMV concentration samples and serial dilutions.

Results: In the direct comparison of the single-plex, duplex and triplex assays, the triplex assay displayed the highest fluorescent signals and lowest Ct values. The single-plex assay displayed the lowest fluorescent signals and highest Ct values, with the duplex assay lying between the other two.

In the system comparison with the panel of 86 potentially CMV reactive samples, System 1 yielded 23 reactive samples (27 %), System 2 13 reactive samples (15 %) and *PoET CMV* 41 reactive samples (48 %). 35 samples were reactive with all three systems. Of those, no samples were exclusively reactive with System 1, 1 sample was exclusively reactive with System 2 and 16 samples were exclusively reactive with PoET CMV.

Summary/Conclusions: Assays with Target-Plexing are more complex than single-plex assays and therefore need to be designed meticulously and are costlier to manufacture.

However, Target-Plexing has different advantages. It compensates for mutations in the genomes of pathogens and also increases the sensitivity of an assay. In blood screening, the sensitivity of an assay if often of particular interest. Highly sensitive assays provide an extra margin of safety in the process of preventing transfusion transmitted diseases.

Transfusion transmitted infections

Hepatitis B (HBV)

P245 | Abstract withdrawn

P246 | A review of anti-HBcore screening at the Irish blood transfusion service

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Background: The Irish Blood Transfusion Service (IBTS) introduced universal blood donor screening for antibodies to Hepatitis B Core antigen (anti-HBc) in January 2002. In 2009, Individual Donation Nucleic Acid Testing (ID-NAT) for HBV DNA was introduced. Currently, all blood donations are screened for anti-HBcore (Abbott Alinity s anti-HBcore), HBV surface antigen (Abbott Alinity s HBsAg) and ID-NAT for HBV DNA (Grifols Diagnostic Solutions Ultrio Elite). Anti-HBc Repeat Reactive (RR) samples are referred to the National Virus Reference Laboratory (NVRL) for HBV confirmatory testing. Ireland is a low prevalence area for HBV infection with an estimated prevalence of less than 0.5%. In 2006, the IBTS introduced a "sample only" collection policy for all non-Irish/non-UK first time donors. These sample only new donors (SONDs) make up 1%–2% of total donors.

Aims: To describe the rate of anti-HBc repeat reactivity in Irish blood donors and to review the confirmatory results of repeat reactive donors.

Methods: All anti-HBc donor results between January 2006 and December 2022 were reviewed to determine the RR rate and the rate of confirmed positivity. RR donors were classified according to their confirmatory testing results; 1-Resolved HBV infection, 2-False positive, 3-Chronic HBV infection (HBsAg positive), 4-Occult HBV infection and 5-Inconclusive.

Results: 2,549,810 samples were screened for anti-HBc between January 2006 and December 2022. 2228 repeat reactive samples were recorded, giving a repeat reactive rate of 0.09%. Of these repeat reactive samples, 38.9% were classified as resolved HBV infection (866/2228), 54.0% were classified as false positive (1203/2228), 2.2% were classified as chronic HBV infection (HBsAg positive) (49/2228) and 4.8% as inconclusive (106/2228). Four donors were classified as occult HBV infection (0.03%). A significantly higher rate of confirmed positive anti-HBc reactivity was observed in SONDs compared to Irish/UK first time donors and repeat donors; 91.4% of repeat reactive SONDs were confirmed positive (resolved, chronic or occult HBV infection) compared to 17.9% of repeat reactive donor samples. Donors with confirmed false positive anti-HBc results are eligible for reinstatement to the active donor panel three months after their reactive donation, if non-specific reactivity persists, they are reinstated a year after the index donation. If the donor has further false positive results after a year, they are permanently deferred. Donors with confirmed past, chronic or occult HBV infection or inconclusive confirmatory results are permanently deferred from donating.

Summary/Conclusions: The combined strategy of the IBTS HBV testing algorithm and the "sample only" collection policy provides the highest safety standards for the Irish patient population. The inclusion of anti-HBc screening in combination with ID-NAT has allowed the identification of four occult HBV infections. Blood donor screening results are consistent with national surveillance data, indicating a low HBV incidence in Ireland, with the majority of HBV infections in Ireland being chronic cases, most of whom migrated to Ireland from HBV endemic countries.

P247 | Investigation of the HBV infection status between vaccinated and unvaccinated blood donors

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Background: Since the neonatal hepatitis B vaccination program has been fully implemented in 1986 in Taiwan, it has achieved remarkable progress in prevention and control of hepatitis B. The positive rate of HBsAg in children has dropped from 10% in 1980 to <1-3% in 1990, and for adolescents it declined from 20-60% in 1990 to <1-6% by 2000. However; 0.01% of people infected with hepatitis B in Taiwan still died of chronic liver disease and cirrhosis in 2020. Hence, HBV-DNA positive blood donors were recruited, and various serological markers of HBV infection were detected to understand their HBV infection status.

Aims: The aim of this study was to compare the production of anti-HBs levels and the time from positive HBV DNA levels to undetectable levels after HBV infection between HBV-vaccinated and HBV-unvaccinated blood donors.

Methods: This study was approved by the Institutional Review Board of Taiwan Blood Services Foundation (TBSF). From 2013 to 2020, blood donors of Taoyuan, Hsinchu, and Miaoli regions in northern Taiwan, who tested positive for HBV-DNA by a nucleic acid amplification test (NAAT), were invited to participate in the study. They were divided into two groups according to whether they have received hepatitis B vaccines. Group 1 (G1) was the unvaccinated group, and Group 2 (G2), the vaccinated group, was the population born after July 1986. With the consent of the blood donors, their serum samples stored before, on the day of HBV positive conversion, and at least 6 months after positive conversion were collected to detect HBV markers such as HBV-DNA, HBsAg, anti-HBc, and anti-HBs, etc.

Results: A total of 99 blood donors participated in the study, including 91 subjects in the G1 group (mean age = 53.5 ± 7.4) and 8 subjects

(mean age = 25.8 ± 3.3) in the G2 group. The results and statistical analyses are as follows: (1) Before HBV-DNA positive conversion, the positive rates of anti-HBs in G1 and G2 groups were 39.6 % (36/91) *vs* 12.5% (1/8), $p = 0.129$; and the positive rates of anti-HBc were 85.7% (78/91) *vs* 25.0% (2/8), $p < 0.01$; (2) After HBV-DNA positive conversion, the anti-HBs positive rate increased to 58.2% (53/91) in G1 group, and 87.5% (7/8) in G2 group, $p = 0.142$. In addition, the anti-HBs levels in G1 and G2 groups were 163.1 ± 279 IU/L and 285 ± 210.6 IU/L ($p = 0.011$), and 78.0% (71/91) subjects in G1 group, and 100% (8/8) subjects in G2 became negative for HBV-DNA ($p = 0.353$); (3) The time interval for HBV DNA to become negative: G1 group was 45.9 ± 24.4 months, while G2 group was about 14.2 ± 15.8 months ($p < 0.01$).

Summary/Conclusions: All subjects in the vaccinated group had undetectable HBV-DNA (8/8, 100%) for an average of about 1.2 years after HBV infection, and the anti-HBs level was significantly higher than that of the unvaccinated group. In contrast, the HBV-DNA negative conversion rate in the unvaccinated group was 78% (71/91), and the negative conversion time was nearly 3.9 years. These data suggest that even though subjects in the vaccinated group were infected with HBV, their serum anti-HBs levels were higher than those in the unvaccinated group, and the antibody production was faster. Therefore, the HBV-DNA negative conversion time of the vaccinated group was shorter than that of the unvaccinated group.

P248 | OBI population analysis and residual risk assessment of blood donors in Hangzhou region

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Background: Occult hepatitis B virus infection (OBI) is a potential threat to blood safty. In previous studies, we found a certain number of OBI individuals in blood donors, but lacking large-scale data and the characteristics of OBI individuals in local blood donors. Besides, the effectivity on reducing the rate of OBI infection as the neonatal HBV immunization implemented in China from 1998 has not been well addressed.

Aims: To obtain the incidence of OBI among blood donors in Hangzhou region, and observe the difference of OBI ratio between immunised and non-immunised blood donors, so as to estimate the residual risk of OBI on blood safety.

Methods: Screening of OBI individuals from about 200 thousand blood donors between January 2018 to December 2019 using ELISA and NAT, supplementary tests were performed on Anti-HBs, HBeAg, Anti-HBe and Anti-HBc by chemiluminescent immunoassay (CLIA). The characteristics of OBI population on age, gender were analysed by GraphPad Prism 5. The questionnaire on hepatitis B vaccination was designed to investigate the gender, age, whether the hepatitis B vaccine was injected, whether the family members had ever infected by hepatitis B, the outcome of the hepatitis B vaccination, and the frequency of blood donation. The residual risk of OBI blood transfusion is calculated according to the positive conversion model of blood donation interval of repeated blood donors proposed by Busch MP.

Results: A total of 370,465 blood donors were detected by ELISA and NAT, and 269 samples were figure out which were HBV DNA reactive and HBsAg ELISA non-reactive, with a detection rate of 7.26/10,000 people (269 / 370,465). In the 269 screened OBI individuals, 207 of them were detected by CLIA for grouping the serum antibody pattern and found 6 of them were HBsAg reactive. Therefore, 201 cases were included in OBI. According to the pattern of serum antibody, anti-HBC and / or anti-HBS positive possessed 47.76% (96 / 201) while anti-HBC and / or anti HBS negative was 52.24% (105 / 201). The 201 OBI individuals including 148 males and 59 females, 82 of them were first-time blood donors (40.80%). Aged 18-25 years group accounted for 3 cases (1.49%), as the proportion of 45-55 years group was the highest and accounting for 93 cases (46.27%). A total of 1,196 questionnaires were completed by 671 males and 525 females, 604of them were first-time blood donors (50.50%), 885 were 18-25 years old (74%), and 597 (49.92%) had been vaccinated against hepatitis B. Only 35 people had family members been infected by hepatitis B virus. Referring to the seroconversion samples and window period formula reported in the literature, the residual risk of HBV transfusion under the serological detection mode was 412.41×10^{-6}.

Summary/Conclusions: In conclusion, the detection rate of OBI in blood donors is about 7.26/10,000, the proportion of the two serum antibody patterns is similar, and the residual risk of HBV is 412.41×10^{-6}. A large sample survey found that the proportion of blood donors aged 18-25 in the detection of OBI was the lowest. Therefore, the hepatitis B vaccination policy implemented in 1998 was instrumental reason of the HBsAg detection rate decreasing in blood donors. However, it should be noted that there is still a low HBsAg infection rate in the 18-24 year group, suggesting that the immunization effect on some personnel was not obvious.

P249 | Abstract withdrawn

P250 | Detection of Hepatitis B infection after the implementation of next generation HBsAg immunoassay for blood donor screening in national blood centre, Thai Red Cross Society

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Background: Hepatitis B related transfusion-transmitted (TT-HBV) risk have been reduced drastically with the improvements in donor selection criteria and blood screening practices. Implementation of nucleic acid testing (NAT) has also reduced window period

(WP) and to certain extent occult HBV infection (OBI) related residual risk . However, infectivity of low viremic HBsAg carriers remains unknown. "*Ekiaby, J Viral Hepatitis,2022*" recently reported the TT-HBV risk of an ID-NAT nonreactive HBsAg positive donation among Egyptian donors to be around 9.3% for RBC (20 ml plasma) and 45.7% for FFP (200 ml plasma) transfusions by modelling estimates and thereby, concluded that blood transfusion from ID-NAT screened HBsAg positive donors is not safe. In our continuous efforts to improve blood safety in Thailand, we had implemented new next generation HBsAg immunoassay, named HBsAg Next Qualitative with higher analytical sensitivity for the routine blood screening at National Blood centre (NBC) and 12 regional blood centres (RBC) since Jan 2022.

Aims: To estimate the prevalence of hepatitis B among blood donors in Thai Red Cross Blood centres after the implementation of Alinity i HBsAg Next Qualitative immunoassay.

Methods: A retrospective study conducted between January 2022 to December 2022 in NBC and 12 RBCs of Thai Red Cross Society and a total of 2,317,579 whole blood donors were analysed. All the collected blood units were initially screened by Alinity i HBsAg Next immunoassay (Abbott Lab, IL) and repeat reactive (RR) were confirmed by HBsAg Next confirmatory assay.

Results: Out of 2,317,579 whole blood donors, 8,693 (0.38 %) were HBsAg Next RR. 1,172 (0.05%) HBsAg RR were concordant with HBV ID-NAT. Higher HBsAg reactive rates were observed among men (72%), first time donors (70%) and among donations performed in a hospital blood banks vs. NBC (0.41% vs 0.1%). There was no significant change observed for HBsAg reactive rates when compared by month at NBC. We also observed 7,159 (0.31%) HBV ID-NAT RR only and unlike HBsAg, this was higher in repeat donors (72%).

Summary/Conclusions: Our findings show substantial HBsAg positive NAT negative yield which could possibly be low viremic HBsAg carriers. This reinforce the fact that use of highly sensitive HBsAg assays besides HBV NAT remains important specially for HBV intermediate to high endemic countries where universal anti-HBc donor screening could not be implemented.

P251 | The beginning of screening of blood donors and blood components for the presence of antibodies to the nuclear antigen of Hepatitis B virus (a-HBcore): The first results

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Background: All blood donors are necessarily tested for the presence of HBsAg in the Kazakhstan. The development of chronic hepatitis B – occult (latent), or HBsAg-negative hepatitis B, has been of interest and the greatest difficulties from the point of view of clinical laboratory diagnostics. A consensus group of experts in 2008 defined occult hepatitis B as a stage of chronic hepatitis B, in which HBV DNA is detected in liver tissue at an undetectable level of HBsAg in blood serum, regardless of whether or not the DNA of hepatitis B virus (HBV) is detected in peripheral blood.

A pilot project to test donors for the presence of antibodies to the nuclear antigen of hepatitis B virus (a-HBcore), showed a high 17.6% occurrence of this marker in donors of our center. Having studied the WHO recommendations and the experience of other countries that screen donors for HBV antibodies, in order to improve the safety of donor components, we proposed to include HBV markers (a-HBcore, a-HBs) in the screening standard of blood donors of the Kazakhstan according to the WHO algorithm (WHO, 2010).

Aims: To determine the prevalence of markers a-HBcore, a-HBs in different groups of donors, to evaluate the dynamics of component write-off based on the results of 4 months after the start of a new screening.

Methods: The study was conducted from October 2022 to January 2023 in the Scientific and Production Center of Transfusiology in Kazakhstan. The samples taken from blood donors were tested for a-HBcore, a-HBs, by immunochemiluminescence analysis on the Architect i2000SR, Alinity i. Statistical analysis was conducted by the Microsoft Excel. Qualitative variables were summarised as absolute frequency and percentages. The normality of the distribution was tested using the Kolmogorov-Smirnov test. Statistically significant results were considered values below $p < 0.05$.

Results: 14951 donations were tested in 4 months from the beginning of screening. The number of donations that were subject to write-off according to the new algorithm was 662 (4.4%). By donor categories, the results were as follows: in primary donors, the frequency of markers (a-HBcore, a-HBs less than 100 mMU/ml) was 3.6%, in repeat donors - 4.8%, in regular donors - 4.0%.

It was observed that the total number of rejected donations decreases (Table), the chi-squared is 7.69 ($p < 0.05$). The monthly dynamics of the share of donors with unacceptable values for a-HBcore and a-HBs markers showed a decrease in all donor categories.

Summary/Conclusions: The inclusion of new markers in the donor blood screening standard increases the safety of donor blood components. Markers (a-HBcore, a-HBs less than 100 IU/ml) are less common among primary donors, compared with regular and repeated ones. By the way, it should be noted that the observation, even for a short period, shows a decrease in the proportion of rejection by new markers.

The introduction of new markers always leads to an increase in the number of write-offs of blood components unsuitable for transfusion, which leads to financial losses. In addition, the withdrawal of donors, including regular ones, creates a burden on the donor department and requires an increase in the cost of attracting new donor personnel to replenish the base.

P251 – Table 1

Period	Category of donors	The number of donors with unacceptable indicators for markers a-HBcore and a-HBs	The number of donors with acceptable results for markers a-HBcore a-HBs	The proportion of donors with unacceptable indicators per 1000 donations
October/ November 2022	primary	29/27	612/832	45/31
	repeat	128/130	2141/2524	56/49
	regular	26/27	385/367	63/52
December, 2022/January,2023	primary	30/32	695/816	41/38
	repeat	97/128	2290/2564	41/48
	regular	14/1	353/369	38/3

P252 | Abstract withdrawn

Transfusion transmitted infections

Hepatitis C (HCV)

P253 | Comparison of performance of two assays for screening Hepatitis C virus in Hong Kong blood donors

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Background: The prevalence of hepatitis C virus (HCV) infection among new voluntary blood donors ranged from 0.051% to 0.130% over the past 10 years. Most HCV infected blood donors are asymptomatic and unaware of their infection. As such, quality-assured HCV screening test is important to prevent transfusion transmission of HCV infection. The Hong Kong Red Cross Blood Transfusion Service has been screening every blood donation for antibodies to hepatitis C virus serologically using Abbott Alinity s Anti-HCV assay (Abbott, Abbott Park, IL) since August 2019. In August 2021, the screening assay transitioned to an improved version (Abbott Alinity s Anti-HCV II assay). All repeatedly reactive samples are subject to confirmatory tests by blotting assay.

Aims: To compare the performance of Abbott Alinity s Anti-HCV and Anti-HCV II assays in terms of specificity, rates of initial reactive (IR), repeatedly reactive (RR) and confirmed positive (CP) results.

Methods: A total of 274,025 donations collected from 1 May 2020 to 25 August 2021 and 279,516 donations from 26 August 2021 to 31 December 2022 were screened for anti-HCV using Alinity s Anti-HCV assay and Anti-HCV II assay respectively. The derived specificities of the Alinity s Anti-HCV II assay was compared with the Abbott product requirements document (PRD) claim. Rates of initial reactive (IR), repeatedly reactive (RR), confirmed positive (CP) of whole blood and apheresis donations were compared statistically by two sample proportion Z-test. A p-value <0.05 was regarded as statistically significant.

P253 - Table 1: Results of IR, RR and CP rates of Alinity s Anti-HCV and Anti-HCV II assay

Alinity s Assay	Study Period	IR rate N (%)	RR rate N (%)	CP rate N (%)
Anti-HCV	1 May 20 - 25 Aug 21	160 (0.058%)	152 (0.056%)	31 (0.0113%)
Anti-HCV II	26 Aug 21 - 31 Dec 22	189 (0.068%)	184 (0.066%)	23 (0.0082%)
		p-value = 0.17	p-value = 0.12	p-value = 0.25

Results: The specificities of Alinity s Anti-HCV II assay was 99.94%, in consistent with Abbott PRD claim (>99.50%). The results of IR, RR and CP rates of Alinity s Anti-HCV and Anti-HCV II assays were listed in Table 1. During the study period, the rates of repeatedly reactive results for Alinity s Anti-HCV and Anti-HCV II assays were 0.056% (152/274,025) and 0.066% (184/279,516) respectively. The rates of initial reactive samples for both assays were also comparable between Anti-HCV (0.058%) and Anti-HCV II (0.068%). Also, the CP rates remained stable between Anti-HCV (0.0113%) and Anti-HCV II (0.0082%).

Summary/Conclusions: The IR and RR rates of the two assays were comparable and both assays exhibited high repeatability. The rate of CP was not statistically different while screened by Alinity s Anti-HCV and Anti-HCV II assay indicating stable prevalence of HCV infections in blood donors during the 2 study periods.

P254 | Risk factors of Hepatitis C infection in repeated donors vary geographically: A nested case-control study

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Background: Geographical differences of the prevalence of hepatitis C virus (HCV) infection were observed in Taiwan. Newly detected HCV RNAemia cases can be found through routine NAT screening in repeated blood donors, and risk factors during the interval of acute infection can be more reliably identified in these cases.

Aims: To identify the risk factors of new HCV infections among blood donors.

Methods: A nested case-control study performed on the donors who had at least two donations during 2013-2022. The donors who were newly detected HCV RNAemia were designated as case group, while those were HCV RNA undetectable during the same period were designated as control group. The controls were 1:3 frequency matched for age, sex, region of residence, and interval between donations. Risk factors of HCV infection, including demographic characteristics, a family history of hepatitis or dialysis, a history of sharing personal items (including toothbrush, razor, or multi-clippers), iatrogenic exposure, acupuncture, tattoo, body piercing, injury for needle stick or sharp vehicles with blood contact, incarcerate, drug use and sexual behavior were collected through face-to-face interview by a well-trained interviewer. Logistic regression was used to estimate odds ratios (OR) for each risk factor stratified by geographic regions (Northern-Central and Southern Taiwan).

Results: A total of 85 cases and 261 controls were enrolled in this study. Risk factors, including a history of sharing multi-clippers (especially manicure, pedicure, or acne care) and receiving high-risk dental treatments from unlicensed practitioners, significantly increased the risk of HCV new infection in donors living in both Northern-Central and Southern Taiwan. The adjusted OR (95% CI) of sharing multi-clippers was 5.1 (1.03-24.9) in Northern-Central Taiwan and 7.7 (1.40-42.4) in Southern Taiwan; while that of receiving high-risk dental treatments was 30.2 (2.23-410.8) and 40.9 (1.21-infinity), respectively. There were geographical differences in risk factors associated with donor HCV infection. In Northern-Central Taiwan, those donors who had lower educational level (*OR* = 3.6, 95%CI:1.22-10.8), who had received any dental treatment in certified dental clinics (*OR* = 3.8, 95%CI:1.35-10.9), and who were healthcare workers (*OR* = 41.3, 95%CI:3.89-438.2) had a significantly higher risk of HCV new infection. In Southern Taiwan, the donors whose family members had a history of hepatitis (*OR* = 3.0, 95%CI:1.05-8.34), sharing razor (*OR* = 4.9, 95%CI:1.14-20.7), and who had a puncture or incised wound exposed with blood contact (*OR* = 10.5, 95%CI:1.01-110.1), had a significantly higher risk.

Summary/Conclusions: This study found that receiving dental treatments from unlicensed practitioners and had a history of sharing multi-clippers are the important exposure for a new HCV infection in both Northern-Central and Southern. We also observed diverse risk factors between regions. This finding would be helpful in developing donor educational materials and donor history questionnaire.

P255 | Abstract withdrawn

Transfusion transmitted infections

HIV

P256 | Evolving deferral criteria for blood donation by men having sex with men from 2016 to 2022 in France: Impact on the HIV risk

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Background: In France, men having sex with men (MSM) deferral for blood donation was reduced in July 2016 from permanent to 12 months since last sexual activity and to 4 months in April 2020. In parallel, quarantine plasma donation by MSM donors with the same deferral rules than for other donors (including no more than one sexual partner in the previous 4 months) was introduced in July 2016 up to March 2022, at which time MSM-specific deferral criteria were removed for all blood donations.

Aims: To assess i) the impact of the MSM deferral from 12 to 4 months on transfusion HIV risk indicators ii) the frequency of HIV-positive donations MSM plasma donors.

Methods: Data were retrieved from the national surveillance of blood donor (BD) population database including the number of donations, the number of BD by donor status (first time or repeat), the number of positives for HIV, HBV, HCV and Syphilis markers. The epidemiological surveillance indicators for 2.5-year periods before (Period 1: October 2017-March 2020) and after (Period 2: April 2020-September 2022) implementing the revised 4-month deferral were compared. We estimated for these two periods the HIV positive donation rate, the fraction of the HIV residual risk (RR) attributed to MSM and the RR for HIV. The RR was calculated using the Incidence Rate–Window Period method. MSM plasma donors were compared to the other plasma and blood donors during the period July 2016-March 2022.

Results: From October 2017 to September 2022, 79 donations were HIV-positive (49 in Period 1 and 30 in Period 2). The rate of HIV-positive donations was estimated at 0.07/10,000 donations in Period 1 vs 0.04 in Period 2, the fraction of the HIV RR attributed to MSM was stable over the 2 periods (56% of men vs 57%), and the HIV RR was reduced from 1/7,800,000 donations in Period 1 to 1/10,500,000 in Period 2. The last modification of MSM criteria in 2022 is too recent to be evaluated, but so far, no negative impact on the HIV-positive donations rate is observed. Between July 2016 and March 2022, a total of 2880 plasma donations were made by 994 MSM donors who would have been deferred otherwise. One donation was HIV-positive: 34.7/100,000 vs 0.6 donations by other donors, relative risk: 61.0 (95% CI:8.5-437.7).

Summary/Conclusions: As when the MSM deferral for blood donation evolved from permanent to 12 months in 2016, our findings showed that the decrease from 12 to 4 months in 2020 had no impact on HIV indicators in BD population. The RR related to HIV remained very low. Plasma donation by donors who would have been otherwise deferred for MSM activity was associated with increased rate of HIV. However, the small number of MSM plasma donors and uncertainty as to their representativeness limits the robustness of infectious epidemiology findings among MSM donors subjected to the same deferral rules than other blood donors. Nevertheless, these results highlight the need to carefully monitor the impact of removing MSM-specific deferral criteria.

P257 | Evaluation of HIV immunoassays for blood donor screening using nationwide real-world data

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Background: Understanding infectious disease assay performance in real-world environment provides valuable insights for both manufacturers and users. National regulations establish that all blood banks in Colombia must conduct supplemental tests on HIV repeat reactive samples. Screening and supplemental test results are sent to the Colombian National Institute of Health (C-NIH) via the National Haemovigilance System (SIHEVI), along with information on donors and donations. The C-NIH analyses the data and publishes open access annual reports. We derived metrics of performance for HIV immunoassays based on this report.

Aims: Estimate and compare specificity (or negative percent agreement) and false reactive rates for HIV screening assays in Colombia during 2021, using nationwide real-world data.

Methods: Confirmatory/supplemental algorithms are evaluated and adjusted periodically in the country. In 2021, blood centers that only used HIV immunoassays for blood screening had to re-test all repeat reactive samples with an alternate 4th generation assay that used a different method or was from a different manufacturer. A reactive result on this alternate assay was considered a possible HIV infection. Conversely, a negative result led to perform a molecular test for HIV-RNA. In blood centers that additionally used HIV-NAT for donor screening, all NAT reactive samples were considered possible infections. As not all repeat reactive samples were tested by a reference standard, we calculated the negative percent agreement (NPA) and the presumptive false reactive rate (FRR) for HIV at each blood center operating in the country, based on the applicable algorithm to determine condition status. To compare differences among assay providers, we calculated and grouped these metrics for 3 Abbott platforms (Alinity s, Alinity i and Architect) and for non-Abbott platforms (CLIA, ECLIA and ELISA based).

Results: In total 897,385 donations were tested for HIV Ag/Ab Combo by 83 blood centers in Colombia, using different platforms. Overall, the HIV NPA was 99.89% (range 99.44%-100%). Almost 64% of these donations were tested by Abbott platforms. Alinity s showed the lowest proportion of HIV false reactive results. On average, non-Abbott platforms had a FRR 3 times higher than Abbott platforms.

Summary/Conclusions: Although a direct assessment of the diagnostic accuracy of HIV screening tests was not feasible, indirect metrics derived from this large database showed relevant differences in assay performance among manufacturers in the country. Highly specific HIV assays are desirable not only to prevent blood product loss but also to mitigate expenses related to supplemental testing and the considerable donor anxiety when additional samples are required.

P257 – Table 1

Platform	Screened donations (%)	HIV Repeat reactive rate (%)	HIV NPA (%)	HIV FRR (%)
Alinity s	48,080 (5.36)	0.07	99.97	0.03
Alinity i	337,755 (37.64)	0.14	99.94	0.06
Architect	187,274 (20.87)	0.14	99.93	0.07
Non-Abbott platforms	324,276 (36.14)	0.33	99.79	0.21
TOTAL	897,385 (100)			

P258 | Abstract withdrawn

P259 | Using pathogen reduction and blood screening to mitigate HIV, HBV, and HCV transfusion-transmitted infections after changing sexual behavior deferrals

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Background: Pathogen reduction technology (PRT) can reduce the risk of transfusion-transmission associated with bacteria, parasites, and viruses including human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). The amotosalen and UVA based PRT is approved for plasma and platelets in Canada. Canada also recently changed its approach to time-based sexual deferrals for Men who have sex with men (MSM) donors. The impact such a change may have on the risk of HIV, HBV, and HCV transfusion-transmitted infections (TTI) is unclear. Using PRT in combination with blood screening may alleviate concerns over the risk.

Aims: To estimate the residual risks of HIV, HBV and HCV TTI associated with labile products after treatment with PRT and blood screening in the absence of sexual deferrals for MSM.

Methods: A probabilistic approach based on Bayesian networks and Monte Carlo simulations was used. A directed acyclic graph containing the variables influencing the risk and their dependencies was adapted to conduct simulations in two stages. In the first stage, independent donors, their infectious state, and their detection state were simulated. Donor data from Canadian Blood Services and Héma-Québec were used to define probabilities for donor profiles, prevalence and incidence. The viral loads of HIV were modeled based on the literature for incident cases, while prevalent cases were modeled based on a national database of infected cases. In the second stage, all test-negative donations were released and pooled to reach the required volume for PRT treatment. The residual risk was the ratio between the number of contaminated products (i.e., those with a viral load of >1 copy per product) and the number of products after PRT. The risk of HIV, HBV, HCV was assessed assuming blood screening was performed using nucleic acid testing (NAT) [Procleix Ultrio Plus (Grifols Diagnostics Solutions Inc.) in minipools of 16 and Cobas MPX (Roche Molecular Systems, Inc.) in minipools of 6] and/or serology testing, and PRT was applied to screened blood products.

Results: For HIV, the residual risk of contamination was <0.1 per million products when combining PRT with Procleix Ultrio Plus NAT and serologic testing, and <1 per million when either Procleix Ultrio Plus NAT or serologic testing was removed. For HBV, the risk was <0.1 per million products when combining PRT with Procleix Ultrio Plus NAT and serologic testing, <0.1 per million products when combining PRT with only Procleix Ultrio Plus NAT, and <1 per million when combining PRT with only serologic testing (all with HBsAg testing). For HCV, the risk was <1 per million products when combining PRT with Procleix Ultrio Plus NAT and serologic testing, but >1 per million when either Procleix Ultrio Plus NAT or serologic testing was removed. Similar results were obtained with the Cobas MPX NAT assay.

Summary/Conclusions: Assuming current sexual behavior deferrals are removed, the residual risk of HIV, HBV, and HCV TTI would be <1 per million products if PRT is used in combination with NAT and serologic testing. Therefore, donor deferrals could be removed in countries using these blood screening assays. The residual risk is lower for HBV when NAT or serologic testing is removed because HBV has lower viral loads, including occult cases, whereas viral loads above the inactivation efficacy of this PRT could be observed after HCV infection. PRT may not be able to replace current HCV testing procedures, although it could be used as an additional safety measure to remove some deferral criteria and improve the inclusiveness of blood donation.

Transfusion transmitted infections

Bacteria

P260 | Comparison of positive rates of bacterial surveillance test on apheresis platelet and buffy coat pooled platelets after implementation of large volume delayed sampling strategy

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Background: The Hong Kong Red Cross Blood Transfusion Service commenced screening all pre-released platelet concentrates (PCs) sampled at no sooner than 36 hours for bacterial surveillance test (BST) using aerobic BacT/ALERT 3D (bioMérieux, Durham, NC, USA) since 1998 (Liu, Vox Sang, 1999). Single-step strategy with large volume delayed sampling (LVDS) at no sooner than 36 hours was implemented on all non-pathogen reduction-treated apheresis platelets (APs) and buffy-coated (BC) pooled platelets in September 2021 pursuant to the 2019 US FDA guidance. PCs with negative results after 24 hours of culture are released for transfusion while the culture bottles are monitored continuously until platelet expiry (5 days).

Aims: To compare the BST positive rates before and after implementation of LVDS on APs and BC pooled platelets.

Methods: Sampling of individual PC was undertaken at 36 to 48 hours after donation (i.e. Day 2). Before LVDS implementation, APs and BC pooled platelets were tested for BST using aerobic bottle only with a sample volume of 2 mL and 4-10 mL respectively, pooled before inoculation. In LVDS, 16 mL sample was taken into a SampLok device with 8 mL inoculated into each of an aerobic (BPA) and anaerobic (BPN) culture bottle. Incubation was monitored by BacT/ALERT 3D system until platelet expiry and platelet units were held for 24 hours before release to hospitals. Initial positive samples were retested by repeat bacterial culture taken from the index PC or associated blood components. Initial positive culture bottles with negative re-culture results were regarded as false positive. Bacterial culture results before and after implementation of LVDS were compared using chi-square test. A p-value <0.05 was considered as statistically significant.

Results: From late September 2021 to December 2022, a total of 19455 units of APs and BC pooled platelets were sampled using LVDS strategy. The false and confirmed positive rates were 0.041% and 0.005% respectively whereas the false and confirmed positive rates of 36243 APs and BC pooled platelets sampled using non-LVDS strategy from July 2019 to September 2021 were 0.028% and 0.014% respectively. There was no statistically difference in both false positive rates and confirmed positive rates before and after implementation of LVDS strategy. The bacteria isolated from the confirmed case by LVDS was *Staphylococcus aureus*.

Summary/Conclusions: LVDS aims to enhance detection of contaminated platelets by delay sampling to >36 hours post-donation and increasing sample volume in both aerobic and anaerobic bottles. Our study revealed that introduction of LVDS in BST was not associated with increase in false positive culture and did not pick up additional true positive PCs.

P260 - Table 1. False and confirmed positive rates of BST before and after implementation of LVDS

False positive rates in terms of percentage of bottles

	Before LVDS (Jul 19 - Sep 21) BPA		After LVDS (Sep 21 - Dec 22) BPA & BPN (*counted as one when only BPA, BPN or both is/are positive)	
	Total, N	False Positive, N (%)	Total, N	False Positive, N (%)
Apheresis platelet + pooled platelet	25,171	7 (0.028%)	19,455	8 (0.041%)
	P = 0.45			

P260 – Table 2

Confirmed positive rates in terms of percentage of platelet units

	Before LVDS (Jul 19 - Sep21)		After LVDS (Sep 21 - Dec 22)	
	Total, N	Confirmed Positive, N (%)	Total, N	Confirmed Positive, N (%)
Apheresis platelet + pooled platelet	36,243	5 (0.014%)	19,455	1 (0.005%)
	P = 0.35			

P261 | Abstract withdrawn

P262 | Syphilis infections among the blood donors in the regional blood center in Poznan, Poland in years 2014–2021

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Background: Syphilis is an infectious disease caused by the bacterium Treponema pallidum. In Poland in years 2014-2021 the total number of10632 people were infected, in the area of Greater Poland Province - 1505. Although the risk of getting infected by the means of transfusion from an infected donor is very low, the platelet concentrate is the the component the transfusion of which bears greater risk of infection. Blood donors - independently of the stage of syphilis - can be a potential source of infection for the recipient of blood and its components. Currently IUSTI recommendations from 2008 (IUSTI: European Guidelines on the Management of Syphilis) require routine testing of certain groups i.e. among the others: pregnant women, blood donors or organ donors. Thanks to compulsory testing of blood donors for markers of syphilis, the risk of infection is even further minimised as this infection is included in the haemovigilance procedures that aim to minimise the risk of occurrence of acute post transfusion adverse reactions.

Aims: The aim is to analyse the number of detected and confirmed Treponema pallidum infections among the blood donors in the area of activity of Regional Blood Donor Center in Poznan, Poland in years 2014-2021.

Methods: We have analysed data regarding testing for syphilis in years 2014-2021 In accordance to the current regulations in Poland screening testing for potential infection using serological methods is performed for every donation - both for the first time donor as well as repeat donors. Blood donations that are reactive must be tested again using the same method in two subsequent tests. If both test results are reactive, the samples undergo confirmatory testing that is performed in the Institute of Haematology and Transfusiology in Warsaw.

Results: In 2014: 8 samples of total 91876 were tested positive (2 - FTD, 6 - RD). In 2015: 19 samples of total 92760 were tested positive (10 - FTD, 9 - RD). In 2016: 11 samples of total 94875 were tested positive (5 - FTD, 6 - RD). In 2017: 21 samples of total 99211 were tested positive (8 - FTD, 13 - RD). In 2018: 11 samples of total 100 331 were tested positive (3 - FTD, 8 - RD). In 2019: 6 samples of total 102 373 were tested positive (3 - FTD, 3 - RD). In 2020: 13 samples of total 90 406 were tested positive (7 - FTD, 6 - RD). In 2021: 16 samples of total 102 995 were tested positive (9 - FTD, 7 - RD). *FTD – first-time donors; RD – repeat donors. The analysis has shown that the population of blood donors in years 2014-2021 still included people with syphilis infection. No trend can be observed in reference to the fact whether the infections were among the first time or repeat donors.

Summary/Conclusions: We have proved that testing blood donors for the Treponema pallidum infection increases the safety of recipients of blood and its components and that obligatory testing of donors is fully justified. It must be reminded that syphilis still remains the problem of social life in many countries including Poland.

P263 | Comparable detection of bacterial contamination in apheresis platelet concentrates suspended in plasma or platelet additive solution E with an automated culture method

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Background: Historically, Canadian Blood Services has manufactured platelet concentrates (PC) suspended in 100% plasma (plasma-PC) and is in the process of implementing PC suspended in platelet additive solution E (PAS-PC). Plasma-PC are screened for bacterial contamination with the BACT/ALERT 3D culture system using a large volume delayed sampling (LVDS) screening algorithm involving PC sampling at ≥36 hours post-collection for inoculation of aerobic and anaerobic culture bottles, and post-sampling quarantine for ≥6 hours.

Aims: To compare detection of bacterial contamination in apheresis PAS-PC and plasma-PC using our LVDS testing algorithm.

Methods: Double apheresis hyperconcentrate units were collected and split into single hyperconcentrates diluted in either PAS-E or concurrently collected plasma. Units were tested for *in vitro* quality markers (eg., volume, platelet concentration, glucose, lactate) and baseline sterility prior to spiking with transfusion relevant bacterial species. Units were inoculated at a target concentration of 30 CFU/unit of facultative *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Serratia marcescens*, and *Klebsiella pneumoniae* or 10 CFU/mL of anaerobic *Cutibacterium acnes*. Spiked PC units were stored under standard conditions for 7 days. At 24-hours post-spiking, samples were serially diluted and plated for determination of bacterial concentration. At 36- and 48-hours post-spiking, samples were taken for BACT/ALERT testing and determination of bacterial concentration. If no growth was obtained, BACT/ALERT testing was repeated at the end of PC storage. Times to detection in the BACT/ALERT system and bacterial concentration were compared between plasma-PC and PAS-PC. The experiments were repeated three times for each species.

Results: *In vitro* quality testing showed that while volume and platelet concentration were consistent between PAS-PC and plasma-PC, glucose and lactate were lower in PAS-PC, which is expected due to donor variation and dilution with PAS. No differences in times to detection were observed for fast-growing *K. pneumoniae* and *S. marcescens* when PC sampling was done at 36-hours vs 48-hours. However, slow-growing *S. aureus* and *S. epidermidis* were detected faster when sampling was performed at 48 hours. *C. acnes* does not proliferate in PC; however, it was interesting to observe that this bacterium was detected faster in PAS-PC compared to plasma-PC,

attaining statistical significance at the 48-hour sampling time. *S. epidermidis* was detected faster in the aerobic culture bottle in plasma-PC compared to PAS-PC at the 48-hour sampling time but no differences were observed with the anaerobic bottle. Importantly, no statistically significant differences were observed in times to detection in the BACT/ALERT system between plasma-PC and PAS-PC at the 36-hour sampling time.

Summary/Conclusions: Transfusion relevant facultative bacteria grow in PC suspended in PAS-E at a similar rate as in PC suspended in 100% plasma, despite the differences of the suspension solutions of the two components (e.g., lower glucose content in PAS-PC). Faster detection of *C. acnes* in PAS-PC may be due to bacteria-platelet aggregation in plasma-PC that reduces the chance for detection. Importantly, this study showed that BACT/ALERT times to detection are not significantly different for any of the bacteria tested when grown in plasma-PC or PAS-PC if screening is done following the LVDS testing algorithm implemented at Canadian Blood Services. Therefore, there is no increase in the safety risk to transfusion patients associated with the change in PC suspension solution from plasma to PAS.

P264 | Abstract withdrawn

Transfusion transmitted infections

Parasites

P265 | Detection of plasmodium in specimens from healthy asymptomatic individuals in Nigeria

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Background: The investigational **cobas**® Malaria for use on the **cobas**® 5800/6800/8800 Systems is a qualitative *in vitro* nucleic acid screening test for the direct detection of *Plasmodium (P. falciparum, P. malariae, P. vivax, P. ovale,* and *P. knowlesi)* ribosomal DNA/RNA (rDNA, rRNA) in whole blood and amplifies two targets.

Aims: To compare *Plasmodium* detection by cobas Malaria with microscopy, antibody, and antigen tests in healthy asymptomatic individuals from a malaria endemic country.

Methods: Two hundred and ten (210) specimens were collected in Benin City, Edo State, Nigeria from August to September 2021 and tested individually by cobas Malaria. An in-house NAT was used as a confirmatory assay. cobas Malaria initial reactives were tested at 1:6 dilution to simulate pools. NovaLisa Malaria ELISA by NovaTec and Malaria Pf/Pv Ag Rapid Test by CTK Biotech were used for antibody and antigen testing respectively.

Results: Of the 210 specimens, 199 were evaluable. The average age of the 199 specimen contributors was 30.8 years with a median of 29.0 (18 – 61); 115 were males and 84 females.

Of the 162 antibody positives, 70 (43.2%) were cobas Malaria confirmed reactive. Among the 15 equivocal and 22 negative specimens, 3 each (20.0%, 13.6%) were confirmed cobas Malaria reactive.

For the 76 cobas Malaria confirmed reactives (Table 2), 4 were antigen and microscopy positive, and no other specimens were antigen or microscopy positive. Sixty-one (61, 80.3%) were reactive in simulated pools of 6. In repeat individual testing, the specimens showed different reactive rates, suggesting variable parasitemia. 92.1% (70/76) were antibody positive vs. 74.8% (92/123) of the remaining specimens.

Summary/Conclusions: There were 76 cobas Malaria confirmed reactive samples among 199 specimens collected from healthy asymptomatic individuals in the rainy season in an endemic country. cobas Malaria detected the 4 microscopy/antigen positive specimens; 72 confirmed reactive specimens were microscopy/antigen negative. These data showed that a molecular malaria assay detecting rRNA/rDNA could more effectively detect current infections in healthy asymptomatic individuals, as compared to microscopy/antigen tests. Apparent variation in levels of parasitemia in the subjects may reflect varying states of acquisition and clearance of infections during the malaria risk season in a hyper endemic region. Among the 162 antibody positives, 43.2% (70) were confirmed reactive by cobas Malaria. The rate was different from that of a non-endemic country, where the PCR reactive rate of confirmed malaria antibody positive blood donors has been reported as under 1% (Kitchen, Vox Sang., 2014).

P265 - Table 1. Results by different test methods from the 199 specimens

	Positive N (%)	Negative N (%)	Equivocal N (%)
Microscopy	4 (2.0)	195 (98.0)	-
Antigen	4 (2.0)	195 (98.0)	-
Antibody	162 (81.4)	22 (11.1)	15 (7.5)
cobas Malaria (confirmed)	76 (38.2)	123 (61.8)	-

P265 - Table 2. Results by different methods on the 76 cobas Malaria confirmed reactives

cobas Malaria (Additional Testing)*	Microscopy**/Antigen	Antibody	*Plasmodium* Species***	Number of Specimens ($N = 76$)
(+)/+/+/+***	+	±	*P. falciparum*	3/1
(+)/+/+/+	-	+	NA	54
(-)/+/+/+	-	+	NA	4
(-)/+/±	-	+	NA	2
(-)/±/-	-	+	NA	2
(-)/-/-/-	-	+	NA	5
(+)/+/+/+	-	-/Equivocal	NA	2/1
(-)/+/±	-	Equivocal	NA	2

* Values in parenthesis were the pools of 6 results
** Species were reported by microscopy
*** The parasitemia for the 4 microscopy positives were 22, 36, 88, and 60 (the antibody negative specimen) parasites/uL
NA - species not detected by microscopy

P266 | Abstract withdrawn

Transfusion transmitted infections

Newly emerging pathogens and other transfusion related pathogens

P267 | Abstract withdrawn

P268 | Hepatitis Delta virus seroprevalence among blood donors with overt and occult Hepatitis B infection in Dalian, Liaoning province, China

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Background: Hepatitis delta virus (HDV) is a small single stranded circular RNA virus, and an obligate satellite of hepatitis B virus (HBV). HDV needs the HBV envelope proteins for assembly of infectious virions, dissemination, and transmission. HDV/HBV coinfection is associated with downregulated HBV replication but with a faster progression of chronic infection to cirrhosis and hepatocellular carcinoma than HBV monoinfection. Although HDV and HBV are transmissible through exposure to infected blood, data about HDV infection in blood donors remain scarce. HDV prevalence data mainly derived from patients with clinically progressed HBV infection that cannot be translated to blood donors showing mostly HBV inactive carriage. Recently, a Chinese multicenter study reported low HDV seroprevalence in HBV co-infected donors from 14 provinces or municipalities but no data were available for Liaoning province in Northeast China.
Aims: To estimate the seroprevalence of HDV among asymptomatic HBV-infected blood donors from Dalian, Liaoning province, and to estimate the rate of HDV infection in a particular subgroup of donors of various geographical origins carrying occult HBV infection (OBI).
Methods: Candidate blood donor samples were collected between 2011 and 2021 and tested for HBsAg and HBV DNA in the Dalian blood center. HBsAg rapid testing (95% LoD: 5 IU/mL) was performed before donation. Post-donation, HBsAg and HBV DNA were tested using two different enzyme-linked immunosorbent assays (95% LoD: 0.2 IU/mL), and mini-pool or individual-donation multiplex nucleic acid test (NAT) (95% LoD: 3-10 IU/mL). Donors tested reactive for HBsAg and/or HBV DNA, and previously characterised OBI donors (Denmark, $n = 1$; Poland, $n = 43$; Spain, $n = 12$; Switzerland, $n = 11$; South Korea, $n = 3$; and South Africa, $n = 54$) were included in the study. Qualified donors with isolated anti-HBc or anti-HBs reactivity were randomly selected too. Plasma samples were tested for HDV IgG using WANTAI HDV-IgG ELISA (WANTAI) or LIAISON XL murex Anti-HDV assay (DiaSorin). Reactive samples were tested further with Hepatitis D antibody (IgG) and Hepatitis D antibody (IgM) assays (Beijing Beier Bioengineering). HDV RNA was tested using an in-house RT-qPCR assay.
Results: Overall, 2,175 donors from Dalian were tested for HDV IgG: 65 HBsAg rapid testing reactive pre-donation, 1,017 confirmed HBV-infected post-donation (507 HBsAg+/HBV DNA+, 33 HBsAg+/HBV DNA-, and 477 HBsAg-/HBV DNA+ [OBI]), 327 NAT initially reactive but non-repeated reactive, 397 anti-HBc+ only, and 369 anti-HBs+ only. Two samples (0.09%) initially tested reactive for anti-HDV IgG (S/CO: 2.3 and 1.8) but were not confirmed reactive on further testing with the other two serologic tests. In addition, one of 124 (0.8%) non-Chinese OBI donors tested reactive to HDV IgG repeatedly. No HDV RNA was detected.
Summary/Conclusions: The retrospective screening of 2,175 samples collected over 10 years showed no HDV seroreactivity in blood donors with confirmed or suspected markers of HBV infection, in Dalian, Liaoning province. These results confirmed the extremely low prevalence of HDV in Chinese blood donors and a low risk of HDV transmission through blood transfusion. In addition, HDV/HBV co-infection appears to play no significant role in the genesis of OBI.

P269 | Anti-SARS-CoV-2 antibody development during the COVID-19 pandemic in Swiss blood donors

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Background: The primary purpose of infection serology surveillance is to study the percentage of a population that has antibodies against a particular infectious disease agent (IDA). The blood donor population has great potential to serve as a sentinel system for the general population. The data presented in this study of a survey of the prevalence of anti-SARS-CoV-2 antibodies in Switzerland, is an illustrative example of this possibility.
Aims: The present study focused on the nearly three-year course of the anti-CoV-2 seroprevalence against the S and NCP protein in Swiss blood donors representing the population of the whole country. The aim was, to describe the course of the antibodies against the nucleocapsid protein (NCP) and spike protein (S) and to differentiate between natural infection and vaccination-induced immunity.

P269 – Table 1: Course of the anti-SARS-CoV-2 seroprevalence of seven consecutive time points over the whole of Switzerland

Time points	Amount donor samples	NCP positive (%)	S positive (%)
Mar 2020	1,953	0.3	0.3
Jul/Aug 2020	2,202	4.3	4.3
Jan 2021	2,147	16.4	16.8
May/Jun 2021	2,319	21.1	55.9
Nov/Dec 2021	2,145	22.0	90.5
Jun/Jul 2022	2,196	74.4	98.5
Oct/Nov/Dec 2022	2,196	83.9	99.0

Methods: A total of 15,158 random blood donor samples from seven different regions of Switzerland, representing the whole of Switzerland from seven consecutive time points from March 2020 to December 2022 were investigated. Serological antibody testing was performed on all samples with the individual Elecsys SARS-CoV-2 total antibody assays against the NCP and the S proteins.

Results: From March 2020 to July/August 2020, the increase in antibody seroprevalence for both NCP and S was only very moderate, up to 4.3 %. In January 2021 the seroprevalence for both proteins was around 16–17%. In May/June 2021, the NCP-seroprevalence was 21.1% and the S-seroprevalence 55.9% respectively, indicating a moderate natural infection rate and already an increase of the antibodies against the S protein, due to vaccination campaign all over Switzerland. The dramatic increase of the antibodies against the NCP-protein (22.0%) since November/December 2021 to June/July 2022 (74.4%), resp. November/December 2022 (83.9%) was simultaneous with the appearance and rapid spread of the Omicron SARS CoV-2 variant in Switzerland and the abolition of the compulsory mask waring in public spaces. At the end of 2022, 99% of the blood donor population already had antibodies against the S protein.

Summary/Conclusions: The present study focused on the three-year course of anti-SARS-CoV-2 seroprevalence against the S and NCP protein in Swiss blood donors representing the whole country, differentiating between natural infections and vaccination-induced immunity. The increase of the antibodies against the S protein was in accordance with the rate of the vaccinated population. The dramatic increase of the antibodies against the NCP protein since beginning the year 2022 was in accordance with the abolition of mask wearing and the spread of the Omicron variant with milder symptoms in the population. These data show how the blood donor population can be used to represent the infection surveillance of the general population of a region or country.

P270 | Abstract withdrawn

P271 | Infection of red blood cell progenitors with SARS-CoV-2 results in dysregulated haemoglobin and iron metabolism.

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Background: COVID-19, an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes acute respiratory symptoms and is reported to affect the vascular system, which may be underlying the systemic symptoms observed in affected patients. However, severe cases were reported to be associated with reduced erythrocyte (RBCs) turnover, low haemoglobin (Hb) levels along with increased total bilirubin and ferritin serum concentrations. Moreover, expansion of erythroid progenitors in peripheral blood together with hypoxia, anaemia, and coagulopathies highly correlates with severity and mortality. We demonstrate that SARS-CoV-2 directly infects erythroid precursor cells, impairs Hb homeostasis and aggravates COVID19 disease.

Aims: Here, we provide the first experimental evidence of disruption of haemoglobin synthesis and iron dysmetabolism directed by SARS-CoV-2 infection of erythroid progenitors.

Methods: RBC precursors derived from peripheral CD34+ blood stem cells of healthy donors were infected in vitro with SARS-CoV-2 alpha variant and differentiated into RBCs. Hb and iron metabolism in more than 20 hospitalized Covid-19 patients and controls were analysed in plasma-reduced whole blood samples using mass spectroscopy, qPCR and western blot analysis. Furthermore, we measured Raman spectra of RBCs from whole blood of severe COVID-19 patients and compared those with healthy and convalescent donors. Raman spectra were automatically acquired and analysed using a special statistical data analysing software.

Results: RBC precursors express ACE2 receptor, CD26 and CD147 at day 5 of differentiation, which makes them susceptible to SARS-CoV-2 infection, but virus is not able to replicate in these cell. qPCR analysis of differentiated RBCs revealed increased HAMP mRNA expression levels, encoding for hepcidin, which inhibits iron uptake. Furthermore, we found significantly changes in spin state of the iron in Hb as well as the tertiary structure shown by the formation of disulfide bridges in samples of COVID-19 patients. In addition, COVID-19 patients showed impaired Hb biosynthesis, enhanced formation of zinc-protoporphyrine IX, heme-CO_2, and CO-Hb as well as degradation of Fe-heme. Moreover, significant iron dysmetablolism with high serum ferritin and low iron and transferrin levels occurred, explaining

disturbances of oxygen-binding capacity observed in severely ill COVID-19 patients. Additionally, COVID-19 patients showed enhanced expression of enzymes and proteins involved in iron and haemoglobin metabolism such as HAMP, FECH, and HMOX-1. Long-COVID patients also showed enhanced formation of CO-Hb.

Summary/Conclusions: Our data identify RBC precursors as a direct target of SARS-CoV-2 and suggest that SARS-CoV-2 induced dysregulation in Hb- and iron-metabolism contributes to the severe systemic course of COVID-19. Because changes in Hb structure may also be significantly involved in the development of Long-COVID symptoms such as fatigue and exhaustion, our findings may open the door for new diagnostic and therapeutic strategies for both intensive care COVID-19 patients and Long-COVID patients.

P272 | Investigation of possible transfusion transmission of the tick-borne bacterium Neoehrlichia mikurensis

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Background: *Neoehrlichia (N.) mikurensis* is an intracellular, gram-negative bacterium widespread in ticks and rodents across Europe and Asia. It targets the vascular endothelium and can lead to severe disease in humans, but may also cause persistent asymptomatic infection. Whereas several related bacterial species of the *Anaplasmataceae* family have been implicated in transfusion-mediated infections, there are no previous reports of transmission of *N. mikurensis* via blood transfusion. However, a recent study of Swedish blood donors showed that 0.7% of them were asymptomatically infected by *N. mikurensis*.

Aims: We present two cases where the risk of transfusion-transmitted infection of *N. mikurensis* was investigated.

Methods: Trace-back and look-back investigations included blood donors, recipients and blood components that were tested for *N. mikurensis* by PCR. A typing strategy based on whole genome sequencing data will be developed for the discrimination between different *N. mikurensis* strains.

Results: Case 1: A female patient in her forties presented with acute haemorrhage in the lower abdomen and underwent extensive emergency surgery, requiring massive transfusion. The postoperative period was complicated by multiple cerebral infarctions where an infectious cause was suspected, and blood tests revealed *N. mikurensis* infection. Although possible that the patient had acquired the infection through earlier tick-bites rather than via the transfusion route, it was decided to investigate the 33 blood donors who had provided blood components to the patient. A total of 31 donors agreed to be tested for *N. mikurensis* by PCR and one turned out positive, a man in his thirties who had not experienced any symptoms. In connection to the testing, he donated blood that was processed into packed red blood cells and fresh frozen plasma which also tested positive by *N. mikurensis* PCR. A look-back investigation involving 13 more patients who had received blood components from this donor 2007-2020 did not detect any additional cases. Comparison of the *N. mikurensis* isolates from the index patient and the positive donor, respectively, is still a work in progress challenged by low genomic intra-strain variation and low bacterial DNA amounts in healthy donor samples.

Case 2: Incidentally, it was discovered that a woman in her fifties who worked in the laboratory was an asymptomatic carrier of *N. mikurensis*. She had been a blood donor for more than 30 years, was in good health and had completely normal inflammatory blood parameters. A look-back investigation encompassing the period 2014-2021 included 28 recipients of blood components from this donor. Nineteen patients were deceased, and the remaining nine patients all tested negative by *N. mikurensis* PCR.

Summary/Conclusions: *N. mikurensis* is an emerging tick-borne human pathogen that can cause febrile disease accompanied by vascular events, as well as persist without symptoms. Our case reports and other recent data show that the bacterium is present among healthy blood donors in Sweden and raise the question of potential transfusion-transmitted infection. A genomic assay for differentiation of *N. mikurensis* strains may provide further answers. Studies regarding the ability of *N. mikurensis* to remain viable under the preparation and storage of blood components are also needed.

P273 | The impact of the COVID-19 pandemic on blood donations at a tertiary university hospital centre

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Background: The occurrence of a new Coronavirus with an exponential rate of infection, and high mortality and morbidity rates, led to the declaration of a "State of Emergency" worldwide. In Portugal, the "State of Emergency" was declared fifteen times between March 2020 and April 2021, comprising a total of 218 days of limited commutes due to lockdown. Despite the restrictions imposed by the Portuguese government, travels to hospitals or similar for blood donations were allowed. Despite the fact that SARS-CoV2 is a respiratory virus, the presence of RNA on blood of asymptomatic individuals was not entirely investigated. Although there is no evidence of its transmission by blood transfusion, facing the risk of transmissibility, the precautionary principle was adopted. For this reason, our Tertiary University Hospital Centre followed the guidelines set by Portuguese Institute of Blood and Transplantation and the Directorate General for Health in order to ensure and maintain a safe therapeutic support of blood components in all hospital requests.

Aims: This study aims to evaluate the impact of COVID-19 pandemic on blood donations at a Tertiary University Hospital Centre during the time period of national contingency.

Methods: A retrospective longitudinal study will be conducted reviewing homologous blood donations from 2018 to 2022. Demographic analysis includes age and gender. Analysis will also focus on first blood donors` retention after pandemic crisis amelioration and the importance of regular donors for blood bank sustainability.

Results: A total of 44,028 blood donations were obtained from 47,088 attendance off voluntary and benevolent donors. Of these, 24,441 (51.90%) were males and 22,647 (48.95%) were females. Blood donors were divided according to their age group, namely: 18 to 24 years-old ($n = 4,502$; 9.56%), 25 to 44 years-old ($n = 16,325$; 34.67%), 45 to 65 years-old ($n = 24,887$; 52.85%), and over 66 years-old ($n = 1,374$; 2.92%). Over the time period concerning this study, our Centre registered donations from 27,520 (83.32%) regular blood givers and from 5,509 (16.68%) new donors.

Summary/Conclusions: At our Centre, there was a significant daily increase in homologous blood donations in 2020 from the same periods of the previous years. We believe this might be a consequence of social awareness of the panorama at that time, with a tendency to decrease from May 2021. Currently, blood donations are still decreasing, placing 2022 as the year with the least number of donations. It is of paramount importance to keep developing public awareness campaigns to illustrate the value of blood donations and how they can save lives. Furthermore, it is also crucial to understand the reason why new donors do not keep donating blood, and to adopt measures to contradict this trend.

P274 | Development of a Mayaro and Yellow fever virus duplex real-time RT-PCR assay for high-throughput testing

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Background: Blood transfusions are one of the most common medical procedures globally. According to the World Health Organization, 118.5 million blood donations are collected per year of which most are administered to patients over 60 and children. Despite saving millions of lives, blood transfusions can also transmit bloodborne infectious diseases, including arthropod-borne virus (arbovirus) infections. Transfusion-associated arbovirus infections are known for dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV), and others. As demonstrated by WNV in the United States of America, those infections can be fatal. Despite the rising threat posed by arboviruses as illustrated by the recent outbreaks of Chikungunya virus (CHIKV), ZIKV, and yellow fever virus (YFV), the implementation of new testing guidelines and the availability of molecular tests for arboviruses optimised for high-throughput testing platforms commonly applied in blood banks are limited. Beyond CHIKV and DENV, the Mayaro virus (MAYV) and YFV pose a potential risk to cause new outbreaks and endanger blood-safety. YFV is particularly known for its high fatality rate of 20-60%, while the recent discovery of MAYV in anthropophilic mosquitoes suggests an increased risk for urban outbreaks of MAYV.

Aims: The aim of this study was to develop and validate new YFV and MAYV real-time RT-PCR assays for their usage on the widely used high-throughput **cobas**® 6800 System.

Methods: Candidate assays for YFV and MAYV were designed by adapting published assays based on the multiple alignments of all available genomic sequences from NCBI database. Locked nucleic acid and 2'-O-methyl modifications were included to improve assay performance in multiplex. Integrated DNA Technologies (IDT) available dyes, double quenched probes, with ZEN internal quencher positioning determined by IDT software, were utilised. The sensitivity and specificity of candidate assays was evaluated using full virus RNA and in-vitro transcripts. After testing on a LightCycler® 480 System using the cobas omni Optimization Kit, the tests were transferred and validated on the **cobas omni** Utility Channel on the **cobas**® 6800 System.

Results: In total, 24 primer and probe combinations were tested for YFV and 128 for MAYV. Of those candidate assays, 2 were selected for each virus for further validation based on the best experimental performance. In both cases, 2'-O-methyl modified forward primers improved assay performance. However, assay performance was often reduced with 2'-O-methyl modified reverse primers (RT primers). The detection limits were lower than 1.5 copies per µl. All assays discriminated between the target virus and closely related viruses.

Summary/Conclusions: Our work shows how previously published assays can quickly be adapted to the established high throughput **cobas omni** Utility Channel. Further, we provide a ready to use protocol for high-throughput YFV and MAYV testing in blood-banks and beyond. The availability of these tests allows rapid upscaling of testing

in the context of outbreak scenarios and enables customised combinations with other assays.

P275 | Abstract withdrawn

P276 | SARS-CoV-2 in Transfusion Medicine: Seroprevalence evaluation and SARS-CoV-2 RNA detection in blood donors in Brazil

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Background: The COVID-19 pandemic caused by the SARS-CoV-2 virus has significantly impacted global healthcare systems, including transfusion medicine. The assessment of SARS-CoV-2 RNA in plasma from blood donors and knowledge of prior exposure to the virus are essential factors that contribute to increased transfusion safety. However, it still needs to be fully understood whether plasma viral load can result in infection, and there are still gaps in understanding the transmission of SARS-CoV-2 through blood components.
Aims: This study aims to assess the seroprevalence of SARS-CoV-2 antibodies and the detection of SARS-CoV-2 RNA in blood donor candidates.
Methods: The analysis was performed in two groups: i) samples from blood donor candidates; and ii) samples from blood donors who provided post-donation information (PDI). Six hundred forty-six plasma samples from blood donors were screened for SARS-CoV-2 antibodies using an enzyme-linked immunosorbent assay (ELISA), and 724 samples were evaluated for SARS-CoV-2 RNA using real-time reverse transcription-polymerase chain reaction (RT-PCR). The establishment of viral load was also determined in positive samples by the standard curve constructed with the RNA reference (NIBSC code 20/146).
Results: The serological analysis represents 4.7% (646/13,824) of the total donations in 2020. The results showed a seroprevalence rate of 4.8% (31/646) among blood donors, indicating that a significant proportion had been exposed to the virus in the first year of the pandemic. RT-PCR detected 1.4% (5/358) of positive SARS-CoV-2-RNA in blood donors samples. These samples belong to January 2022 during the Omicron wave. No detectable SARS-CoV-2-RNA was observed during the gamma wave ($n = 323$ plasma samples tested) or 43 blood donors' plasma samples screened from PDI. Furthermore, the viral load was calculated in five positive samples showing quantification of 9.3x10e4 copies, suggesting a low viral load.
Summary/Conclusions: This study provides essential information regarding SARS-CoV-2 RNA detection in plasma from blood donor candidates, contributing to increased transfusion safety and public health. Studies related to the viral load are still needed in plasma, which will allow the real impact of SARS-CoV-2 transfusion medicine. Our findings highlight valuable information for developing transfusion strategies during the ongoing pandemic.

P277 | Abstract withdrawn

P278 | Lack of COVID-19 transmission through granulocyte transfusion: A case report

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Background: According to the FDA, there have been no known cases of SARS-CoV-2 transmission through blood transfusion. Nevertheless SARS-CoV-2 RNA has been detected in the plasma of pre-symptomatic and asymptomatic blood donors who reported COVID-19-like symptoms or diagnosis after donation, raising the concerns for possible transfusion transmission. In addition to screening and deferring donors with recognizable risks, most blood centers have procedures for retrieving and discarding untransfused blood products from donors who provide post-donation information (PDI) of developing clinical symptoms of, or testing positive for COVID-19. Granulocyte transfusions (GTXs) pose a much higher theoretical risk of disease transmission, as products must be administered fresh with minimal delay to severely immunocompromised recipients, many times before any PDI can be collected to trigger product retrieval. We report a case of a severely immunocompromised GTX recipient, who received granulocytes collected from a donor who became symptomatic and COVID-19 positive 24 hours after donation.
Aims: Case report of a patient who received granulocytes from a donor who was subsequently diagnosed with COVID-19.
Methods: PDI of the implicated granulocyte donor and the medical record of the recipient were retrospectively reviewed.
Results: A 23-year-old female diagnosed with acute lymphoblastic leukemia in May 2022 who had achieved minimal residual disease negative complete remission in June 2022 was hospitalized for severe foot and arm pain in December 2022. Prior to the hospitalization she was receiving delayed intensification therapy which resulted in acquired pancytopenia. On admission she was found to have WBC of 0.3 K/uL and absolute neutrophil count of 0.1 K/uL. Three days into the hospitalization, she was found to have disseminated mucormycosis with lung, brain, and soft tissue involvement. Consequently, she began antifungal medications amphotericin B and isavuconazole. After one week on those medications, chest CT showed worsening of the lung infiltrate and her absolute neutrophil count remained at 0, and she was started on daily granulocyte transfusions. The fourth granulocyte unit was collected from a 33-year-old female donor who was healthy at the time of donation. The fresh product was transfused 3 hours following collection and transfusion was well tolerated. The donor then reported COVID-19 positivity with rapid antigen test 24 hours after donation developing fatigue and sore throat the evening of the day of donation, and congestion the morning after the day of donation. PDI was shared with the clinical team. The recipient was monitored closely and did not develop any new symptoms from baseline concerning for COVID-19. Four days after the implicated granulocyte transfusion,

the recipient also tested negative by PCR for COVID-19. Due to the improvement of her baseline symptoms and lack of new COVID-19 symptoms, additional COVID-19 tests were not performed. The patient received 4 additional granulocyte units, and her WBC and absolute neutrophil counts improved 9 days after starting granulocyte transfusions. Her symptoms and infection slowly improved, and she was discharged 7 weeks after admission.

Summary/Conclusions: Transfusion of fresh granulocytes from a donor who became symptomatic and COVID-19 positive shortly after donation did not result in COVID-19 transmission in a severely immunocompromised recipient. This case confirms that blood transfusions are unlikely to transmit SARS-Cov-2.

P279 | SARS-CoV-2 seroprevalence in blood donors: Three years of Canada-wide monitoring

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Background: Blood services around the world have informed public health policy during the SARS-CoV-2 pandemic through sero-surveillance. Sero-surveillance data are used to plan and evaluate the impact of interventions such as vaccination policies and implementation or scaling back of restrictions. By January 2022 when Omicron became the dominant variant with milder symptoms, public health case and contact testing was scaled back. Waste-water surveillance at sentinel sites provided an indication of increasing infections, but only sero-surveillance could monitor the infection rate.

Aims: To monitor infection and vaccination mediated SARS-CoV-2 antibodies in blood donors over the course of the pandemic.

Methods: From April 2020 to the present cross-sectional samples of blood donors at all Canadian Blood Services (CBS) locations were included (all provinces except Quebec). From April to December 2020 the Abbott SARS-CoV-2 antibody assay detected IgG nucleocapsid antibodies (anti-N). From January 2021 onwards the Roche Elecsys anti-SARS-CoV-2 antibody assays detected total antibodies to spike protein (anti-S) and anti-N. Seroprevalence was standardised to population-level demographics and adjusted for assay characteristics using the Rogan-Gladen equation.

Results: Up to January 2023 663,073 samples were tested. Anti-N seroprevalence was low over 2020 (less than 2%). In 2021 anti-N increased from 2.24% (95% CI 2.08, 2.41) in January to 6.39% (95% CI 6.01, 6.76) in December. With vaccine roll-out the percentage anti-S positive increased from 2.80% (95% CI 2.60, 3.00) in January to 98.58% (95% CI 98.34,98.82) by December 2021. With the emergence of the Omicron variant in 2022 anti-N seroprevalence increased to 76.72% (95% CI 76.25, 77.19). In January 2023 anti-N positivity was highest in 17-24-year old's (86.55%; 95% CI 85.46, 87.63), racialized donors (81.95%; 95% CI 80.97, 82.94) vs white (75.44% 95% CI 74.91, 75.98), and those in materially deprived neighbourhoods (78.49%; 95% CI 76.91, 80.07 vs 75.44 95% CI 74.51, 76.38).

Summary/Conclusions: Anti-S seroprevalence reflected high uptake of vaccine in donors. SARS-CoV-2 seroprevalence due to natural infection was low until 2022 but despite vaccination increased rapidly when the Omicron variant dominated. Racialization and material deprivation are important predictors of higher infection rates. Ongoing monitoring of seroprevalence has been important for public health policies over the pandemic. Monthly reports were provided to national and provincial public health departments. A national seroprevalence rate incorporated seroprevalence test results from other studies with the majority of data points from blood donor sero-surveillance.

P280 | Serological detection of SARS-CoV-2 vaccine breakthrough infections in US blood donors

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Background: Many countries conducted SARS-CoV-2 cross-sectional seroprevalence studies using detection of anti-Nucleocapsid (N) antibodies (Ab) to estimate the prevalence of individuals with previous infection over time. Anti-N Ab assays can be used in countries that employed COVID-19 vaccines that induce anti-Spike (S) Ab. However, some studies have reported poor sensitivity of anti-N Ab assays in detecting vaccine breakthrough infections (VBTIs), even when using assays with high sensitivity in detecting infections in unvaccinated people. Using serological testing supplemented by donor surveys, Vitalant and the American Red Cross are following a large repeat donor cohort (RDC) of 142,612 donors with the aim of estimating population incidence of primary infections, vaccine breakthrough infections (VBTIs) and reinfections over time.

Aims: The primary aims of the study were to 1) to derive an Ortho total Ig anti-N assay cutoff with improved sensitivity while maintaining high specificity and 2) validate the sensitivity of the manufacturer's recommended cutoff as well as the revised cutoff to detect VBTIs, using survey reported swab confirmed VBTIs.

Methods: We used receiver-operating characteristic (ROC) curve analysis to derive an optimal cutoff for the Ortho anti-N assay. True negatives were pre-pandemic samples ($N = 1{,}448$); true positives were Ortho anti-S and Roche anti-N positive samples identified in the National Blood Donor Seroprevalence Study ($N = 371$). Sensitivity for detection of 4,484 VBTIs was validated using RDC donors who responded to detailed surveys on vaccination and infection and 1) reported COVID-19 vaccination; 2) made a subsequent donation that tested S-reactive N-nonreactive; and 3) donated again ≥14 days after a reported swab-confirmed infection. Specificity for VBTI could not be estimated owing to high rates of undiagnosed VBTIs in survey respondents.

Results: ROC curve analysis gave an optimal Ortho anti-N cutoff of 0.395 (sensitivity = 98.7%, specificity = 98.7%, AUC = 0.99). Sensitivity for detection of swab confirmed VBTIs in different periods, age strata and by symptom status are shown in the table. Overall sensitivity was 93.9% using the standard cutoff and 95.6% using the revised cutoff, a statistically significant improvement. Sensitivity was reduced in donors 65 and older and with asymptomatic infections.

Summary/Conclusions: These results indicate robust detection of swab confirmed VBTIs using the standard threshold of the Ortho anti-N Total Ig assay. Although statistically significant, the incremental improvement in sensitivity using the revised cutoff is small. Further study of the durability of anti-N Ab detection following VBTI is in progress.

P280 – Table 1 Sensitivity of Ortho anti-N total Ig assay for detecting swab confirmed VBTIs

	N	Manufacturer's cutoff % (95% CI)	Revised cutoff % (95% CI)
Overall	4,484	93.9 (93.2,94.6)	95.6 (94.9,96.1)
Delta (Jul-Dec 2021)	1,437	95.1 (93.8,96.1)	96.2 (95.1,97.0)
Omicron (Jan-Sep 2022)	3,047	93.4 (92.5,94.2)	95.3 (94.5,96.0)
Under 65	3,091	95.0 (94.2,95.7)	96.2 (95.4,96.8)
65+	1,393	91.5 (90.0,92.9)	94.3 (93.0,95.4)
Symptomatic	3,676	94.5 (93.7,95.1)	96.2 (95.5,96.7)
Asymptomatic	808	91.6 (89.5,93.3)	92.9 (91.0,94.5)

P282 | Transmission of variant Creutzfeldt-Jakob disease through blood transfusion and plasma-derived products: a narrative review of observed and modelized risks

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Background: Secondary transmission of variant Creutzfeldt-Jakob disease (vCJD) can occur through blood transfusion or receipt of plasma-derived products. However, published reviews on this topic are outdated, focused on a single country or product type, or did not comprehensively review how vCJD may impact the safety of the blood supply.

Aims: We reviewed existing data on observed and modelized risks of transfusion-transmission of vCJD.

Methods: We conducted a non-systematic, narrative review of relevant articles in MEDLINE. The literature was searched by three independent authors using keywords related to prion disease transmission by blood transfusion, epidemiological studies, and risk models, without restrictions on publication date. The results were supplemented with references cited by relevant articles. The references identified by each author were pooled, duplicates were removed, and the final set was selected based on consensus.

Results: To date, five patients are suspected to have acquired clinical vCJD or a vCJD infection after receiving a blood- or plasma-derived product from a donor who later developed clinical vCJD. All were transfused with non-leukodepleted red blood cells in the United Kingdom (UK) between 1996 and 1999, before the adoption of universal leukodepletion; three were methionine homozygous (MM) at codon 129 of *PRNP*; and two were methionine-valine heterozygotes (MV). Two UK-based, descriptive cohort studies evaluated the risk of vCJD among recipients of vCJD-implicated factor VIII concentrate ($N = 787$) and recipients of UK-sourced immunoglobulin ($N = 75$). These studies found no case of clinical vCJD over ~13 years of follow-up. Ten modeling studies were identified, including seven that reported on the risk of having a vCJD-contaminated donation, six on the risk of infection, and 6 on the risk of clinical vCJD. The risk of having a contaminated donation was generally <23 per million donations, that of infection was generally <10 per million transfusions or doses, and that of clinical vCJD was generally <2 per million transfusions or doses. Several countries have reassessed or are reassessing the need for vCJD deferral policies. Animal studies support that vCJD has preferential tropism for leukocytes and that leukoreduction is effective in reducing (but not eliminating) the risk of transmission. Prion reduction filters have been developed but are not used anywhere, possibly because of conflicting results in animal studies and the substantial costs of implementing this technology amid a waning vCJD epidemic. Despite initial concerns of a second vCJD wave driven by non-MM individuals, there has been only one autopsy-confirmed case of clinical vCJD in an MV individual. Further, the high prevalence of PrP^{sc}-positive appendices observed in the UK (1 in 2028 individuals in the Appendix-II study) may reflect dietary exposure to the bovine spongiform encephalopathy agent rather an ongoing, active infection. According to expert consensus, should a second wave occur, no more than a few cases may arise.

Summary/Conclusions: No cases of transfusion-associated vCJD have been reported since the adoption of universal leukodepletion in the UK in 1999. Furthermore, descriptive cohort studies and modeling studies suggest the risk of having a contaminated donation is minimal. Initial concerns of a second, more significant wave have yet to materialize despite the high prevalence of PrP^{sc}-positive appendices in the UK. Therefore, the ongoing trend to reassess or (in some countries) fully withdraw vCJD deferral criteria seems justified and may significantly expand the donor base.

Immunohaematology

Red cell immunohaematology–Serology

P283 | Abstract withdrawn

P284 | Evaluation of a novel monoclonal anti-Vel phenotyping reagent.

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Background: Vel is a high frequency antigen in the UK population, Vel- individuals are rare (approximately 1:4000). Anti-Vel antibodies are predominantly IgM and fix complement and can result in severe transfusion reactions. Anti-Vel does not normally cause Haemolytic Disease of the fetus and Newborn (HDFN) as IgM antibodies do not cross the placenta and Vel antigens are weakly expressed on fetal red cells. When anti-Vel is encountered, it is extremely challenging to provide compatible blood for transfusion.

In Wales, in 2021 and 2022, two separate patients were identified with anti-Vel during routine antenatal antibody screening. Antibody titres in both cases were not considered to be significant for HDFN. However, the possibility of blood transfusion at delivery was carefully planned. The Welsh Blood Service (WBS) donor database identified only two suitable blood donors. This number has significantly reduced in last 10 years due to the 'retirement' of existing Vel- donors and the

suspension of high frequency antigen donor screening. Suitable blood donors were identified by the English Blood Service (NHSBT) and imported to Wales to provide cover for both deliveries.

In recent years, the systematic screening of blood donors for Vel- donations has decreased in Wales due to the absence of suitable anti-Vel typing reagents available. These two patients highlighted the need to escalate high frequency antigen screening as a priority.

Aims: The aim of the study was to evaluate the suitability of a novel anti-Vel monoclonal antibody for blood donor screening using both manual and automated techniques. Sanquin have developed an anti-Vel monoclonal antibody and the WBS assessed the suitability of this reagent for antigen screening by manual techniques and automated techniques.

Methods: The anti-Vel was tested by manual techniques:

- Gel card (Bio-Rad NaCl/Enz gel cards)
- Gel card (Grifols DG gel cards)
- Gel card (Sanquin Cellbind)
- Direct Agglutination

Automated Techniques:

- Beckman Coulter Olympus PK 7300 (using bromelised cells in PBS)
- Immucor NEO IRIS

A total of 400 random blood donors were tested, 10 examples of known Vel- samples and three examples of known Vel weak samples were tested (all confirmed historically by the International Blood Group Reference Laboratory).

Results: All manual techniques produced consistent results with neat anti-sera and diluted sera up to 1 in 4 dilution. Both automated technologies failed to produce definitive positive reactions when tested versus random blood donors (assumed to be Vel positive).

Summary/Conclusions: Preliminary results are encouraging, the use of this anti-Vel is suitable for manual antigen screening. The optimum methodology for high throughput screening would be use of automated blood group analyser. Further studies to be performed on the PK 7300 using non-bromelised cells in a high concentration of albumin.

P285 | Prevalence of antigen CW (RH8) in a Portuguese blood donor population

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Background: Antigen C^w (RH8) is one of the 55 antigens in the RH system, located in the first extracellular loop of the RhCE protein. It has an estimated prevalence of 2% in the general Caucasian population, and up to 7–9% in the Latvian, Laplander and Finnish populations. Both C^w (RH8) and the low prevalence antigen C^x (RH9) are antithetical to the high prevalence MAR (RH51). Contrary to many RH antigens, anti-C^w does not seem to be clinically significant, as there are no reports of acute or delayed haemolytic reactions in the available literature. It is significant, however, in obstetric medicine, with various reports haemolytic disease of the foetus and the newborn, ranging from mild to severe. Commercial anti- C^w reagents are not available in many locations. As such, prevalence studies are lacking and there are no reports available regarding the prevalence of Cw antigen in the Portuguese population.

P285 - Table 1. RH phenotype distribution of C^w positive donors

Phenotype	Total
D+ C+ c- E- e+ C^w+	18
D+ C+ c+ E- e+ C^w+	21
D+ C+ c+ E+ e+ C^w+	3

Aims: To present the first estimate of the prevalence of C^w antigen in the Portuguese population.

Methods: Electronic blood donor records were searched for every RH phenotype performed from April 2021 to April 2022. Only individual phenotypes were considered and not donation phenotypes, as to exclude duplication of data. The Rh phenotyping was performed by hemagglutination in gel cards using monoclonal antibodies against D (RH1), C (RH2), E (RH3), c (RH4), e (RH5), K (KEL1) and C^w (RH8). (Grifols Diagnostics S.A., Barcelona, Spain).

Results: Rh phenotyping was performed in 3983 individual donors. A total of 42 donors tested positive for the C^w antigen, which results in a prevalence of 1.05%. All the donors were RHD positive. The prevalence of RHD positive phenotype was 80.24%. Table 1 shows the phenotype distribution of C^w positive donors.

Summary/Conclusions: The prevalence of antigens within a population helps estimating the occurrence of alloantibodies. To our knowledge, this is the first study conducted in the Portuguese population. We found a C^w antigen prevalence of 1.05%. This is nearly half of the estimated prevalence of the general white European population. While blood for transfusion can easily be found due to the low prevalence of the antigen, it is clinically significant in pregnant women and attention must be paid to exclude this antibody in a routine antibody screening. Antibody identification panels may not always include these low prevalence antigens. Therefore, knowing the prevalence of said antigens in a population aids in the identification efforts.

P286 | Frequency and specificity of irregular red blood cell antibodies in patients with congenital haemoglobinopathies in Albania

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Background: Haemoglobinopathies are among the most widespread inherited diseases worldwide. In Albania there are about 300,000 carriers of haemoglobinopathies or 8% of the population. Therefore, the

evaluation of the frequency of alloimmunization and autoimmunization in patients with transfusion-dependent haemoglobinopathy in our country is of critical importance for both transfusion physicians and clinicians.

Aims: The purpose of the study is to evaluate the frequency and specificity of allo- and auto-antibodies in patients with congenital haemoglobinopathies in Albania, who regularly receive blood transfusions, and to analyse the factors that can influence on the creation of these antibodies.

Methods: The study included 120 patients with congenital haemoglobinopathies who regularly receive transfusions in the Department of Pediatric Hematology at QSUNT, Tirana. The antibody screening test was performed for all the patients, and in case of positive results, antibody identification was performed with the 11-cell panel. Direct Coombs test was also performed on all samples. Laboratory data were supplemented with data from patients charts to evaluate the role of clinical and demographic variables in the frequency of alloimmunization.

Results: 11 (9.2%) patients presented irregular antibodies to erythrocytes, all clinically significant. A total of 18 alloantibodies against erythrocytes were identified. The most frequent alloantibody was Anti-K identified in 8 cases (44.4% of total antibodies), followed by Anti-D in 4 cases (22.3%), Anti-Kpa in 3 cases (16.7%), Anti-C in 2 cases (11.1%) as well as in one case Anti-c was identified (5.5%). Anti-K in 3 out of 8 cases was found combined with Anti-Kpa, while in one case it was found combined with Anti-c. Anti D was found in one case combined with anti-K and in another with Anti-C. In total, 7 out of 11 patients developed combined antibodies. Out of 120 patients included in the study, 25 resulted with positive DCT. In 4 out of 25 cases positive DCT is associated with positive ICT, of which only in one case we have data that a splenectomy was performed. Meanwhile, 10 of the 25 patients with positive DCT underwent splenectomy.

Summary/Conclusions: Following the strict rules of Rhesus and Kell compatibility, without occasional exceptions, significantly reduces the creation of new alloantibodies and autoantibodies. The creation of a national registry for patients with haemoglobinopathies and the improvement of testing techniques using molecular ones would help to fully evaluate the factors of alloimmunization. Meeting the needs of blood through regular donations is necessary for the implementation of the compatibility rules in transfusion and for the prevention of the formation of antibodies in our patients.

P287 | Implementation of erytra automated analyser for blood group typing in an blood donor setting: experience and first results

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Background: According to the Swiss guidelines, the ABO blood group and RH1 antigen have to be tested on all donations. First time donor ABO typing includes the use of two different anti-A and anti-B clones and reverse grouping using test cell of blood group A1, B and O. In addition, testing includes the RH (antigens RH2, RH3, RH4 and RH5) and KEL1 phenotyping. Since 2012, our institution has conducted these tests on the 7300systems (Beckman Coulter) using antisera from various manufacturers (Medion Grifols, Bio Rad, and Immucor). Different commercial platforms with the corresponding highly sensitive and specific reagents were evaluated and finally the Erytra system and the reagents from Grifols were selected. Erytra is a fully automated immunohematology analyser with high-throughput and –performance capacities for pretransfusion testing and blood group typing. The design of the Erytra is intended to be used with 8-column DG Gel cards.

Aims: We describe the experience we encountered during the implementation of the new workflow for the blood group typing in our routine blood donor-screening laboratory. Issues confronted during the change from the previous unidirectional to the bidirectional workflow (blood donor software – analyser) and description of the performance of the system during the first 8 months are presented.

Methods: ABO/RH1 grouping and RH/KEL1 phenotyping were performed according to the defined profiles on the analyser. All reagents used (gel cards, red cells, anti-sera) were from Medion Grifols. The samples with weak or unclear results were further investigated in our national immunohematology reference laboratory.

Results: The workflow with Erytra is well standardised without bottlenecks or resource constraints obtaining a high productivity. Many manual manipulations encountered with the previous systems including reagent supply and preparation of solutions have been eliminated. Results are continuously transmitted, which is a significant improvement for urgent samples. Furthermore, the continuous output of results eliminates the waiting time for batch results.

During the method verification process, concordant results between the Erytra and the reference methods (on PK 7300 system) were obtained in all 300 samples (100%). From the 49,940 donations (July 2022 to January 2023), 8,237 were from first time donors. From these donors nine phenotypes and 38 ABO with weak/doubtful positive reactions and 55 RH1 results with weakened reaction strengths had to be confirmed in the national immunohematology reference laboratory.

Summary/Conclusions: The Erytra analyser shows a high and reliable sensitivity and specificity in our blood donor screening setting. The capacity and velocity to cope with the high workload in our laboratory routine is very satisfactory. Due to the bidirectional workflow, human handling errors are practically eliminated. Urgent samples can be processed immediately. The result transmission is automated, rapid, safe and constant. There is less maintenance downtime and only minimal user maintenance than with our former blood typing analyser. Employee satisfaction is high and the working conditions are safe and attractive. During the first eight months, the Erytra analyser including all new reagents used was found to be highly suitable for our blood donor screening laboratory. No discrepancies were found in the 38 ABO and 55 RH1 confirmations. By using the DG Gel Double Pheno card, which is used for the first donors, we observed that one of the anti-C clones is more sensitive in the presence of the RH8

antigen or for example a RH2 variant, as clearly attenuated reactions were detected.

P288 | An improved approach to detecting the variant RhD - DVI in donors

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Background: The detection by direct testing of the partial D variant DVI in the donor population is useful for categorizing the individual for donation purposes as RhD positive. The DVI variant is a frequently encountered partial D type in Europe. The use of a reagent that can distinguish these individuals eliminates unnecessary additional testing to classify their red cells as D positive.

Aims: A new prefilled column-based test reagent comprised of a monoclonal blend was evaluated for performance to detect RhD as well as the DVI variant and compared with an additive column-based test reagent that detects RhD and the DVI variant. The reagent testing was performed across a variety of platforms using fully automated, semi-automated, and manual test methods.

Methods: Three sites participated in the studies. Testing compared a new prefilled Anti-D(DVI) column (anti-D clones RUM1 and P3X21223B10) (Ortho BioVue® System - Anti-D(DVI)) (BDVI) with the additive test method of Ortho Sera™ Anti-D(DVI) (anti-D clone ESD1M) (OSDVI) reagent using a BioVue Reverse Diluent cassette. Testing was done on 8742 donor and cord blood samples. A subset of these samples was selected for variant D antigen expression including DVI+. Two study sites evaluated 5676 samples tested across the fully automated Ortho Vision® and Ortho Vision® Max platforms, semi-automated Ortho Optix® Reader and manually using an Ortho® Workstation with a visual read of the reaction. The third site evaluated 3066 samples on the Ortho Vision platforms. Results were deemed discordant when a negative result was found with one reagent method compared to a positive result with the comparator. A tube-based test using BioClone® Anti-D was used to evaluate discordant results as a judge of final disposition. Concordance was defined at ≥ 99.4% with a one-sided 95% lower confidence limit (LBCI).

Results: Of the 5676 samples tested from the two combined sites, there were 4976 positive and 692 negative results with both test reagents, with 8 tests positive with BDVI versus negative with OSDVI. These eight samples tested with the tube method demonstrated positive reactivity and were included as concordant positive samples for 100% agreement with a 99.95% agreement at a 95% LBCI. Two samples were identified as DIVa, one DNU and five weak D. All DVI+ selected samples were reactive with both test reagents. Data from the third site was analysed separately and consisted of 3066 samples, with 2561 positive and 505 negative concordant results giving 100% agreement with a 99.99% LBCI.

Summary/Conclusions: These studies, comparing the two reagents formulated to detect the variant D (DVI), demonstrated that the new Anti-D(DVI) reagent, performed as expected. The BioVue System Anti-D(DVI) test reagent demonstrated reactivity and specificity with routine RhD-positive samples along with detection of the DVI variant and other variant D examples. Testing of donors with a reagent that is prefilled in a column that routinely detects the DVI variant and detects other weak D examples will facilitate improved efficiency and productivity of the laboratory.

P289 | A case of anti-G in pregnancy

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Background: In Rh blood group system, the G antigen is absent from red blood cells lacking the D and C antigens, but is present in nearly all D- or C- positive red blood cells. For regular transfusion, it is not necessary to distinguish anti-D and anti-C from anti-G. However, it is crucial during pregnancy since initial antibody testing can misidentify anti-G as anti-D and anti-C. When a patient is genuinely a candidate for anti-D immunoglobulin (RhIG) prophylaxis, the misleading presence of anti-D will prevent the patient from receiving RhIG.

Aims: Patients who tested positive for anti-D or anti-G need to be monitored closely because they are at risk of having haemolytic disease of foetus and newborn (HDFN). Therefore, precise identification enables clinicians to appropriately manage the patients.

Methods: This poster shall precede a case of a 29 years old woman, gravida 4 para 1+2 at 21 weeks of pregnancy was observed during her antenatal visit. Her previous miscarriages was at 12 weeks of pregnancy in 2018 and 10 weeks of pregnancy in 2019. In 2020 patient was wrongly labelled as Rh positive hence no RhIG was administered post labour. Her child has history of neonatal jaundice and underwent phototherapy, however no exchange transfusion was done. She has history of motor vehicular accident in 2010 which required 3 units of packed red blood cell transfusion.

Her blood group is B with rr (dce/dce) phenotype. A Direct Coomb's test was performed using a gel technique (ID-Card "LISS/Coombs") and revealed to be negative. Antibody screening (3-cell panels, Bio-Rad ID-DiaCell I-II-III Asia) was positive (2+) by gel technique (manual method). Antibody identification (11 cell panel, Bio-Rad ID DiaPanel, ID DiaPanel-P, gel technique) revealed the presence of anti-C and anti-D specificities. In view of the presence of combined anti-D and anti-C, the presence of anti-G was suspected and further confirmed by double adsorption and elution using R2R2 (DcE/DcE) and rr' (dce/dCe) cells, which was performed by the tube technique.

Results: Anti-D can result in severe HDFN as the D antigen is highly immunogenic. Anti-C may cause HDFN to certain extend. Contrary to anti-D and anti-C, the anti-G rarely achieves high titre that could endanger the foetus. However, several investigations asserted that HDFN could be caused by anti-G, with high anti-G titre indicative of

moderate to severe HDFN. Anti-G identification is a time-consuming and very complex process that interests curious of immunohematologists and involves difficult workups of double adsorption and elution procedures. Through the use of the differential adsorption and elution techniques, we were able to corroborate the case of anti-G with anti-D and anti-C in our investigation.

Summary/Conclusions: This study emphasises the significance of identifying anti-D, anti-C, and anti-G in an antenatal woman with an initial antibody screening that is positive for anti-D and anti-C in order to manage HDFN and administer prophylactic RhIG. Pregnant women who have anti-G, but without anti-D need prophylactic RhIG to prevent RhD HDFN. Red blood cells that are G, D, and C antigen negative should be offered to individuals who have these antibodies if transfusion is needed.

P290 | Association of ABO blood group with COVID-19 severity among patients admitted at Eka Kotebe General Hospital, Addis Ababa, Ethiopia

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Background: The Corona Virus Disease of 2019 is an emerging infectious disease outbreak that was later declared a global pandemic in 2020. It is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The illness brought on by COVID-19 can range from being asymptomatic to being fatal. It has caused millions of deaths worldwide to this point. Ethiopia is one of the nations with the most COVID-19 cases. The number of deaths attributable to this disease varies greatly between various nations and regions. It is hypothesised that the presence of certain ABO blood groups may be indicative of increased severity or complications in COVID-19 patients. However, there has been no specific study conducted on the association of blood group and COVID-19 severity in the local context so far. Hence, this study was performed, where the association between blood type and the overall severity of the disease caused by COVID-19 was observed.

Aims: Was to assess the association between ABO blood group and COVID-19 severity among patients admitted at Eka General Hospital, Addis Ababa, Ethiopia.

Methods: A hospital based retrospective cross-sectional study was conducted using a systematic random sampling technique, and data were collected using the ODK data collection tool. Descriptive statistics such as frequency, percentage, mean, standard deviation, median, and interquartile range were used to summarise the results. An ordinal logistic regression model was used to assess the association between blood group and severity of COVID-19. The basic assumptions and adequacy of the model were evaluated. Multivariate analysis was performed to assess and screen out significant independent variables. P-value ≤ 0.05 was considered as statstically significant association.

Results: In the study, 355 patients' medical records were included. According to the study, blood group O predominates with a magnitude of 37.5% (133). The study found that the magnitude of blood groups A,O,B, and AB among those with severe COVID illness was 68.3%, 65.4%, 62.7%, and 54.2%, respectively. There was no association between ABO blood group and severity of COVID-19. However, the result showed that pregnancy and the presence of complications were statistically significant for the severity of COVID-19.

Summary/Conclusions: The investigation did not show a significant association between blood groups and COVID-19 disease severity.

P291 | Abstract withdrawn

P292 | A rare report of H-partially deficient, non-secretor phenotype from India. An Ah like the Réunion and unlike a Para Bombay: Clarifying misinterpretations

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Background: In 1961, the A_h phenotype was first described in a patient as partial suppression of A in a Bombay variant. Later in 1982, forty-two H-deficient individuals but with detectable amounts of A and/or B antigens on erythrocytes with anti-H antibodies were found on Réunion Island, off the East Coast of Africa called the Rèunion Oh, A_h, and B_h phenotypes. Prior to this only two largest series of H-deficient Bombay phenotype were published in the Indians of Bombay and South Africa.These reunion type phenotypes have not been reported since then to the best of our knowledge and may lead to misinterpretations when encountered in the laboratory settings of low income (LIC's) and low middle income (LMIC's) countries.

Aims: We report the first case of H-partially deficient non-secretor-the A_h phenotype in an elderly woman from India that do not fit into the common Bombay, or the Para Bombay series of H-deficient phenotype. We arrived at a conclusion only after series of unclear findings and help from the International Blood group reference laboratory (IBGRL), NHS, Blood and Transplant, Bristol.

Methods: A 64-year-old $G_6P_6L_5$ female was admitted at our tertiary care health centre,later diagnosed as acute disseminated encephalitis with post-infectious demyelination. A request of PRBC was received in view of 5.8 gm/dl and haematocrit of 16.2% at our blood centre. The pre-transfusion tests indicated forward/cell grouping as A Rh 'D' positive whereas reverse/serum grouping as "O" with a 4+ reaction with O cells. Type II discrepancy was ruled out and type IV was evaluated. IAT was pan-reactive (4+) using extended red cell panel. Red cells showed a negative reaction with Anti H that raised the suspicion

of lack of H antigen, but the presence of a 4+ agglutination with Anti-A dismissed our approach towards the more commonly encountered Bombay phenotype and the presence of Anti H antibody. Saliva testing was not possible due to patients' condition but the patient was Le^b negative. A strong suspicion of a clinically significant high-frequency IgG type antibody was established. Phenotype matched blood from our rare blood donor registry was also incompatible (4+) in addition to the samples from siblings and children of the patient. To resolve the ambiguous findings and find compatible blood type,patient's blood sample was sent to IBGRL for analysis.

Results: The final report from the IBGRL confirmed the presence of A_h phenotype with strong Anti-H reacting strongly at 37^0C present in the plasma. H-deficient blood group phenotypes are classified as H-deficient non- secretors (Bombay Oh), H-deficient secretors (Para Bombay), and H-partially deficient non-secretors (O_h reunion, A_h and B_h, AB_h). Tests with Anti-H antisera is significant in identifying these phenotypes.With the advent of potent monoclonal antisera, a strong reaction is observed even on weak antigen expression that explains the 4+ reaction with Anti-A antisera in our cell grouping, misinterpreting the antibody as an unknown high-frequency antibody instead of Anti H. Hence, such discrepancies must be approached systematically with a possibility of H-deficient phenotypes that have a high propensity of being found in India.

Summary/Conclusions: These H-partially or completely deficient phenotypes are rare and requires robust testing for identification. Moreover, assigning correct notations is important as most literature from LMIC and LIC uses A_h and B_h for Para Bombay phenotypes that needs correction. Hence, to enable identification we have initially compiled and highlighted the major characteristic differences between different H-deficient phenotypes available from literature and subsequently deviced an illustrative diagnostic laboratory approach using red cell, serum and saliva testing to identify these H-deficient phenotypes even in resource limited settings where molecular testing is not readily available.

P293 | Abstract withdrawn

P294 | Risk of clinically significant anti-D in Sickle Cell Disease patients with partial D transfused with D-positive red blood cells

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Background: RH diversity in patients with Sickle Cell Disease (SCD) contributes to the high rate of Rh alloimmunization. There are a plethora of D variants caused by genetic variations within the *RHD* gene that alter epitopes of the RhD protein, categorized as partial D. The identification of partial D can be of clinical importance because carriers of partial D antigen may develop clinically significant anti-D when transfused with D-positive red blood cell (RBC) units.

Aims: To identify which SCD patients with partial D are at risk of clinically significant anti-D production, we investigated anti-D immunization in a cohort of partial D SCD transfused with D+ RBC units. We also accessed the clinical significance and the persistence of the alloantibody produced.

Methods: A retrospective study was performed in a cohort of 94 SCD patients with partial D antigen who received at least three D+ RBC units. All patients had complete serological and molecular analyses. Number of D+ RBC transfusions, anti-D identification and anti-D persistence were also recorded. A lookback on the donors and transfusions for those patients including laboratory and clinical findings was performed. Laboratory and clinical findings were also used to evaluate the clinical significance of the alloantibodies produced.

Results: Partial D phenotypes of these patients included DAR, DOL, DAU3, DIVa, DIIIa, DVa and Weak partial D 4.0 and DAU0. Nineteen (20.2%) patients produced anti-D: 3 DAR, 2 DOL, 3 DAU3, 2 DIVa, 3 DIIIa, 3 DVa, 1 Weak partial D 4.0 and 2 DAU0. Laboratory and clinical evidence of a delayed haemolytic transfusion or decreased survival of transfused RBCs and persistence of the anti-D produced were associated with partial D (DAR, DOL, DAU3, DIVa, DIIIa, DVa). Anti-D produced by the patients with DAU0 and Weak partial D 4.0 were not persistent and not clinically significant.

Summary/Conclusions: SCD patients with partial D (DAR, DOL, DAU3, DIVa, DIIIa, DVa) are more likely to develop clinically significant and more persistent anti-D and therefore may benefit from prophylactic D- RBC units or *RHD* genotype-matched transfusions. Our results also suggest that patients with D encoded by *RHD*DAU0* with no evidence of missing epitopes and Weak partial D 4.0 are not prone to develop clinically significant anti-D.

P295 | Comparison for antibody identification testing between conventional tube method and column agglutination technique (CAT) by a fully automated analyser

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Background: Our blood center averages about 820,000 donation per year. Testing around 2500 donors each day for the presence of blood group antibodies and identification (AbID) when positive has traditionally been performed using a tube test method (TTM). However, significant turnaround time (TAT) and hands-on-time (HOT) to complete this testing with limited staff has led us to evaluate a different approach to testing using a fully automated test system.

Aims: This study was designed to compare results of the TTM and automated AbID test methods, TAT and HOT for AbID to recognise the best option for our blood center to improve productivity and efficiency while maintaining routine capability to detect and identify blood group antibodies when present.

Methods: The tube test method employed a room temperature method with a 5-minute incubation time/read and a separate LISS based test

with a 15-minute incubation at 37C with a Polyspecific AHG and read only at AHG. The automated test by column agglutination technique (CAT) using the ORTHO VISION® Max Analyser employed both a room temperature phase and AHG phase of testing using ORTHO BioVue® System Cassettes (BioVue Neutral Cassette and BioVue AHG Polyspecific; Anti-IgG, C3d Cassette) evaluating both 3% red cells with BLISS (a LISS based additive for BioVue tests) and 0.8% red cells prepared in low ionic strength red cell diluent. Each sample was compared across the three methods using the same lot of panel cells.

Known sample population

Eighty-two samples, sixty-two with previously identified blood group antibodies from fresh frozen plasma were tested. These known antibody samples included a variety of blood group antibodies. Twenty of the samples were known negative samples. Samples were grouped in sets of ten and AbID tested on the same day by all three test methods at both phases.

Random sample population

100 random samples that initially tested positive by CAT on the VISION via an antibody screen were tested by all three AbID methods. The same testing approach was used as for the known samples. TAT (minutes), HOT (minutes) and process steps (number) were evaluated and measured for each test method and phase of testing.

Results: The twenty known negative samples were negative across all three methods. The TTM detected antibody in all 62 samples, while the 0.8% CAT method detected 61 of 62 and the 3% CAT method, 55 of 62.

In the 100 random samples, the TTM detected and identified 63 samples with antibody present. The automated 0.8% CAT method using demonstrated reactivity in 74 samples while the 3% method detected antibodies in 52 samples.

TAT measurements revealed a 7-13% reduction in the RT test by CAT methods as compared to TTM and with greatest reduction using the AHG CAT methods with a 40-52% decrease compared with TTM.

HOT evaluation demonstrated that only one minute is required to execute the fully automated CAT based tests compared to TTM at RT (10 min.)/AHG (24 min.), which is a 90 to 96% time saving in actual human interaction to complete the testing.

The number of process steps were 10 per automated CAT method as compared to the RT TTM (31) and AHG tube (66) methods.

Summary/Conclusions: Based on the comparison of the three methods the 0.8% automated CAT method on ORTHO VISION Max produced the best capability of detecting and identifying blood group antibodies as compared with the TTM and 3% automated CAT method. Additionally, the reduction in TAT of 7% and 40% using automated testing along with the decreased HOT of greater than 90% using the 0.8% CAT automated method versus TTM made our decision to move to fully automated testing an easy one with the impact it has on productivity and efficiency.

P296 | Significance of determination of the Rh phenotypes and providing phenotype-matched blood to cancer patients: A retrospective analysis from a tertiary care oncology centre in North India

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Background: Multiple reports are available from different parts of the globe indicating the incidences of alloimmunization and blood transfusion related reactions, which emphasises on the need for phenotyping and providing antigen matched safe blood. Antibodies to the Rh system are the most implicated in these cases. The need for phenotyping and providing antigen matched blood is even more significant in Oncology patients who undergo multiple blood transfusions and thus can develop alloantibodies against antigens of the minor blood group systems

Aims: To determine the frequency of Rh & Kell antigens & phenotype for both donors & patients with an aim to emphasise the importance of use of Rh/K phenotype cross matched PRBC units to minimise the alloimmunization & transfusion reactions

Methods: 10,000 blood donors and 4,000 patients were investigated between Oct 2017 to July 2019. Each donor unit was tested for blood grouping, antibody screening & Rh Kell antigen Phenotyping and the blood unit was issued after patient's blood grouping, antibody screening by 3 cell panels & Rh Kell antigen phenotyping followed by cross matching with a Rh/K matched phenotype RBC unit

Results: 9452 donors were D positive (94.5%) while 548 tested D negative (5.5%). Overall Rh & K antigens frequencies in donors were: 'e' (98%) > 'D' (94.5%) > 'C' (86.6%) > 'c' (57.5%) > 'E' (18.8%) > K (0.98%). Among patients, 3762 tested D positive (94.05%) and 238 tested D negative (5.95%). Overall Rh and K antigens frequencies in patients were: 'e' (98.5%) > 'D' (94.05%) > 'C' (90.2%) > 'c' (51%) > 'E' (18.2%) > K (1.8%).

Summary/Conclusions: Our study has given us more clarity on the prevalence of major Rh antigens in our donor as well as patient populations, highlighting the similarities as well as differences. This variance holds a great significance, since such donor units when transfused into patients may lead to alloimmunization and adverse transfusion reactions. Hence, determination of Rh phenotypes and providing phenotype matched blood will help prevent such events.

P297 | Analysis of red blood cell alloantibody frequency in the Korean population

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Background: The frequency of red blood cell (RBC) alloantibodies can vary significantly among different ethnic groups. However, studies on this issue are often limited in their ability to adequately represent the transfusion candidate population due to their reliance on data from only a few tertiary hospitals.

Aims: The aim of this study is to investigate the frequency and distribution of RBC alloantibodies among Koreans using data collected from a large referral laboratory.

Methods: We analysed the results of unexpected antibody identification tests performed on both inpatients and referrals at a major tertiary hospital from January 2012 to December 2021. Unexpected antibody identification tests were performed with ID-DiaPanel (Bio-Rad), ID-DiaPanel-P (Bio-Rad), and Resolve Panel A (QuidelOrtho). The number of unexpected antibody identification tests performed annually in Korea was obtained from the Health Insurance Review & Assessment Service statistics.

Results: Over the ten years, 17,012 unexpected antibody identification tests were performed, of which 2,799 (16.5%) were conducted on inpatients while 14,213 (83.5%) were referrals. Referrals from medical institutions smaller than secondary general hospitals accounted for 25.4% (12,025/47,284) of domestic claims. The most commonly identified RBC alloantibodies in inpatients were anti-E (37.3%), anti-c (15.4%), anti-Le^a (14.5%), anti-M (9.2%), anti-Le^b (5.3%), anti-C (5.3%), anti-P1 (4.9%), anti-e (4.7%), anti-D (4.2%), anti-Fy^b (3.8%), and anti-Jk^a (2.1%). Among 72.3% (10,275/14,213) of referrals excluding pregnant women, frequent alloantibodies were anti-E (31.8%), anti-c (11.2%), anti-Le^a (9.1%), anti-Le^b (5.8%), anti-M (5.1%), anti-C (4.3%), anti-P1 (4.0%), anti-e (3.7%), anti-D (1.7%), anti-Jk^a (1.3%), and anti-Fy^b (1.2%). Pregnant women accounted for 27.7% (3,938/14,213) of referrals, and the most frequent alloantibodies were anti-E (18.1%), anti-Le^a (13.0%), anti-M (10.9%), anti-c (6.9%), anti-Le^b (6.0%), anti-P1 (3.4%), anti-C (2.6%), and anti-e (2.5%). Although anti-D was found in 18.4% of pregnant women, most of the cases were related with Rh immune globulin administration.

Summary/Conclusions: We were able to identify the frequency and distribution of RBC alloantibodies in a large Korean population with high representativeness. These results will help develop strategies for safe transfusion.

P298 | How effective are automated systems in antibody identification?

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Background: The Red Cell Reference Laboratory at Health Sciences Authority receives over 3,000 referrals each year, from 20 hospitals across the country. This number increases at a rate of 7% every year. Although automated systems have been well in-place for routine pre-transfusion tests such as blood grouping and antibody screen, the feature for antibody identification has been largely ignored. Adoption of such a feature may help hospitals identify straight-forward antibodies, without the need for referrals and longer waiting times. Despite these advantages, hospitals have mostly refrained from implementing antibody identification on their automated system for various reasons, such as the lack of familiarity and resources. The complexity of tests, in certain cases, may also extend beyond the designed capability of their instrument. Scenarios involving patients with multiple antibodies, autoantibodies, and those on immunotherapy such as anti-CD38, are examples that would leave the process largely unresolved.

Aims: The current practice where hospitals refer all cases with a positive antibody screen for identification, may no longer be the best approach. This unnecessarily burdens the reference laboratory with repeat investigations, sometimes comprising of nothing more than straight-forward antibodies which automated systems in hospitals are already well equipped to handle. In order to recalibrate practices, there is a need to

assess the capabilities of automated systems against antibodies routinely identified, in order to provide a clearer basis for a more pragmatic approach.

Methods: A retrospective study using cases from 2022, was theoretically applied using routine reagents (i.e., antibody screening and identification panels) procured by our laboratory between Jan and Mar 2022. Antibodies from these cases were assessed on the rule-in and rule-out algorithms specific to the automated system used. Cases with antibodies successfully identified, and the remaining excluded, were tabulated.

Results: Out of the 3,335 cases referred to the Red Cell Reference laboratory in 2022, 2,678 (80%) were identified with a single antibody, 540 (16%) with 2 antibodies, 98 (3%) with 3 antibodies, 17 (0.5%) with 4 antibodies and 2 (0.06%) with 5 antibodies. Of those identified with a single antibody, 1,411 (42%) cases were deemed to have a clinically significant alloantibody, while 1,267 (38%) of those were identified with an alloantibody of lesser clinical significance. When the theoretical algorithm of an automated antibody identification system was applied to these cases, most of the clinically significant alloantibodies could be identified using a minimally required rule-of-2 principle. Only anti-Mia (23%) and anti-Dia (0.1%), which lacked antigen representation on the identification panels used, could not be identified by the system. In cases involving only a single antibody identified, the presence of other clinically significant alloantibodies

could be fully, or partially excluded (with less than 2-homozygous examples), in >90% of the time.

Summary/Conclusions: The task of antibody identification varies widely, in terms of complexity as well as the resources and expertise needed to resolve them. The use of automated systems may well expedite the process by reducing the need for referrals. Cases with previously identified antibodies which mostly require exclusion of newly developed antibodies, can be assessed against current resources for suitability. Cases with straight-forward antibodies can potentially be resolved without the need for referrals or additional blood samples from the patient. Cases that are not resolved would also benefit from the additional information gained, especially when provided along with the referral. Manufacturers of
these systems can also significantly improve the process by incorporating red cell reagents capable of identifying anti-Mia. The resolution of anti-Mia, without the need for referrals, would most certainly increase the likelihood of adoption among hospitals, where anti-Mia remains the most frequently detected antibody.

P299 | Prevalence and specificity of red blood cell alloantibodies identified among immunised Polish patients (2019-2021)

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Background: Detection and identification of red blood cell (RBC) alloantibodies is a part of routine pre-transfusion testing, followed by patient/donor crossmatching. This procedure can be time consuming in patients with RBC alloantibodies against multi-specific or high-frequency antigens. For multiple blood recipients, the shorten path to select of potentially compatible donors is to search a blood donor registry with extended phenotypes. In 2021, within the Polish Ministry of Health policy program "Ensuring the self-sufficiency of the Republic of Poland in blood and blood components for the years 2021-2026", creation of such donor registry has been planned. Its essential element was the recognition of the Polish patients' needs.

Aims: 3-year analysis of prevalence and specificity of alloantibodies identified among Polish patients in the period 2019 -2021 to estimate the transfusion-related requirements of alloimmunised Polish recipients.

Methods: In Poland, the identification of RBC alloantibodies is performed by Blood Transfusion Centers (BTCs). 17/21 of Regional BTCs participated in data collection and completed the forms prepared by the Institute of Hematology and Transfusion Medicine (IHTM) referring to the number of patients with alloantibodies and antibody specificities (whenever possible), identified between 2019 and 2021. The forms were forwarded to IHTM for analysis of data.

Results: In a 3-year period, all participating BTCs reported 22 038 alloimmunised patients with RBC alloantibodies and 9 BCTs reported their specificities identified in 5934 (41.1%) patients who potentially required blood transfusion. Single antibody specificity was observed in 79.9% of patients and multi-specific antibodies in 20.1% (16.87% with two, 2.70% with three, 0.45% with four, 0.07% with five, and 0.02% with six specificities). Over a half (55.7%) of all antibodies detected in the analysed group were clinically significant and directed against Rh antigens (23.9% anti-E; 16.6% anti-D, 9.1% anti-C, 5.1% anti-c, 1.0% anti-e), and 19.9% were anti-K antibodies. Antibodies to antigens from the Kidd (Jk^a, Jk^b), Duffy (Fy^a, Fy^b), and MNS (M,N,S,s) systems were detected in 19.7% of patients (6.4% anti-M, 5.2% anti-Jk^a, 4.2% anti-Fy^a, 2.1% anti-S, 0.8% anti-Jk^b, 0.6% anti-Fy^b, 0.2% anti-s, and 0.2% anti-N). Other specificities of red cell alloantibodies were detected in the remaining 4.8% of patients, and 5.5% of them were clinically significant or sometimes clinically significant alloantibodies against high-frequency antigens such as: anti-k(cellano), anti-Kp^b, anti-LW^a, anti-Yt^a, anti-Lu^b, anti-Lan, and other including low-frequency antigens: anti-Yt^b, anti-Kp^a. In 20% of multi-alloimmunised patients, antibodies against antigens from the Rh and Kell systems coexisted with the antibodies against antigens from Kidd/Duffy/MNS systems.

Summary/Conclusions: Over 75% of alloimmunised patients required compatible blood units in D, C, c, E, e and K antigens. For about 20% of immunised patients, blood should be additionally selected in extended phenotype including Jk^a, Jk^b, Fy^a, Fy^b, S or s antigens. This data will be used to create a donor registry facilitating supply of antigen-negative blood, especially to patients with multi-specific alloantibodies or antibodies to high-frequency antigens.

P300 | Paroxysmal cold haemoglobinuria in a pediatric patient with concurrent warm and cold autoantibodies

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Background: Pediatric autoimmune haemolytic anaemia (AIHA)is a rare and potentially fatal disease without clearly defined criteria for diagnosis of the four subtypes: cold agglutinin syndrome (CAS), warm AIHA, mixed AIHA, and paroxysmal cold haemoglobinuria (PCH). Laboratory evidence of haemolysis, direct antiglobulin test (DAT) results, and the Donath-Landsteiner (DL) test provide a basic diagnostic framework, which is complicated in practice by low prevalence, atypical presentations, and variation in diagnostic techniques and interpretation. The presence of multiple concurrent autoantibodies creates a diagnostic dilemma and complicates clinical management decisions.

Aims: This report aims to highlight a novel presentation of three concurrent autoantibodies in a pediatric patient with critically severe haemolytic anaemia.

Methods: The clinical case is reported from a retrospective review of combined laboratory data, clinical management, specialist consultation, and available literature.

Results: A young pediatric patient who presented with hematuria and jaundice following a recent respiratory illness was shown to have a haemoglobin of 3.6 g/dL, along with elevated indirect bilirubin and immature reticulocyte fraction. Laboratory evaluation showed a DAT positive for C3 only, PCR panel positive for rhinovirus and respiratory syncytial virus, and viral serology positive for mycoplasma IgM and IgG. With a presumptive diagnosis of CAS, judicious red blood cell transfusion using a blood warmer increased haemoglobin to 6 g/dL. Confirmatory testing sent to an immunohematology reference laboratory revealed microscopic agglutination on DAT with IgG and weak eluate pan-agglutination consistent with a warm autoantibody. Glucocorticoids were administered and the blood warmer was discontinued for warm AIHA. Five additional transfusions were required over four days as haemoglobin continued to decline, leading to clinical reversion to suspected CAS. Subsequent testing demonstrated the cold autoantibodies had a maximum anti-I titre of 4:1 and no evidence of thermal amplitude, weakening the evidence for clinical significance. Consultation with the institutional transfusion medicine specialist prompted collection for the DL test, which demonstrated a definitive anti-P IgG biphasic haemolysin consistent with a diagnosis of PCH. Following a return to cold aversion and permissive anaemia, haemoglobin remained above 7 g/dL and the patient was discharged in stable condition.

Summary/Conclusions: Piecemeal evaluation for the etiology of AIHA may lead to diagnostic anchoring or oscillation, as evidenced in this case of an atypical presentation of three unique concurrent autoantibodies. Precipitous and severe anaemia following respiratory infection in a patient under 5 years old is a classic presentation for PCH, but it was complicated by an initial DAT positive for C3 with positive mycoplasma IgM characteristic of CAS and subsequent DAT positive for IgG with a pan-agglutinating eluate characteristic of warm AIHA. Although low anti-I titre, lack of thermal amplitude, weak in vitro warm reactivity, and inadequate glucocorticoid response indicted these two etiologies, they served as distractions and delayed testing for PCH. Thorough diagnostic evaluation of severe AIHA in the pediatric setting is essential and benefits from cooperative evaluation between transfusion medicine specialists, immunohematology technicians, and treating clinicians.

P301 | A case of RhD antigen blocking in a newborn with severe haemolytic disease of the newborn (HDFN)

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Background:

In the presence of haemolytic disease of the newborn due to a maternal antibody, correct antigen determination of the corresponding antigen in the newborn is absolutely required for plausibility testing of the cause of haemolysis. False antigen negativity may lead to further diagnostic testing which in the worst case can cause delay of adequate therapy of the newborn. Antigen blocking is a rare phenomenon caused by maternal IgG antibodies saturating neonatal red blood cell (RBC) antigens. This may result in false negative typing using monoclonal IgM reagents.

Aims: We report a case of Rhesus D antigen blocking in a neonate with severe haemolytic disease of the newborn caused by high titre maternal (G III, inadequate Rhesus D prophylaxis at G II) anti-D (4096), beside an anti-Jk^a (64) and anti-C (2).

After early delivery (week 36 + 0) due to haemolysis and reduced fetal mobility the newborn needed top-up transfusions (ccddee, Jk^a-). Initially, the neonate was typed Ccddee, Jk^a positive and the direct antiglobulin test was strongly positive (IgG 4+, C3d 3+). The eluate showed anti-D, anti-Jk^a and anti-C.

Methods: To elucidate the discrepancy between the declared Rhesus phenotype (Ccddee) and eluate specificity (anti-D), a sample (neonate EDTA heel blood) was sent to our reference laboratory. Pheno- and genotype analyses were performed using standard techniques including two different saline reactive anti-D antisera (Grifols, Duedingen, Switzerland) and commercially available PCR-SSP kits (inno-train, Frankfurt, Germany). The neonatal Rhesus phenotype was reevaluated serologically after dissociating the maternal IgG ab from the RBC by EGA treatment (EDTA Glycine-Acid Kit, Immucor, Dreieich, Germany). Direct antiglobulin test was performed using a polyspecific anti-human globulin card (BioRad, Cressier, Switzerland) before and after EGA treatment. Standard serological methods for antibody identification were applied (gel-card; BioRad, Cressier, Switzerland) on heat eluted neonate erythrocytes. Clinical and laboratory findings of the neonate and postnatal therapies (top-on transfusions and supportive therapy), as well as data on mother's antibody-titres and prior pregnancies were kindly provided to us by the physicians in charge.

Results: Untreated red blood cells were clearly typed as Rhesus D negative, an observed mix field reaction with anti-C was consistent to the top-up transfusion (ccddee, Jk^a negative) directly after birth. Three consecutive EGA treatments revealed an additional mixed field reaction with anti-D, predicting two separate RBC populations, namely CcD.ee and ccddee. The initially strongly positive direct antiglobulin

test (IgG 4+, C3d 3+) decreased markedly after EGA treatment (1+), as expected. A subsequent genotyping confirmed the suspected serological typing as CcDdee, Jk^a positive. Due to prolonged anaemia the newborn received a total of three top-up transfusions (ccddee, Jk^a negative), directly after birth (Hb 64 g/L), on day 9 (65 g/L) and day 28 (84 g/L). Additionally, intensive photo- and O_2-therapy were given to treat hyperbilirubinemia and low saturation levels.

Summary/Conclusions: Here, we present a case of Rhesus D antigen blocking by maternal Anti-D in a neonate initially mistyped as RhD negative. After dissociation of maternal high titre IgG by EGA the Rhesus D positive phenotype of neonatal RBC became detectable and was confirmed by genotyping. Timely consideration of antigen blocking in cases with severe fetal anaemia in the presence of high titre maternal antibodies helps to rapidly establish a diagnosis and to initiate the necessary potential life-saving therapy.

P302 | Comparison of column agglutination technique and tube test for ABO antibody titration and crossmatching

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Background: ABO antibody (Ab) titration is an important test for successful ABO-incompatible solid organ transplantation and is available on various immunohematology autoanalyser these days. The indirect antiglobulin crossmatch (IAC) is also one of the most important pretransfusion tests for patients with clinically significant unexpected Abs. Our laboratory in 2019 in Korea was the first to introduce Erytra Eflexis (Diagnostic Grifols, Barcelona, Spain, abbreviated as Eflexis). However, there is a paucity of comprehensive studies evaluating ABO Ab titration and crossmatching using DG Gel Coombs Card (Diagnostic Grifols abbreviated as Coombs card).

Aims: The aims of the study were to compare automated ABO Ab titration using the Eflexis and conventional tube test (CTT) and to compare manual IAC using Coombs card with CTT.

Methods: ABO Ab titrations were performed by CTT and on the Eflexis using patient samples that were not treated with dithiothreitol. IgM was defined by ABO Ab detected by the immediate spin phase of CTT or by the Eflexis using DG GEL Neutral card. Total Ab was defined by CTT IAC or by the Eflexis using Coombs card. The titres were converted into log steps of 2 ($n = 20$ for each A, B, O blood group). A reference range for ABO Ab titre using the Eflexis was determined using samples from health checkup patients ($n = 30$ for each A, B, AB blood group) and was defined as the lower limit of the 95th percentile of each range and higher. In 40 cases that had pre-identified Abs by an Ab screening test using the Eflexis, the manual IAC was performed using a Low Ionic Strength Salt Solution (LISS) for CTT or using Coombs card. The hemagglutination strengths (0∼4+) for the two techniques were compared by paired t-test. Donor red cells used for crossmatch tests had been already screened by phenotyping to have the corresponding antigens to the patient's identified Abs.

Results: The average titre for IgM anti-B from group A by the CTT vs Eflexis was significantly different, 3.2 vs 2.4 ($p < 0.001$), and those of anti-A titres from group O samples were also significantly different, 4.5 vs 5.5 for IgM ($p = 0.001$) and 6.3 vs 7.3 ($p < 0.001$) for the total Ab. There were no significant differences for total Ab anti-B from group A, anti-A from group B, and anti-B from group O. The reference ranges were anti-B in group A ≥1:2, anti-A in group B ≥1:8, anti-B in group O ≥1:8 (IgM) and 1≥:32 (total Ab), and anti-A in group O ≥1:16 (IgM) and ≥1:32 (total Ab). In the IAC card crossmatch comparison, the average hemagglutination strength was 1.65, which was significantly stronger than that of the LISS-IAC of CTT of 0.50 (P<0.001).

Summary/Conclusions: In this study, the reference range of IgM or total Ab of anti-B and anti-A for each ABO blood group was determined using the Eflexis. The study showed slight differences in the ABO Ab titrations between the Eflexis and CTT depending on the blood group or type of ABO Ab. It appears beneficial to switch from CTT to the Coombs card method for IAC crossmatching because of the high sensitivity of the Coombs card. These results are expected to help laboratories using Erytra Eflexis and DG Gel Coombs Card.

P303 | Summary of blood group serology proficiency testing in a Chinese transfusion laboratory

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Background: Automachine was widely used in the transfusion laboratory, so the classical tube methods is rarely in the routine test.

Aims: To assessment the blood group serology proficiency of the classical tube methods in Chinese transfusion laboratory to deal with the difficult problem.

Methods: We issue four kind product of blood group serology routine test to 65 laboratories. Titration, sensitivity analysis, weak antigen analysis, score of agglutinative reaction strength and the specimen's control.

Results: 62 feedback report specimen's control is on the control (the serum control and the red cell control attenuation rate less than 30% in logistics). Titration: the reference answer is 256(tube IAT). The geometric mean is 268 in tube IAT and 830 in CAT (Column agglutination test). Sensitivity analysis: 26.23% laboratories can test the 0.011 IU(International Unit), 19.67% laboratories in 0.022IU, 27.87% in 0.044IU, 4.92% in 0.055IU, 16.67% in 0.11IU, 8.2% in 0.165IU and 1.64% all negative. There are 9.84% laboratory goes fake positive. Weak antigen analysis: 74.19% laboratories get all the weak antigen, 3.23% leak detection some weak antigen, 22.58% go wrong with fake positive. Score of agglutinative reaction strength: The low error rate of the agglutinative score happened in the strong period and in the weak period, the high error rate of the agglutinative score happened in the middle period.

Summary/Conclusions: In the titration test, 72.13% laboratories get the median result, little higher than the reference answer. The result of using CAT is higher than using tube IAT. In the sensitivity analysis test, IAT's error range is more than the CAT. Some laboratories detect

the lower concentration, maybe using enhance serological method in IAT. In weak antigen analysis, 22.58% go wrong with fake positive and 74.19% laboratories get the right answer, it maybe that we had over treated the B substance on the O cell. In the Score of agglutinative reaction strength: There was significant difference between different laboratories in the middle reaction period. In the middle reaction period, CAT is stronger than tube IAT in half to 1+. Blood group serology proficiency testing is extension of the EQAS, to assessing the basic operation: such as titration, judgement weak antigen and antibody, score the agglutinative reaction strength etc. If participate laboratories should be unified standard in routine operation, to improve uniformity result in these laboratories.

P304 | Factors affecting red cell alloimmunization rates in oncology patients: Experience from a major tertiary care oncology centre

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Background: Multitransfused patients such as thalassemia major, aplastic anaemia, sickle cell disease, and hematological malignancies are at a high risk of developing alloantibodies. Determining alloimmunization rate in oncology patients is significant considering its impact on post transfusion red cell survival.

Aims: - To estimate the incidence of red cell alloantibodies in the oncology patients

- To analyse the frequency and specificity of red cell alloantibodies in patients

- Management of transfusion needs of patients with red cell alloantibodies.

Methods: This was a retrospective observational study approved by Institutional Ethics Committee (IEC) conducted in the Department of Transfusion Medicine (DTM) at a tertiary care oncology centre. Results of blood grouping and antibody screening of 46081 patients over 18 months were reviewed. Initial grouping and antibody screening was performed by Column Agglutination Technique (CAT). Blood grouping and antibody screening (using commercially available 3 cell panel) were repeated by CAT method or Conventional Tube Technique (CTT) in case of positive antibody screen. Antibody identification was performed using commercially available 11 cell panel and the panel results were interpreted using an Antigram by "cross out" or "exclusion" method. Patients with autoantibodies and blood grouping discrepancies were excluded from the study. Analysis was done to find out correlation between alloimmunization and factors like age, gender, blood group, diagnosis, obstetric and transfusion history. After the identification of the antibody, AHG (Antihuman Globulin) crossmatch compatible and/or antigen-negative units were selected for transfusion.The overall analysis of the data was descriptive with results presented as percentage for categorical data. Chi-square test was applied to see the relationship between different variables.

Results: A total of 221 samples were found positive for antibody screening and a detailed immunohematological workup was done in 106 samples with the identification of 110 antibodies as 5 samples showed multiple antibodies. The incidence of alloimmunization was found to be 0.48% (221/46081). The incidence of alloimmunization in females were more [0.38%, 77/20370] compared to males [0.11%, 29/25710] ($p < 0.00001$). The incidence of alloimmunization was higher in Rh-D negative blood group compared to Rh-D positive group [0.21 vs 0.13] ($p < 0.00001$). The frequency of alloimmunization in solid organ malignancies was 87.7% (93/106) and that in hematological malignancies was 12.3% (13/106). Maximum alloantibodies were found against the Rhesus (Rh) blood group system i.e.,50.9% (56/110) followed by 32.1% against Lewis blood group system (34/106). Among the anti-Rh antibodies, anti-D and anti-E had higher frequency with 19% each, followed by anti-c with 9%. No statistically significant correlation was found between alloimmunization and history of previous transfusions.

Summary/Conclusions: The alloimmunization rate observed in the oncology patient population in current study was lower compared to other studies probably due to the altered immune status of the patient population. Maximum alloantibodies were detected against Rh blood group system followed by Lewis blood group system. Including antibody screening and identification as a routine practice may improve safety of the management of transfusion needs of multitransfused patients.

P305 | Pediatric sickle cell disease: A Portuguese blood bank experience

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Background: Sickle cell disease (SCD) is a genetic red blood cell (RBC) disorder characterised by chronic haemolytic anaemia and acute vaso-occlusion events (VOE). RBC transfusion remains a crucial treatment for SCD, both improving oxygen delivery and preventing VOE. Despite its benefits, RBC transfusion carries many risks, such as alloimmunization, whose prevalence is much higher in SCD. Several alloimmunization risk factors have been proposed, including antigenic differences between the predominantly caucasian blood donors and african SCD patients, blood group polymorphisms and altered immune response. Regarding the demographic changes in Portugal and the subsequent increase in SCD prevalence, it is extremely important to optimise the management of these patients, preferably through a multidisciplinary team.

Aims: Our study aims to analyse transfusion needs, alloimmunization prevalence, and autoantibody presence in pediatric patients with SCD.

Methods: Retrospective analysis of medical records of SCD patients admitted to the pediatric hematology department at Hospital Dona Estefânia, Centro Hospitalar Universitário Lisboa Central, Portugal from January 2020 to December 2021.

Results: Forty SCD patients were included with a total of 110 hospitalizations. 35 patients (88%) had a known expanded RBC phenotype. 29 patients (73%) had prior RBC transfusions, 5 of them (13%) receiving over 20. Three patients (7.5%) had prior positive results on irregular antibody screening (IAS) for antibodies of known specificity. One had a naturally occurring anti-M and another had an anti-D autoantibody, both undetectable during our study. The third patient had an autoantibody and an anti-C alloantibody, both still detectable. Twenty-four patients (60%) received 139 transfusions during hospital stay. 11 patients (28%) were given between 1-2 transfusions, whereas the most transfused patient received 34. All patients received RBC matched for ABO, D, C/c, E/e and Kell antigens. Four *de novo* alloantibodies (anti-Jka, anti-M, anti-Lua and anti-Jsa) were detected in 3 patients, resulting in an 8% alloimmunization prevalence, at a rate of 3.0 alloantibodies per 100 transfusions. 1 of these patients had 2 alloantibodies (anti-M and anti-Lua) and all of them had autoantibodies.

Summary/Conclusions: The relatively low prevalence of alloimmunization in our study may be attributed to the exclusive use of RBC transfusions matched for C, c, E, e, and K antigens according to our Institutional Policy. Two of the alloantibodies identified (anti-Jsa and anti-Lua) target low-frequency antigens, which usually do not pose challenges on RBC selection. The best strategy to prevent alloimmunization would be to match transfused RBCs as closely as possible, but this is not always feasible. However, the high prevalence of the Rh variant phenotype in this population raises the question of whether RBC genotyping would be beneficial. Among the alloimmunised patients, 1 received 17 transfusions prior to alloimmunization, while the other 2 received only 1-2 transfusions. This suggests that the inflammatory state may be more significant to the alloimmunization risk than the total number of transfusions, as other studies have shown. Additionally, the 3 of them presented concomitant autoantibodies, which has also been identified as an important risk factor. As some antibodies may undergo evanescence after primary response, such as anti-Jka, the implementation of a centralised registry of IAS results would be an important measure in preventing delayed haemolytic transfusion reactions.

P306 | Abstract withdrawn

P307 | Evaluation of reagents and techniques to detect RhCE variants by serologic routine testing

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Background: RhCE variants are more prevalent in Afro-descendent individuals and mainly related to the *RHCE*ce* allele**.** The identification of RhCE variants is carried out exclusively by molecular techniques; however, serological findings, such as diminished RhCE antigen expression, are important to recognise a variant antigen and guide the molecular investigation.

Aims: The aim of this study was to evaluate which monoclonal antisera and techniques are capable to detect the most common RhCE variants associated with the *RHCE*ce* allele in the serological routine in a population of Afro-Brazilians.

Methods: Twenty red cell samples from blood donors and 6 samples from patients previously characterised by molecular testing were routinely analysed for RhCE phenotype using tube, gel and microplate with a set of monoclonal antisera that included the clones MS-24 and MS8011531019 (Anti-C), MS-33 and MS8011531019 (Anti-c), MS-260, MS-80, MS-906, MS-80 and MS -258 and MS80115310119 (Anti-E), MS-16, MS-21, MS-63 and MS80115310119 (Anti-e) from BioRad, Lorne, Fresenius, Immucor and Grifols.

Results: *RHCE*ceJAL* variant associated with the partial c phenotype present in 6 samples reacted weakly with all antisera, with exception of MS-63 that showed negative reaction. Partial e phenotype caused by *RHCE*ceJAL* present in 5 samples, showed a decreased expression with all monoclonal antisera and techniques. Two samples with the *RHCE*ceMO* allele that encodes the partial e and partial c phenotype showed reduced reactivity in tube with all anti-c and anti-e clones but with normal reactivity in gel test. A single sample with the *RHCE*cEJU* variant allele showed weak expression of the E antigen with all tested antisera in tube, except for clone MS-80, which did not show reactivity. Four samples with the *RHCE*ceAR* allele associated with the partial c and partial e phenotypes, showed weak expression of c and e antigens with clones MS-33 and MS-21,MS-16,MS63. Five samples with the *RHCE*ceVS.01* allele associated with the partial e phenotype, reacted weakly (2+) only in tube and one sample with the same allele associated with the partial c antigen, showed weak expression only with clone MS-33 . Three samples with the *(C)ceS* haplotype showed weak C expression with clones MS-24 and MS80115310119.

Summary/Conclusions: Our results show the importance of keeping the tube test in the serological routine and the identification of clones capable of recognizing RhCE variants. Detection of decreased RhCE antigen expression in serologic routine testing can help guide the molecular investigation. This practice may be useful to recognise RhCE variant antigens by serologic routine testing and thus avoid Rh alloimmunization in polytransfused patients, especially those with sickle cell disease.

P308 | Abstract withdrawn

P309 | Evaluation of clinical performance of ABO and RhD blood grouping rapid test

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Background: ABO and RhD Blood Grouping Rapid Test (OABD-402), manufactured by Hangzhou Alltest Biotech Co., Ltd., China is a solid-phase, cassette test for ABO and RhD blood group determination. The test is intended for professional use to detect blood group antigens A, B, and RhD from whole blood at the patient's side. The cassette contains IgM monoclonal anti-A, anti-B, and anti-RhD coated particles, which react with the corresponding antigen on the surface of the red blood cells (RBCs). Antigen-positive RBCs are captured on monoclonal antibodies and observed as a red signal, indicating a positive result. If corresponding antigens are not present on RBCs, a color change into red does not occur, since RBCs are washed away after adding a washing buffer. Various anticoagulants can be used for blood collection: EDTA, heparin sodium, sodium citrate, and potassium oxalate.

Aims: The aim of the study was to evaluate the suitability and performance of the ABO and RhD Blood Grouping Rapid Test (OABD-402), according to the manufacturer's validation protocol.

Methods: We compared the results obtained with routine and rapid ABO and RhD testing. According to the standard operating procedures, routine blood group testing was performed using fully automated immunohaematology (IH) analysers Erytra (Grifols, Spain). After completion of routine testing, the ABO and RhD Blood Grouping Rapid Test was performed on the residual blood sample, and both methods' results were compared. The study was performed at the Slovenian Institute for Transfusion Medicine after the approval of the Medical Ethics Committee of the Republic of Slovenia. Special requirements for the samples selected for the study were given by the study sponsor and are based on the criterion from the Commission Decision of 7 May 2002 on common technical specifications for *in vitro*-diagnostic medical devices (notified under document number C(2002) 1344). A Kappa consistency test was performed to statistically evaluate the conformity between the two methods.

Results: A total of 520 blood samples were tested, of which 401 were blood donors, 102 were patients and 17 were newborns. Obtained results with calculated conformity rates are presented in Table I (for ABO) and in Table II (for RhD). In four samples, there were discordant results for ABO blood grouping. In all four samples, the first result of the rapid test indicated blood type AB, but after identifying the discrepancy and repeating the rapid test, the result was correct (A, B, O). There were no discordant results for RhD grouping.

Summary/Conclusions: A Kappa value of 0.988 was obtained for ABO analysis and a Kappa value of 1 was obtained for RhD analysis. According to our results, the accuracy of the ABO and RhD Blood Grouping Rapid Test (OABD-402) was 99.23% for ABO and 100% for RhD determination.

P309 - Table 1: Number of samples with results of ABO testing

Routine test / Rapid test	A	B	AB	O
A	276	0	0	0
B	0	104	0	0
AB	1	1	33	2
O	0	0	0	103
Accuracy	**99,64%**	**99,05%**	**100%**	**98,10%**
All together accuracy: **99,23%**				

P309 - Table 2: Number of samples with results of RhD testing

Routine test / Rapid test	RhD-postive	RhD-negative	Total
RhD-postive	413	0	413
RhD-negative	0	107	107
Relative sensitivity		**100%**	
Relative specificity		**100%**	
Accuracy		**100%**	
Kappa		**1**	

P310 | Abstract withdrawn

P311 | Analysis of clinical application of direct and indirect antiglobulin test

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Background: The detection of antibodies directed against red blood cell (RBC) antigens is critical in pretransfusion compatibility testing. It is one of the principal tools for investigating potential haemolytic transfusion reactions and immune haemolytic anaemias. In addition, it aids in detecting and monitoring patients who are at risk of delivering infants with haemolytic disease of fetus and newborn (HDFN).

The antiglobulin test is a method of demonstrating the presence of antibody or complement bound to Red Blood Cell membranes by the use of anti-human globulin to form a visible agglutination reaction.

The antiglobulin test is divided into two types: the direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT). The IAT looks for antibodies in the patient's blood, whereas the DAT looks for antibodies or complement linked to the patient's RBCs, indicating in vivo sensitization.

Aims: The aim of this study was to list out the causes of Direct and Indirect Antiglobulin test positive cases, to determine monospecific

Red Cell bound antibodies in the DAT positive cases and to identify the clinically significant Alloantibodies in Patient's serum.

Methods: This was Hospital based Prospective study done in a tertiary care teaching hospital for 7 months. A polyspecific direct antiglobulin test was performed on 507 patient samples using BIORAD Coombs gel card. In the positive DAT cases, further investigation using specific DC screening column agglutination cards was carried out.

A commercial 3-cell ID Dia cell antigen panel was used for the antibody screening. If antibody screening with 3-cell antigen panel was positive, an extended 11 cell panel was used for antibody identification.

Results: Among 507 samples, 41 samples (8%) were DAT positive, 56 samples (11%) were IAT positive and 23 samples (5%) were both positive. Positive DAT were seen in AIHA (76%), drug-induced haemolytic anaemia, HDFN (22%), investigation of haemolytic transfusion reactions (1%), Passenger Lymphocyte Syndrome. DAT was typically positive with anti-IgG antisera in warm AIHA (62.5%). A patient with Cold Agglutinin Disease showed a positive Antiglobulin test for anti-C3d (10.4%). Amongst IAT positive sample, Anti-D (22.7%)>Anti-E (13.9%)>Anti-M (11.3%) was found in our study.

Summary/Conclusions: Clinical conditions resulting in positive DAT includes AIHA, Drug-induced Haemolytic Anaemia, HDN, investigation of haemolytic transfusion reactions and Passenger Lymphocyte Syndrome. In our study, maximum number of DAT was positive for AIHA (diagnostic hallmark) followed by Systemic Lupus Erythematous and multiple transfusions. Anti-IgG monospecific DAT was found in Warm AIHA. A patient with Cold Agglutinin Disease showed a positive DAT for Anti-C3d. IAT was most coomonly found positive in Rh incompatibility followed by cases of multiple transfusion. Both IAT & DAT were positive in Haemolytic Anaemias.

P312 | The complexity of two faced Anti-M : A case series

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Background: Anti-M is a naturally occurring antibody of the MNS group system usually reactive at below room temperature and regarded to be clinically insignificant.

Aims: We describe three cases of clinically significant anti-M in HTAR; one 'immunizing' type and other two 'naturally occurring' and review the literature.

Methods: Case series.

Results: Case 1: 22 year old male with underlying aplastic anaemia on regular blood transfusion, admitted for symptomatic anaemia with haemoglobin of 4.4g/dl. Two unit of packed red cells were requested and the blood group was typed as A Rh (D) positive. However, all the 10 red cell units were incompatible. Direct antiglobulin test (DAT) and autocontrol was negative. Antibody screening was positive and antibody identification showed patient has anti-M antibody. Two units were crossmatched with M antigen negative packed cell was fully compatible and supplied to patient.

Case 2: 3 year old boy treated for Tuberculosis meningitis, was planned for right external ventricular drain. His haemoglobin was 10.5g/dl, thus 2 units of packed cell was requested and there was no previous history of transfusion. However, all the 6 red cell units were incompatible. DAT and autocontrol was negative. Antibody screening was positive and antibody identification showed patient has anti-M antibody. He was then supplied with 2 units M antigen-negative packed cell which was fully crossmatch compatible.

Case 3: 10 month old girl,was admitted for severe Respiratory Syncytial Virus (RSV) Pneumonia and iron deficiency anaemia. Her haemoglobin level was 7.8 g/dL. Thus, decision for 2 unit packed cell transfusion was made. The ABO grouping showed Group A with extra reaction at A1 cells. DAT and autocontrol was negative. Antibody screening was positive and antibody identification showed patient has anti-M antibody. Phenotyping showed patient was negative for M antigen. Using M antigen-negative reagent red cells for reverse grouping we confirmed the patient's blood group was A Rh(D) positive. The patient was then supplied with 2 units M antigen-negative packed cell which was fully crossmatch compatible.

Summary/Conclusions: Anti-M has varied presentation, it occurs individually as non complement activating, IgM antibody and complement activating IgG antibody, existing alone or in combination. Anti-M may be naturally occurring or immune mediated due to exposure in a previous pregnancy, transfusion, or transplantation. These are of blood banking interest as it causes ABO discrepancy in reverse grouping and crossmatch incompatibility at 37° C. Anti-M is more commonly found in children than adult, often in the setting of infection. The abundant sialic acid on glycophorin A, on which MN antigens are present some infected host may evoke so called naturally occurring anti-M during immune response to invading pathogens. Although allo anti-M usually exhibits 'transfusion-related anti-M attenuation', the significant appearance of the antibody in an immunologically compromised patients may be due to the failure of immune accommodation. In these reported case series, this clinically significance antibody urge the necessity of careful evaluation of each case of anti-M present to ensure safe transfusion practice.

P313 | Abstract withdrawn

P314 | Enzyme-only antibodies and discontinuation of enzyme crossmatch: Slovenian experience

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Background: At the Slovenian Institute for Transfusion Medicine, as part of routine pre-transfusion testing patient plasma is screened for the presence of irregular antibodies by indirect antiglobulin test (IAT). Additionally, for every issued unit of red blood cells (RBC) and

P314 - Table 1: Antibody specificity and patient transfusion history

Specificity	No. of patients	Transfusion history
anti-E	14	5 patients never received a transfusion
anti-C^w	4	2 patients never received a transfusion
anti-Le^a	4	3 patients never received a transfusion
anti-Le^b	1	never received a transfusion
anti-P1	1	never received a transfusion
anti-Lu^a	1	received a transfusion
SUM:	25	12 patients never received a transfusion

granulocytes serological crossmatch (XM) is performed. For more than 30 years, we performed crossmatch in two techniques in parallel: in LISS/Coombs (L/C) and NaCl- enzyme (bromelain) technique. The reason for the additional enzyme XM was enhanced detection of low titre RBC alloantibodies, which could be undetectable in the standard L/C technique.

Aims: Due to the high percentage of enzyme-only antibodies that we were observing over the years and the lack of evidence of their clinical significance, we decided to abolish enzyme XM as a part of the routine testing. Before final abolition, we had a transitional period of additional screening and investigating of enzyme-only antibodies.

Methods: During 5 months period, we monitored for enzyme-only antibodies by adding to our routine antibody screening (Serascan Diana 4, Grifols, and ID-DiaCell, Biorad) indirect test with papain-treated RBC (IT-P; Serascan Diana 4P, Grifols, and ID-DiaCell P, Biorad). Enzyme-only antibodies were defined as reactivity with IT-P and no reactivity with IAT. As usual, all positive results were investigated further by determining antibody specificity (Identisera Diana P, Grifols, and ID-DiaPanel-P, Biorad). Antibody specificity was concluded either as a specificity against a specific antigen, as antibodies of undetermined specificity, where both positive and negative results were obtained with tested cells, or as an enzyme interference, where panreactivity was obtained with all tested cells including enzyme-treated patient RBC in autocontrol. Furthermore, we investigated whether patients with positive results received transfusion prior to testing and if in the subsequent six months, antibodies became detectable in the L/C technique.

Results: During 5-months period, we performed 4972 screenings with IT-P of which 489 were positive (9.8%). Among all positive results, only 25 had obvious specificity against a specific antigen: 14 cases of anti-E, 4 cases of anti-C^w, 4 cases of anti-Le^a, and one case of anti-Le^b, anti-P1, and anti-Lu^a were detected (Table 1). In the 6-months period after the detection of specific antibodies, none of the specificities became detectable in the L/C technique.

Summary/Conclusions: With higher test sensitivity, we detected more enzyme-only antibodies either with low or without clinical significance. Since many researchers have been reporting a lack of clinical significance of such antibodies, enzyme methods are being gradually excluded from routine testing worldwide. After the adjustment period, we completely stopped performing enzyme XM, which reduced laboratory workload, reduced testing costs, and speed up the testing process with no risk to patient safety. Since enzyme XM discontinuation more than one year ago, we have not observed a single case of haemolytic reaction due to enzyme-only antibodies.

P315 | Multidisciplinary management and outcome of a pregnant women with H deficient red cells (Bombay phenotype)

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Background: Phenotypes with H-deficient red cells are rare (1/10.000 and 1/1.000.000 individuals, in India and Europe respectively). Homozygosity for inactivating mutation in FUT1 gene, that encodes the fucosyl-transferase, responsible for biosynthesis of H, results in red cells with no H antigens (hh). The hh red cells lack the precursor of A and B, typing as group O. Individuals hh non-secretor (Bombay phenotype) can produce anti-H, anti-A and anti-B. Anti-H is clinically significant and can cause severe HTRs and HDFN. In these cases, compatible blood is very difficult to find and autologous blood donation and transfusion is recommended in some situations.

Aims: A case of a 33-year-old Chinese female (G3A0P2) is presented. She was known as RHD positive and in the analytical study of the first trimester of her current pregnancy, performed in her reference hospital, presents a positive irregular antibody screening and a panagglutination in the identification panel. With the suspicion of a rare blood group/antibody against a high incidence antigen, samples of the patient were send to the blood transfusion center (BTC), with the aim of identifying and deciding the best strategy to safeguard both, mother and child health.

Methods: Patient samples were analysed for: 1) ABO-RhD grouping (tube, Inmuclone-Immucor and Totem Diagast antisera, respectively); 2) Direct Coombs (DC)(poli-specific, IgG and Cd3, gel Biorad); 3) antibody screening and identification panels in different media (IA) (gel, 11 cells, Liss-Coombs, BioRad; 11 cells, papain, Grifols; 20 cells, PEG, Immucor); 4) Crossmatch testing (CT) between patient plasma and 2 Bombay donors red cells samples (gel, Liss-Coombs, BioRad); 5) Erythrocyte genotyping (EG) (InnoTrain, Diagnostic GMBH and IDCOREXT Grifols); 6) Molecular study of FUT1 and FUT2 gens by amplification of exons 4 (FUT1gene) and 2 (FUT2gene) (In house Next Generation System (NGS); 7) Further testing using alloadsorption and titration for Anti-H were also determined

Results: BG resulted O RHD positive, and DCT negative. All identification panels resulted pan-reactive at 37°C, with negative auto-controls. EG allowed discarding the following high incidence antigens in the patient sample. CT resulted negative in both case, highly suspicious of Bombay phenotype in the patient. This result was confirmed with the NGS study (2 null alleles in FUT1 gene, and in FUT2 no variant H-/Hw+ alleles were detected). Patient's plasma was treated with 0.01 mol/L dithiothreitol (DTT) at pH 7.3 discarding the presence of IgG and concluding the IgM condition of the plasma antibody. Allo-adsorptions with iso-phenotype erythrocytes was negative discarding other alloantibodies. Anti-H titre was 1/8. Regarding the low frequency of hh red cells units, a plan to be prepared for transfusion support during pregnancy and delivery was discussed (BC and hospital obstetric and hematology teams), deciding a PBM strategy based in auto-donation. The patient haemoglobin (Hb) was 11.2g/dL. She was treated with intravenous iron (Ferinject; 500mg) and Erithropoyetin (Binocrit, 40.000IU), and a second dose of both, 2 weeks later. Afterwards, Hb was 11.2 substituting Binocrit by Aranesp (300 mcg) for two additional weeks, reaching Hb 12.8g/dL and allowing the patient first autodonation, and the second, two weeks later (patient Hb 12.8g/dL). Twenty days later a scheduled caesarean was performed without any incident. Following delivery, the patient Hb was 12g/dL. No transfusion was needed and the 2 units were cryopreserved in the BC.

Summary/Conclusions: Anti-H can cause HDFN and supposes a challenge in blood transfusion, especially in the context of a pregnancy. A multidisciplinary approach involving BTC, hospital transfusion service and the early referral of the patient to a feto-maternal medicine unit, combining a PBM strategy, a planned delivery and the provision of compatible units (autologous/allogeneic) is needed.

P316 | Five cases of Piperacillin-induced immune haemolytic anaemia

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Background: Piperacillin has the broadest activity of all penicillins, and is therefore a widely used antibiotic. In addition to the frequently known adverse reactions such as diarrhoea, headache or vomiting, piperacillin is known to cause massive haemolysis in rare cases, often observed in patients with cystic fibrosis. Due to its rarity, drug-induced immune haemolysis (DIIHA) by anti-piperacillin antibodies is often overlooked or misdiagnosed, e.g. as autoimmune haemolytic anaemia.

Aims: To describe common laboratory findings and medical history in five patients who developed immune haemolysis after administration of piperacillin.

Methods: Records of five patients with suspected haemolysis and a positive direct antiglobulin test (DAT) after (re-) administration of piperacillin were reviewed. Standard serologic tests for detection of red blood cell (RBC) antibodies were applied by gel card technique (antibody screening, indirect antiglobulin test (IAT), papain treated cells and DAT (BioRad, Switzerland)) and the Capture® technique (Immucor, USA). RBC-bound antibodies were eluted by using the standard acid elution method (BAG, Lich, Germany).Testing for drug-dependent antibodies was performed in the presence and absence of piperacillin and its ex vivo metabolites (urine samples of patients treated with piperacillin).Piperacillin was dissolved (1 mg/ml) in 0,9 % saline.

Results: All patients had severe underlying diseases. Four of five patients were male. All patients received at least one unit of RBC in temporal relation to the drug-induced immune haemolysis. One patient required massive transfusions throughout the stay at the intensive care unit. Lactate dehydrogenase was elevated in four of the five patients and haptoglobin was low in four samples and was not determined in one patient. The haemoglobin level was low in all five patients and ranged from 2.40 to 4.80 mmol/l.Serologic investigations showed strongly positive DATs in all patients, whereas the DAT was either negative or only weakly positive in previous investigations. During haemolysis, all samples were positive with anti-IgG. In addition, three patients had positive reactions with anti-C3d. Crossmatches were generally incompatible. No RBC-bound autoantibodies nor alloantibodies were detectable after acid elution. Positive reactions in the IAT and with papain-treated cells revealed an auto-anti-e in three samples and panagglutination in the other two cases.Tests for drug-dependent antibodies confirmed red cell antibodies in the patient plasma reacting positive in the presence of piperacillin or its metabolites.In three of the five cases specific drug or metabolite dependent antibodies could also be detected in the eluate.

Summary/Conclusions: Piperacillin-induced haemolysis should always be considered when (re-) administration of piperacillin results in haemolysis in combination with a positive DAT. Autoantibody-like reactions in the indirect antiglobulin test with negative eluates are highly suggestive of a DIIHA.Patients with DIIHA must never receive the causing drug again because re-exposure could result in life-threatening haemolysis.In order to not overlook drug-induced immune haemolysis, a precise medical history is essential when sudden haemolysis occurs.

P317 | Abstract withdrawn

P318 | The evaluation of the improvements in efficiency and performance of two automated titration systems

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Background: Titration is a critical tool in determining the concentration of antibody present and apply their impact in treatments of patients receiving transfusion and transplantation. However, the

testing has been utilised through tube-based tests employing manual dilution with the potential of performance error. Additionally, significant technical time is invested through manual activity by medical laboratory scientists.

Aims: Evaluation was undertaken to demonstrate the efficiency and performance value of the automated titration at room temperature (RT) and indirect Antiglobulin test (IAT), utilising automated instrument systems with the capability to automate the serial dilution procedure and titration serological testing process.

Methods: Thirty donor samples drawn in EDTA from 10 group A, 10 group B and 10 Group O individuals. The samples were tested by titration at RT and IAT phases providing 40 results. The testing was compared on two automated testing systems using doubling serial dilutions (DIL) on the ORTHO VISION® Max (OVM) and the BioRad IH500 (IH500). The OVM used ORTHO BioVue® System Cassettes (Reverse Diluent and Anti-IgG) with 0.8% Affirmagen A1 and B reagent red blood cells. The IH500 used ID-Cards (NaCl and Coombs Anti-IgG) along with ID-Diacell A1 and B reagent red blood cells. Test results on OVM using three endpoint (first 0.5, last 0.5 and 1+) reactivity was compared to the 1+ endpoint of the IH500 test results. Additionally, to evaluate efficiency and productivity, both turnaround time (TAT) and hands-on time (HOT) in minutes (min.) were compared between the two systems.

Results: For the last 0.5+ titre endpoint, OVM RT was higher by 1 (DIL) 20%, 2(DIL) 30%, 3(DIL) 40%, 4(DIL) 7.5% and equivalent in 2.5% of the results. OVM IAT was higher by 1 (DIL)32.5%, 2(DIL)25%, 3(DIL)20%, 4(DIL)7.5%, 5(DIL)2.5% and equivalent in 5% and lower in 7.5% of the results. For the first 0.5+ titre endpoint, OVM RT was higher by 1 (DIL) 25%, 2(DIL) 50%, 3(DIL) 15%, and equivalent in 10% of the results. OVM IAT was higher by 1 (DIL) 47.5%, 2(DIL) 25%, 3 (DIL) 10%, and equivalent in 10% and lower in 7.5% of the results. For the 1+ titre endpoint, OVM RT was higher by 1 (DIL) 47.5%, 2(DIL) 15%, and equivalent in 27.5% and lower by 1(DIL) 10% of the results. OVM IAT was higher by 1 (DIL) 37.5%, 2(DIL) 7.5%, and equivalent in 42.5% and, lower by 1(DIL) 10%, 3(DIL) 2.5% of the results. TAT for one antibody for both RT and IAT (A or B) was 56 min. on OVM compared to 60 min. on IH500 while two antibodies (A and B) was 88 min. on OVM versus 100 min. on IH500. HOT for 1 antibody was 2.23 min on OVM and 1.33 min. on IH500. For two antibodies, the HOT was 3.36 on OVM and 3.00 on IH500.

Summary/Conclusions: Automation of titration testing of both OVM and IH500 improves overall efficiency, productivity and minimises potential for error by reducing manual steps. OVM demonstrated a better TAT, though HOT was marginally better for IH500. The automation process enhances confidence in result outcome by providing a defined end point and showed a substantial shortened TAT which enhances throughput of larger volumes of titration test workload.

P319 | Abstract withdrawn

P320 | Abstract withdrawn

P321 | Red cell alloimmunisation among haemoglobinopathy patients following Rh- and Kell-matched red cell transfusion in Jazan region of Saudi Arabia: A multi-centre study

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Background: Alloimmunisation remains a major complication of blood transfusion among sickle cell anaemia (SCA) and thalassemia patients, and is often associated with several transfusion adverse outcomes, such as acute and delayed haemolytic transfusion reactions (HTRs), and shortened RBC survival. The prevalence of RBC alloimmunisation in SCD and thalassemia patients shows great variations among different populations worldwide, ranging from as low as 2.6% to as high as 76%, which appears to be associated with disparities in race, age, underlying clinical conditions and transfusion protocols. Since 2012, the recommended transfusion protocol for patients with SCD or Thalassaemia in Jazan region has been the selection of RBC units that are extended-phenotype-matched for RhC, E, c, e, and K antigens and compatible in the indirect antiglobulin (IAT) crossmatch.

Aims: To determine the prevalence of alloimmunisation to RBC and alloantibody specificities among SCA and thalassemia patients in three major hospitals in Jazan region of Saudi Arabia.

Methods: A cross-sectional, multicentre study was conducted from January to December 2019 in the blood banks of three hospitals in the Jazan region – namely, King Fahd Central Hospital, Prince Mohammed Bin Nasser Hospital and Samtah General Hospital. The study population comprised all registered patients with SCD and thalassemia and transfused at least once during the study period. Patient demographic, ABO and Rh groups, alloimmunisation status, and specificities of detected alloantibodiest data were collected from the blood bank records. Descriptive statistics were used to summarise and describe the significant differences of collected data.

Results: During the study period, a total of 1,027 registered patients with haemoglobinopathy were identified. Among the identified patients, 483 (47%) were males, and 544 (53%) were females. Of the 1,027 patients included, 906 (88.2%) had SCD, while 121 (11.8%) had thalassemia. The most common blood group was O (59.9%), followed by A (29.7), B (8.6%) and AB (1.8%). Most of the patients included in the study were D-positive (95.5%), and only 4.5% were D-negative. The antibody screen test was positive in 78 (7.6%) of the 1,027 patients, who developed a total of 108 red-cell alloantibodies. Of the 906 patients diagnosed with SCA, 71 (7.8%) had one or more alloantibodies, while 7 (5.8%) patients of the 121 identified thalassemia patients developed alloantibodies. The alloimmunisation rate in the two patient groups did not differ significantly ($p = 0.42$). Most alloantibodies detected in our study were directed to antigens of the Rh (49.1%) and Kell (25.0%) blood-group systems. Anti-E and anti-K were the most prevalent antibodies (25.9% and 24.1%, respectively), followed by anti-c (13.0%), anti-C and unknown specificity (6.5% each), anti-Fy^a and anti-S (5.6% each). The proportion of the 78 alloimmunised patients who

developed a single antibody was 78.2% (61). Conversely, seven (9.0%) and eight (10.3%) of SCD patients developed two and three alloantibodies, respectively, while three (2.5%) of them developed four antibodies. None of the thalassemia patients developed multiple antibodies.

Summary/Conclusions: The overall rate of alloimmunisation among patients with haemoglobinopathy in Jazan who routinely transfused with Rh and Kell phenotype matched RBC was 7.6%, which is low compared to other areas in the country and worldwide. The knowledge of most encountered alloantibodies in our population will aid in selecting the most appropriate antigen-negative red cells. Further research is needed to explore factors associated with residual risk of alloimmunisation in these patients.

P322 | Risk of new alloimmunization in patients on DARA treatment using tube LISS-IAT method

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Background: Daratumumab (anti-CD38, DARA) has proven to be highly efficacious for relapsed and refractory multiple myeloma (MM) and, lately for solid tumours and autoimmune diseases (Lancmann, Transfusion Med, 2018). It has been recognised that anti-CD38 interferes with pretransfusion testing by binding to CD38 on RBCs and causing panagglutination in indirect antiglobulin test (IAT), cross-matches and often direct antiglobulin test (DAT), thus complicating the management of such patients who often require a blood transfusion. In order to optimise laboratory management in these patients it is important to establish ABO, Rh, Kell, Kidd, Duffy, MNSs and antibody status prior to commencing treatment with DARA.

Aims: As pretransfusion work-up of MM patients has been our daily routine, we wanted to evaluate if patients treated with DARA are in a higher risk of developing new alloantibody while performing cross-matches and IAT in tube LISS-IAT.

Methods: A retrospective study of the complete serological profile and transfusion history of 66 patients treated with DARA was carried out in the Croatian Institute of transfusion medicine from 2018-2022. Routine pre-DARA testing included ABO/D typing, RBC phenotype or genotype, DAT, IAT and antibody identification in column agglutination technology (CAT) (Bio-Rad, DiaMed GmbH, Switzerland and Ortho Clinical Diagnostics, USA). Extended phenotyping for C, c, E, e, Jka, Jkb, Fyb, M, N, S, s was performed by monoclonal IgM typing reagents in the absence of RBC transfusion in the prior three months. Otherwise, RBC genotyping was performed. During the DARA treatment tube LISS-IAT was used for cross-matches with pheno/genotypically matched RBCs and IAT, mostly in parallel with CAT-IAT.

Results: A total number of blood samples received in the study period was 195. Around 244 antibody screens in column agglutination technology and 136 in tube technique were performed for 66 patients treated with DARA. There were 33 (50%) female and 33 (50%) male patients included in the study. Out of 66 patients referred to our laboratory, 45 (68%) were transfused with 234 phenotypically matched RBCs. All 66 patients (100%) demonstrated a positive IAT (≤1+ to 2+) in CAT with all panel cells after receiving therapy. The duration of positive IAT varied from 1 to 6 months, and the interference disappeared after the cessation of the therapy in all patients. Direct antiglobulin test was positive (≤1+) in 40/66 (61%) patients. Prior to commencing DARA therapy, 3 patients had preexisting RBC alloantibody. The specificities of these alloantibodies were anti-E, anti-K and anti-C. No new alloantibodies were detected during the follow-up period.

Summary/Conclusions: Transfusion management of patients treated with monoclonal antibody (MAb) therapy can be challenging and sometimes delay transfusion support. Our study proved panagglutination with all panel RBCs with low and variable agglutination intensity. Some patients presented with positive DAT, whereas others remained negative, which is in concordance with recent studies (Carreno-Tarragona G, Transfusion Med, 2019). Panagglutination remained positive for 1 to 6 months after drug cessation. No new alloantibodies were detected among patients on DARA therapy, which means that the policy of transfusing them with pheno/genotypically matched RBCs, for at least Rh and Kell, and if possible Kidd, Duffy and MNSs, and performing IAT/cross-matches in tube LISS-IAT is a safe and effective policy.

P323 | Abstract withdrawn

P324 | Daratumumab in transfusion medicine in University Hospital Dubrava

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Background: Daratumumab is a monoclonal antibody used in the treatment of multiple myeloma in University hospital Dubrava since July 2019. It is a targeted therapy that works by binding to a protein CD38, which is found on the surface of myeloma cells but also on the red blood cells. There lies the importance for transfusion medicine because it interferes with antibody detection and crossmatch. Our approach was to do pre-transfusion testing for every patient before the start of the treatment with daratumumab. The testing included type and screen (IAT), direct antiglobulin test (DAT) and phenotyping/genotyping of blood groups. If serological testing was possible, extended phenotyping included: C, c, E, e, C^w, K, Fy^a, Fy^b, Jk^a, Jk^b, M, N. S and s. If not, samples were sent to genotyping to reference laboratory for immunohematology. For the treatment with red blood cells (RBCs) we used extended-match RBCs if available. If not, the samples were sent to a reference laboratory capable of removing anti-CD38 interference.

Aims: Our goal was to see how many patients were treated with daratumumab in University hospital Dubrava and what were their characteristics and the need for RBCs.

Methods: We analysed the characteristics and the need for RBCs of all the patients treated with daratumumab in University hospital Dubrava until the end of 2022 (the onset being July 2019).

Results: From July 2019 until the end of 2022 there was a total of 58 patients treated with daratumumab, 34 male and 24 female. Patients´ age ranged from 54 to 86 years ($M = 69,5$). The blood type distribution was as follows: O RhD pos 14 (24%), O RhD neg 5 (9%), A RhD pos 19 (33%), A RhD neg 3 (5%), B RhD pos 8 (14%), B RhD neg 3 (5%), AB RhD pos 6 (10%), AB RhD neg 0 (0%). Molecular testing in a reference laboratory was needed in 15 (25,9%) patients. We discovered an alloantibody in 1 (1,7%) of the patients (anti-Fy^a specificity). 4 (6,9%) patients had the pre-transfusion testing done in another university hospital centre. Transfusion of RBCs was needed in 27 (46,6%) of the patients. 27 patients received 206 units of RBCs, minimum per patient 1, and maximum was 37 units.

Summary/Conclusions: Thanks to good collaboration with hematologists, we had the opportunity to perform pre-transfusion testing including extended phenotyping in almost all of our patients. Only in 2 (3,45%) patients was daratumumab given before immunohematology tests were done. For the patients that had the testing done elsewhere (4 of 58) we didn´t get that information on time - together with the request for RBCs, which delayed the transfusion of RBCs. Basically, understanding of daratumumab related problems in transfusion medicine is needed in all fields of medicine, not only in hematology, and timely communication is crucial for solving major problems caused by daratumumab in transfusion medicine.

P325 | Split personalities: Making sense of unexplained mixed-field reactions

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Background: Mixed-field (MF) reactivity is where both agglutinated and unagglutinated cells are observed in the same test tube or card, and is most often explained by the presence of discrete cell populations with differing phenotypes. Chimerism is a term given to the presence of two distinct genotypes in an individual that originated from different zygotes, often after transfusion or transplantation. Some individuals, however, are born with two genotypes – so-called "natural" chimeras. In contrast, mosaicism is the presence of two genotypes that originated from the same zygote due to somatic mutation. If these mutations occur in haematopoietic stem cells, erythrocytes derived from the mutated clone may lack one, or more, blood group antigens expressed by the remainder.

Aims: To describe the investigation of two such samples that were received by the RCI laboratory in Bristol, UK; and to discuss the implications of these phenomena on routine transfusion testing and blood selection.

Methods: Both samples showed MF reactivity in card with anti-D and anti-C reagents (Bio-Rad, USA). Patient histories were obtained, but no evidence of recent transfusion or SCT was found. A variant D panel (ALBAclone, Alba Bioscience, UK) was attempted on both samples; but no variant was identified. Both samples were analysed by Flow Cytometry using FITC-conjugated anti-D (Brad-3, IBGRL, UK) to confirm the MF reactions were due to a dual population of cells. Extended phenotyping for additional red cell antigens was also employed.

Results: Patient 1 had been seen previously by the referring Hospital, and grouped as D Positive. Flow cytometry showed two clear populations, one D Negative (67%) and the other D Positive (33%). No MF reactivity was seen with any other red cell antigens. Patient 2's history could also not explain the MF reactivity; but a previous sample received in 1999 had also shown unexplained MF reactions. Again, flow cytometry showed two distinct populations – 51% D Positive in this case. MF reactions were also seen with anti-Fya and anti-Fyb typing sera.

Summary/Conclusions: Resolving unexplained MF reactions rely on a robust patient history, including prior transfusion or transplants. If these are excluded, natural chimerism or mosaicism can be inferred by the current and historical results. If the patient grouped clear in the past but is now MF, mosaicism is more likely (especially if the patient has a haematological diagnosis). If typing for multiple blood group antigens encoded by genes at different loci yields MF results, chimerism would seem more likely. Mosaicism was inferred with Patient 1 given her history of a concluded D type. The patient's original phenotype would appear to be R1r (DCe/dce). Loss of heterozygosity caused by complete deletion of the *RHD-RHCE* gene locus is most likely, resulting in a clone of phenotypically rr (---/dce) cells. With Patient 2, the MF reactions also seen with Duffy typing sera coupled with the patient's history of past anomalous D typing results would suggest he is a natural chimera. In confirmed cases of natural chimerism or mosaicism, supplying blood should not be a concern. In mosaicism, the antigen being "lost" is well established as a self-antigen; and in chimeras the immune system tolerates both phenotypes. However, as these phenomena are hard to conclusively prove, it may be pertinent to practice caution and select antigen negative cells in cases with ABO discrepancies, patients with atypical red cell antibodies, and in cases of complete antigen loss.

P326 | Time to start routine antenatal RhD immunoprophylaxis in Serbia

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Background: Anti-D antibody is still the most frequently detected clinicaly significant antibody in the population of pregnant women in Serbia and can lead to very serious forms of haemolytic disease of the fetus and newborn. According to the current recommendations, every pregnant woman should come for the first control and antibody screening as early as the 12th week of pregnancy, but there are a large number of those who come much later or just before giving

birth. In such cases, anti-D antibody could be determined when it is too late for timely administration of antenatal RhD immunoprophylaxis. A large number of studies have shown that for prevention of immunization of RhD-negative pregnant women, the most effective application of RhD immunoprophylaxis is in the 28th and 34th week of pregnancy and, if necessary, after delivery by applying an additional dose. In our country, it is a routine practice to apply RhD immunoprophylaxis only after birth, but not necessarily before.

Aims: The aim of this study was to determine how often does immunization of pregnant women occur as a result of immunization events of current pregnancy.

Methods: Data analysis for the period from year 2020 to 2023 was performed at the Institute for Blood Transfusion of Serbia at the Department for Immunohematological testing.

All pregnant women who came during this period to have their blood type determined, were screened for red cell alloantibodies using the gel method (ID-Card Coombs anti-IgG, BioRad, DiaMed GmbH, Switzerland) and commercially prepared reagent red blood cells (ID-DiaCell, BioRad, DiaMed GmbH, Switzerland) as well as with enzyme treated reagent red blood cells (ID-DiaCell P, BioRad, DiaMed GmbH, Switzerland). If the antibody screening is positive by one of these two methods, the specificity of the antibody is also determined by the gel method in an appropriate manner (ID-DiaPanel or ID-DiaPanel-P, BioRad, DiaMed GmbH, Switzerland).

Results: In the years 2020, 2021 and 2022 there were 6.138, 6.237 and 5.828 antibody screenings done, respectively. In the period from year 2020 to 2023, of all pregnant women tested, in 490 of them a positive antibody screening was detected. In all these cases, specificity of antibodies was determined and presence of one or more antibodies confirmed. Anti-D antibody was confirmed in 61 (12.5%) of 490 pregnant women with positive antibody sccreening. In 36 of them it was detected as a result of immunization during previous pregnancies but in 25 (41%) of them, it was detected for the first time in the third trimester of pregnancy, meaning these women were immunised during actual pregnancy.

Summary/Conclusions: This research pointed out that there is a large number of pregnant women still being immunised during pregnancy and there is a possibility of lowering the number of immunizations by applying antenatal RhD immunoprophylaxis. Following the example of developed countries, legislation should be passed according to which antenatal RhD immunoprophylaxis would become mandatory. Antenatal immunoprophylaxis is needed to lower the prevalence of RhD immunization even further.

P327 | Automated anti-D titration with calculated titre score

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Background: Through UK Serious Hazards of Blood Transfusion (SHOT) reporting it has been identified that anti-D detected in pregnancy could be mis-categorised. This mis-categorisation has contributed to missed antenatal follow ups for women with immune anti-D, leading to neonates being affected by haemolytic disease of the fetus and newborn (HDFN).

Due to these SHOT findings the British Society for Haematology (BSH) published revised guidelines for antenatal testing in blood transfusion in 2016. This guideline made a recommendation to measure Anti-D concentration, when detected in pregnancy, by continuous flow analysis (CFA). Within the UK, NHS Blood and Transplant (NHSBT) Red Cell Immunohaematology (RCI) laboratories are the only centres currently able to process this test.

Evans et.al (Transfusion Medicine, 2021) performed a multi-centre comparative study evaluating the results obtained from NHSBT to an automated titre score method performed on the Ortho Vision platform. Within this study a relationship between the two tests was demonstrated resulting in the recommendation of the titre score method, with a cut of defined at 35. This can be used as an alternative in-house screening tool to differentiate between passive and immune anti-D.

Aims: Due to a recent change in immunohaematology analyser, we wanted to demonstrate correlation between our new system, the IH500 from Bio-Rad, to the Ortho Vision platform by performing a direct method comparison. Part of the validation was to establish uncertainty of measurement for the technique to inform a safe titre cut off value.

Methods: 122 samples were tested on both the IH-500 platform and Ortho Vision platform using the on board serial dilution methods native to each. The titre score value was calculated using the method described by Evans et. al.

A comparison of the obtained titre scores was performed and statistical significance calculated using Pearson's correlation coefficient.

50 replicates of NIBSC Anti-D standard were used to calculate uncertainty of measurement.

Results: The IH-500 showed a strong correlation in anti-D titre score results (r(120):0.99) with the Vision and demonstrated reliable results with an uncertainty of measurement of 1.82 – equivalent to approximately 6% at a titre score of 32.

Summary/Conclusions: We demonstrated that Anti-D titre score as an in-house screening tool to differentiate between passive and immune anti-D can be implemented on the IH-500. Results correlated strongly with our validated method on the previous platform (the Ortho Vision).

In the UK, it is a requirement of our accreditation service that uncertainty of measurement is considered and applied. Therefore, the original titre score cut off should be adjusted to 32 on the IH-500. Organisations must appraise uncertainty of measurement within their own setting and apply this to the original recommended cut off (34).

The application of a decision-making algorithm is of vital importance to aid interpretation of these results. This algorithm must incorporate the following factors: whether any additional antibody specificities are present, whether anti-D was detected prior to injection of anti-D immunoglobulin, whether the patient has received anti-D immunoglobulin within the last 8 weeks. If there is any doubt as to the origin of the detected anti-D, a safe approach is to refer the sample for CFA to NHSBT RCI.

P328 | Implementation of automated serological crossmatch process for PRBC transfusion in a hospital blood transfusion service

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Background: Khoo Teck Puat Hospital (KTPH) is a 795-bed general and acute care hospital, serving the northern sector of Singapore since June 2010. The Blood Bank at the Department of Laboratory Medicine in KTPH functions to provide blood transfusion services to the patients in KTPH as well as the neighboring Yishun Community Hospital.

With the increase in clinical services provided by the hospital over the years, the number of packed red blood cells (PRBC) transfusions in the hospital has increased by 44.6%, from 5013 units in 2011 to 7251 units in 2019. The total number of serological crossmatches performed also increased by 47.8%, from 5490 in 2011 to 8115 in 2019.

Conventionally, abbreviated and full crossmatches were performed using the tube technique. However, with the significant increase in workload, the limitations became more apparent - the need for increased manpower, increased risk of human error in testing and transcription of results and inter-individual variability in crossmatches performed among staff (e.g. red cell suspension concentrations prepared by different staff may be varied, resulting in different antigen to antibody ratio when performing crossmatches).

Aims: The aim of this study is to validate the analytical performance and overall efficiency of the ORTHO VISION (OV) analyser (Ortho-Clinical Diagnostics, United States) in performing automated abbreviated and full serological crossmatches and perform interface testing to ensure transmission of results from OV analyser to the Blood Bank LIS middleware, eTrace Line (MAK-SYSTEM, United States).

Methods: A concordance study was performed to compare the serological crossmatch results in 51 samples analysed using the tube method and OV analyser, including 31 compatible and 20 incompatible crossmatches involving various blood groups and antibody types.

For interface testing, crossmatches were requested for the patients on eTrace Line before loading the samples into the OV analyser to test the transmission of orders for patients' samples and types of PRBC products as requested on eTrace Line.

Results: There was 100% concordance observed between the serological crossmatches performed by the tube method and OV analyser. A higher degree of sensitivity in detection was observed on the OV analyser as compared to the tube method.

eTrace Line successfully received the crossmatch results and reserved the compatible PRBCs electronically. For crossmatches with incompatible results with a degree of 0.5 or higher, the tested units were not reserved by eTrace Line.

Summary/Conclusions: With increasing workload demands, automated serological crossmatching has proven to not only save time and reduce manpower demands, but also ensures patient safety with increased sensitivity, uniformity and accuracy.

The total number of serological crossmatches increased from 8115 in 2019 to 10176 in 2022. Despite the increase, the manpower requirement stayed the same; since the time taken to do an automated serological abbreviated crossmatch is about 33% faster, as it takes 10 mins, when compared to the tube abbreviated crossmatch which takes about 15 minutes. This has resulted in reduced manhours required even after the anticipated surge in workload post-pandemic.

Reservation of PRBCs of wrong blood groups or non-antigen negative (for patients who require antigen negative blood) was prevented by patient order checks within the OV analyser. If the wrong patient sample was loaded, the analyser would not proceed with the crossmatch, prompting staff to investigate.

Automated serological crossmatching also increases 'walkaway' time, allowing staff to handle other tasks while the analyser performs the crossmatches.

The implementation of an automated serological crossmatch helped to enhance patient safety in blood transfusion while creating a hassle-free workflow for the laboratory staff.

P329 | Missed delayed haemolytic transfusion reaction: revisited

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Background: Multiple transfusions to patients expose them to various complications of blood transfusion including alloimmunization against red cell antigens, especially in thalassemia. One complication of alloimmunization is delayed haemolytic transfusion reaction (DHTR). Delayed haemolytic transfusion reactions (DHTRs) may occur when there is an antigen mismatch between transfused red blood cells and recipient antibodies where sensitized red blood cells are cleared by macrophages or complement activation leading to immunoglobulin G (IgG) mediated haemolysis. It occurs after alloimmunization to an RBC antigen(s) after a transfusion. Over time, the patient's antibody levels fall to undetectable levels. Subsequent re-exposure of the recipient to red blood cells that possess the antigen triggers an anamnestic response and subsequent haemolysis.

Aims: The study was conducted to assess the antibody implicated in delayed haemolytic transfusion reactions

Methods: A total of 19 episodes of delayed haemolytic transfusion reaction occurring in 17 patients were analysed. The patients were given ABO matched cross match compatible red blood cell units. Delayed haemolytic transfusion reaction was considered when there was failure in rise of haemoglobin after transfusion, presence of jaundice, increase in the levels of bilirubin from baseline and the event occurring between 5 to 12 days of transfusion.

Results: The median time between red cell transfusion and diagnosis of delayed haemolytic transfusion reaction was 7 days. Out of the 19 episodes in 15 episodes there was no change in haemoglobin levels after red cell transfusion where as in 4 episodes the haemoglobin fell below the pre transfusion levels. The post transfusion direct

antiglobulin test was positive in 3 events. The total antibodies implicated were 9 (Anti Jka: 6, Anti Jkb: 2, Anti Fya: 2, Anti Fyb: 1, Anti S: 1, Anti s: 1, Anti E: 2, Anti e: 1, Anti c: 1). Significant jaundice was noted on physical examination. Laboratory data showed mean fall in haemoglobin (0.8 g/dl) and increased bilirubin (1.6 mg/dl).

Summary/Conclusions: The initial symptoms of delayed haemolytic transfusion reaction are often missed as in most of cases; no antibody is identified during testing. Delayed haemolytic transfusion reactions can be asymptomatic or mimic other conditions and may be misdiagnosed. In patients with known clinically significant antibodies, confirming on each pre-transfusion sample tested, that the evaluation for the presence of additional clinically significant antibodies has been correctly performed and appropriately phenotype red blood cell unit is issued. Failure to recognise this entity could lead to inappropriate treatment and future transfusions reactions

P330 | Abstract withdrawn

P331 | Performance evaluation of a new direct Coombs card based on column agglutination system

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Background: The Direct Antiglobulin Test (DAT) with polyspecific AHG is a routine immunohematology test used to identify red blood cells (RBCs) coated *in vivo* with immunoglobulin and/or complement. It aids in the diagnosis of warm and cold immune haemolytic anaemias. Monospecific reagents differentiate warm from cold immune haemolytic anaemia. A new Direct Coombs DG card was developed with anti-Ig, -IgM, -IgG, -IgA, -C3c, -C3d, and -C4 reagents to broaden the analysis of the DAT. The DG Gel card format allows visualisation of result and uses a proprietary low ionic strength solution. Low ionic strength DATs are known to detect low affinity bound autoantibodies, representing a 'super' Coombs technique (*Blood* 2017;129:2971-9).

P331 – Table 1

DG Gel DC Scan Plus (Diagnostics Grifols)	DC-Screening I card (Biorad) Anti-IgA		DC-Screening I card (Biorad) Anti-IgM	
	Positive	Negative	Positive	Negative
Positive	42	2	37	1
Negative	2	1090	3	1095
Total	1136		1136	
PPA	95,45% (86,37%)		92,50% (81,74%)	
NPA	99,82% (99,42%)		99,91% (99,57%)	
OPA	99,65% (99,20%)		99,65% (99,20%)	

P331 – Table 2

DG Gel DC Scan Plus (Diagnostics Grifols)	DC-Screening I card (Biorad) Anti-C3c		Tube reagent (Medion Grifols) Anti-C4	
	Positive	Negative	Positive	Negative
Positive	29	7	25	2
Negative	2	1098	0	1073
Total	1136		1100(1)	
PPA	93,55% (81,05%)		100% (88,71%)	
NPA	99,37% (98,81%)		99,81% (99,42%)	
OPA	99,21% (98,62%)		99,82% (99,43%)	

(1) 36 samples were not tested with the Medion Anti-C4 due to lack of reagent availability.
% in () represent lower 95% Confident Bound

Aims: To evaluate the sensitivity and specificity of a new Direct Coombs DG card using the DG Gel DC Scan Plus card system.

Methods: A total of 1143 blood samples were tested: 1035 were from patients (530), pregnant women (177), newborns (119), and donors (209), with another 108 samples sensitized *in vitro* with immunoglobulins and complement factors. All samples were processed manually with the DG Gel DC Scan Plus cards and, in parallel, with the DC-Screening I card (BioRad) or with an anti-C4 tube reagent (Medion Grifols). DG Gel DC Scan Plus cards contain anti-Ig (polyvalent), anti-IgG, anti-IgM, anti-IgA, anti-C3c, anti-C3d and anti-C4 reagents. In parallel, BioRad's DC Screening I cards contain the anti-IgG, anti-IgM, anti-IgA, anti-C3c and anti-C3d reagents. Agglutination was graded as from 0 (negative) to ±, w+, 1+, 2+, 3+, 4+ (positive). Discrepancies were investigated according to the internal practices of the reference laboratory including alternative monospecific reagents for gel card and tube techniques.

Results: Seven out of the 1143 samples were excluded from the analysis due to a positive negative control. The lower confidence bounds for negative percent agreement (NPA) and overall percent agreement (OPA) exceed 95% with anti-IgA, -IgM, -C3c and -C4. However, the lower 95% confidence bounds for the positive percent agreement (PPA) were 86.37% for anti-IgA, 81.74% for anti-IgM, 81.05% for anti-C3c and 86,16% for anti-C4 (see Table). The disagreement included 19 discrepancies; 4 anti-IgA, 4 anti-IgM, 9 anti-C3c, and 2 anti-C4.

Summary/Conclusions: The high NPA between the DG card anti-IgA, anti-IgM, anti-C3c and anti-C4 reagents and the reagents of reference system demonstrated that the DG card is noninferior to an existing DAT gel card. The PPA had a lower agreement. Twelve of 19 DG card positive discrepancies were supported by the clinical evaluation of immune haemolytic anaemia, and with an independent institutional evalution for laboratory evidence of immune-mediated haemolysis. The addition of anti-IgM, anti-IgA, and anti-C4 to the new DG card expands the breadth of laboratory diagnostic tests used to evaluate immune-mediated haemolytic anaemia.

P332 | Abstract withdrawn

P333 | Eryptosis due to IgA autoantibodies, Donath-Landsteiner antibodies and IgG/ IgM alloantibodies

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Background: Eryptosis has been shown to be an independent pathway of haemolysis in cold agglutinin disease and IgM warm autoimmune haemolytic anaemia (wAIHA), in addition to extravascular and intravascular haemolysis. IgM autoantibodies (aabs) activate the complement system to sublytic terminal complexes C5b/C6, C5b-C7 and C5b-C8 that trigger eryptosis shown by externalization of phosphatidylserine (PS) on red blood cells (RBCs). Since eryptotic cells are removed rapidly from circulation by phagocytosis, eryptosis is most like underdiagnosed.

Aims: The question remains whether antibodies other than IgM aabs may induce eryptosis. We, therefore, studied patients with immune-haemolysis due to other autobodies and alloantibodies for eryptosis.

Methods: Six patients with eryptosis are presented in this series. RBCs of four patients with haemolysis due to antibodies were directly inspected for eryptosis. In two cases, frozen serum samples from patient with autoantibodies were incubated with O RBCs from healthy donors to induce eryptosis. Eryptosis was detected by binding annexin-PE to externalized PS and performing flow cytometry analysis as described before.

Results: We could detect eryptosis on RBCs in two patients after incompatible transfusion against alloantibodies (Anti-Le(b) and Anti-D/Anti-C, respectively), in one child with IgG Donath-Landsteiner aabs after viral infection, one pregnant woman with IgG and IgA warm aabs and two patients with warm AIHA only due to IgA aabs.

Summary/Conclusions: Our data indicate that eryptosis, together with extravascular and intravascular haemolysis, may also occur in cases other than IgM- wAIHA or CAD. In patients with IgA aabs, a complement independent pathway seems to be responsible for eryptosis. We most likely suspect shear forces that compromise membrane stability and facilitate Ca^{2+}influx.

P334 | Haemolytic transfusion reaction due to anti-A1 in a patient with chronic myelomonocytic leukaemia post allogeneic haematopoietic stem cell transplantation

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Background: Allogeneic haematopoietic stem cell transplantation (a-HSCT) is an established treatment for many haematologic malignancies and it may be carried out despite major ABO incompatibility between donor and recipient. For example, if the donor is type A and the recipient type O, it is expected that successful transplantation should result in the loss of anti-A and allow for the transfusion of red cells of type A. Anti-A1 is typically a cold antibody, i.e., it is primarily IgM and reacts optimally at room temperature. It is generally considered clinically insignificant. We describe a case of a patient with chronic myelomonocytic leukaemia who received a-HSCT from type A donor and developed a moderately severe haemolytic transfusion reaction due to anti-A1.

Aims: To elucidate the reason for the patient's transfusion reaction.

Methods: We conducted a retrospective analysis of the clinical and laboratory data of the patient, including blood typing, antibody screening and cross-match. Furthermore, on a new set of samples additional tests, including A-subtypes, titration of IgM and IgG anti-A, cross-match with donors of type A1, A2 and O were carried out.

Results: The patient, a 58-year-old male, blood type O, received a-HSCT from an unrelated blood type A donor in 2022. Seven months later, the patient was found to type as blood type A, i.e. with a 4+ with anti-A, however, also with a 2+ reaction with A1 test cells.

Since the titre of IgM anti-A was 1, he was concluded to be blood type A and his transfusion requirement was set to type A or O red cells. Based on a computer cross-match, typing 4+ with anti-A, he received most of a unit of type A red cells after which he developed a moderately severe haemolytical transfusion reaction. The symptoms were chills, fever (up to 39.5°C) and biochemical signs of haemolysis, as well as an absence of any rise in blood haemoglobin, despite a transfusion of two thirds of a unit of red cells. He was treated with fluids, antihistamines and corticosteroids. He made a full recovery within 24 hours. Serological investigation demonstrated the transfused unit to be serologically incompatible at 37°C IAT; it was A1+ and the patient was A1−. On a new set of samples 72 hours after the transfusions, the patient was found to be compatible with A1− red cells, DAT negative. Immunoglobulin M and IgG anti-A (A1+ test cells) titres were 2. In conclusion, the patient had received an a-HCST from a type A2 donor and had developed a warm reacting, IgM and IgG anti-A1. This anti-A1, IgM titre 1 clearly had clinical significance.

Summary/Conclusions: Our case highlights the potential clinical significance of even low titre anti-A1 in group O patients who undergo a-HSCT with type A2 stem cells. As such a conclusion of patient's ABO type post transplantation must await the disappearance of anti-A. In the present case, the anti-A1 is probably persistent even with full engraftment. In this and in similar cases, transfusion of A2 red cells should be feasible. Information on a-HSCT donor A-subtype is relevant for the post-HSCT follow up and planning of transfusion regimen.

P335 | Abstract withdrawn

P336 | Use of Deacetylase for conversion of group A1 red blood cells to acquired B test cells for quality control purposes

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Background: The acquired B phenomenon was first described in 1959 when weakly reactive B antigen was observed in blood samples from seven unrelated patients, previously typed as group A. Anti-B was present in serum and only A and H antigens were detected in saliva from the secretors. This phenotype is most often associated with patients suffering from malignancies where the integrity of the gastrointestinal wall is compromised. It is thought that bacterial enzymes can cause conversion of the immunodominant blood group A sugar, *N*-acetylgalactosamine, into a structure that highly resembles the blood group B sugar structure, galactose. This can lead to potential errors in blood grouping. Although acquired B is considered transient, it is vital that these patients are not falsely typed as B, especially in emergency situations. Thus, regulatory agencies require that commercial monoclonal anti-B is nonreactive with acquired B red blood cells (RBCs). However, these RBCs are not readily available since they are patient-derived.

Aims: Our objective was to explore the possibility of producing acquired B RBCs by enzymatic conversion, for quality control purposes.

Methods: Anonymised donor A_1 RBCs were subjected to enzymatic digestion by *Fp*GalNAcDeAc (Rahfeld et al. Nature Microbiology 2019). Group B and O RBCs were tested in parallel. RBCs at 50 % haematocrit in phosphate buffered saline were incubated with enzyme at two different concentrations, 0.05 µg and 0.25 µg. Incubation was performed at 37 °C for one hour under gentle agitation. After subsequent wash steps, 1 % suspensions were prepared and tested in parallel with untreated RBCs with monoclonal anti-A, anti-B, anti-B (ES-4 clone), as well as a panel of human plasma samples. Standard serological techniques including gel column agglutination (Biorad, Switzerland) and rapid tube testing were used to evaluate the efficacy of the enzyme treatment. *Fp*GalNAcDeAc-treated cells were frozen by our routine glycerolisation method at -80 °C and thawed for testing to evaluate stability.

Results: Specific deacetylation of A antigen on the A_1 RBCs was achieved at both enzyme concentrations. Enzymatically modified A_1 RBCs reacted 2+ with clone ES-4 but not with all other anti-B in routine use, both in gel cards and in tubes. Group B and O RBC controls reacted as expected. The reactivity was maintained upon freezing and thawing. In crossmatch tests, 1-3+ reactions were observed in 17/26 group A plasma with the modified RBCs but not with their untreated controls. Anti-A reactivity in 6 group B plasmas was completely abolished in 4/6 samples, where 2 samples reacted only weakly. Interestingly, 2/3 group AB plasmas reacted strongly with the modified RBCs.

Summary/Conclusions: Using a recombinant deacetylase, we have demonstrated the feasibility of creating acquired B RBCs from readily available donor A_1 RBCs, thus providing a reagent for quality assurance of monoclonal anti-B. These RBCs can be frozen once treated, thus providing a reliable source of this unusual but clinically important phenotype.

P337 | Abstract withdrawn

P338 | Cephalosporins induced haemolytic anaemia: a case study

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Background: Cephalosporins are known by causing haemolytic anaemia (HA). A ten-year-old child was treated with cefuroxime and ceftriaxone and 4 days after its onset presented severe immunomediated haemolytic anaemia (minimum Hb 2,6g/dL, 4 days after admission with Hb 12,9g/dL), characterised by malaise, dizziness, acute marked pallor, sensation of heat, tachycardia (110bpm), acute low back pain.
Aims: To prove Patient's haemolytic crisis was caused by cefuroxime and ceftriaxone. The red blood cells (RBC) treated with cefuroxime was thought to be the cefuroxime´s haemolytic mechanism, and Patient´s serum in the presence of ceftriaxone and a suspension of red blood cells not treated, proved the haemolytic mechanism of ceftriaxone.
Methods: Female child, 10 years old, with the following personal history: Lymphangioma of the left parotid lymph node, with no indication for surgery due to associated vasculo-nervous risk, with several hospitalisations for overinfection and several courses of antibiotic therapy, including ceftriaxone and clindamycin, with no apparent adverse reaction; Hashimoto's thyroiditis, obesity, hypertriglyceridemia, hypercholesterolaemia and steatohepatitis. Initial stabilisation was achieved with volume expansion with repeated crystalloid infusions and later transfusion support 1 unit CE ORh-, inotropic support and anticonvulsant treatment. She was subsequently transferred to the Paediatric Intensive Care Unit (PICU) of another hospital. Antibiotic therapy was changed to quinolone (ciprofloxacin/levofloxacin) associated with clindamycin with good tolerance.
Our investigations due to the severe immunomediated haemolytic anaemia included the study of red blood cells (O RBC) and the Patient´s serum by R.T. (room temperature), 37°C and indirect antiglobulin test (IAT). Patient´s serum was tested against cefuroxime treated RBCs. It was also tested against untreated RBCs in the presence of ceftriaxone.
Results: The autologous control was positive either by IAT or by RT. However, Patient's serum showed positive by IAT, but negative by R.T. The Patient's serum did react with O RBCs in presence of ceftriaxone and complement, and with cefuroxime -treated RBCs.
Summary/Conclusions: After antibiotic therapy was changed to quinolone, the post-transfusion Hb control was 24h and 48h later, 7.7 and 9.1 g/dL, respectively. Improvement of the haemoglobinuria was observed throughout hospitalisation, with normalisation of the renal function approximately 12 hours after the initial episode. Diuresis was always maintained. Always vigilant, without new convulsive episodes or other neurological complaints.
Combined with Patient's history, clinical data, and laboratory results, we strongly suspected the HA was caused by ceftriaxone and cefuroxime (cephalosporins).

Immunohaematology

Red cell immunohaematology–Molecular

P339 | Genotype analysis of JR blood group and development of amplification refractory mutation system PCR in Korean population

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Background: The JR blood group system (ISBT JR 032), which has one antigen, Jr^a, is generally known as a high prevalence antigen in most population. Jr(a-) phenotype has been reported to be relatively higher in Japanese and Asian population. Jr (a-) phenotype prevalence was reported 0.07 percent in Japanese blood donors, but, it has been found to be relatively rare in other populations. Jr^a antigen is encoded by *the ATP-binding cassette, member G2 (ABCG2)* gene on chromosome 4q22.1. Jr(a-) individuals can be incidentally identified by the production of anti-Jr(a) antibodies and confirmed by the presence of two null alleles of *ABCG2*. The mutation site in *ABCG2* is different according to ethnic group and c.367C>T has usually been found in Asian population.
Aims: It is difficult to find Jr^a negative blood donor for compatible transfusion in general inventory of Korea because the Rare Blood Program in Korea (KRBP) does not include JR blood group system.

P339 – Table 1 RBC treated with Cefuroxime and Patient´s serum:

	Treated RBC	Untreated RBC	Autocontrol
RT	0	0	weak
IAT	weak	0	weak

P339 – Table 2 - Untreated RBC with Ceftriaxone and Patient´s serum:

		Patient´s Serum +Ceftriaxone	Patient´s Serum+ Ceftr+ Complement	Patient´s Serum+PBS
Ceftriaxone	0		weak	0

Therefore, we analysed genotyping of *ABCG2* polymorphism using direct sequencing in healthy people and compared frequency with estimated prevalence from public databases. Additionally, we performed, simple and economical method, tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique to genotype single nucleotide polymorphisms (SNPs) to differentiate heterozygous for a single *ABCG2* mutation.

Methods: We have analysed the genotype frequency of *ABCG2* null allele (c.376C>T, rs72552713) from 300 national healthy population cohort using direct sequencing, comparing with calculated frequencies from public genomic databases. Additionally, we developed a simple and robust genotyping method, tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique that can be adopted in clinical setting.

Results: MAF of East Asian was 0.023 and it was estimated that heterozygote and homozygote for variant were 0.0354(3.5%) and 0.000529(0.053%). We assumed that 12.9 individuals in 300 Korean people would heterozygote for *ABCG2*. In our study, we found c.367C>T nucleotide change of 14 DNA samples in Sanger sequencing and it was 4.67% (14/300).There was no homozygote for the mutation causing Jr (a-) phenotype by direct sequencing, but we calculated homozygous variant frequency (0.001%). In tetra-primer ARMS-PCR, allele-specific primers produced exact bands predicting SNP allele. Total 14 mutants and other 26 wild type samples, which were confirmed in Sanger sequencing, were tested and it showed distinct band pattern in 137bp (C allele) and 165bp (T allele). While wild type samples revealed only 137bp band in tube 2, it showed 165bp band in tube 1, 137bp band in tube 2 in case of all mutant type samples.

Summary/Conclusions: In this study, we performed genotyping of JR blood group antigen polymorphism using direct sequencing and tetra-primer ARMS-PCR. The frequency of MAF (c.367C>T) was 4.67% in Korean and the estimated prevalence of homozygote for variant allele was estimated 0.001%. Though, it is still very important to define JR blood group because anti- Jr^a antibody can cause severe transfusion reaction, alloimmunization. Therefore, application of tetra-primer ARMS-PCR can be a useful solution to detect null alleles of *ABCG2* causing Jr(a-)phenotype in common laboratory.

P340 | Genetic diversity of *RHD* in Brazilians

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Background: : Rh is the most polymorphic blood group system with an increasing number of variant alleles, and this complexity is especially evident in multiethnic populations. Knowledge of *RH* variants in these populations is essential to improve transfusion safety. RhD variants can be detected during serological routine by reduced expression of the RhD antigen or discrepancy in results.

Aims: The purpose of this study is to describe *RHD* diversity in individuals from Southeast Brazil with weak D phenotype or discrepancies in the serological tests.

Methods: A total of 588 samples were included in the study, of which 489 were selected due to reaction ≤3+ in microplate typing using anti-D RUM-1 and D175+D415 (Immucor NEO®); 76 had a discrepancy in D status between different donations, with a D-negative result in the previous donation and D-positive in the current donation; and, 23 were typed as D-positive with anti-D detected in serum. All samples were genotyped using PCR-RFLP, Multiplex-PCR and Sequencing.

Results: Significant allelic diversity was shown, with 38 different types of variant *RHD* alleles. The most frequent variants with reduced RhD antigen expression were *RHD*DAR1.02* (31%), *RHD*DAR3.01* (21%), *RHD*01W.3* (15%), *RHD*01W.2* (9%) and *RHD*01W.38* (7%). In the samples with discrepant D typing results, 79% were characterised as *RHD*01W.38*, followed by *RHD*11* (14%), *RHD*DAR1.2(1063A)* (3%) *RHD*01W.3* (3%) and *RHD*DEL1* (1%). The most common variants involved in anti-D alloimmunization were *RHD*DIVa* (35%), *RHD*DIIIc* (13%), *RHD*DAR1.02* (13%) and *RHD*DIIIa* (9%).

Summary/Conclusions: Our results show the diversity of *RHD* alleles present in Southeast Brazilian RhD variants. This study also shows a high prevalence of weak D type 38 and *RHD*DAR1.02* emphasizing the mixed background of the Brazilian population and highlights a significant number of partial antigens with a risk of alloimmunization. This is particularly relevant as these data could provide the means for reducing RhD alloimmunization in this population.

P341 | Rare *RHD* variants in blood donors

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Background: In the last decades molecular determination of blood groups, particularly within the Rh system has expanded considerably. Different variants of RH genes in donors when undetected can cause alloimmunisation in RhD-negative recipients.

Aims: The aim of the study was to detect distinctive molecular mechanisms for RhD negative phenotype within donors.

Methods: DNA was isolated from 600 serologically RhD-negative unrelated blood donors with C+ and/or E+ who routinely donate blood at Institute for transfusion medicine of Republika Srpska. Molecular testing included initial screening for *RHD*, *RHD* exon scanning, determination of clinically most relevant weak *RHD* alleles. The FluoGene assays used are based on PCR-SSP (PCR-sequence specific primers) with the results evaluation by fluorescence reading on the FluoVista instrument (*Inno-train Diagnostik*). All the samples with an *RHD* variant were subsequently examined by adsorption/elution

technique (human anti-D – in house production, eluted by DiaCidel solution for acid elution, *Bio-Rad Laboratories*) in order to confirm or dismiss the DEL expression of the respective *RHD* allele.

Results: In 5/600 (0,83%) donors in total three different *RHD* alleles were determined – *RHD*01N.03*, *RHD*01EL.44* (*RHD*DEL44*) and *RHD*05.05* (*RHD*DV.5*).

Four donors with the rare hybrid alleles (*RHD*01N.03* and *RHD*01EL.44*) were afterwards confirmed as RhD-positive by adsorption/elution, thus confirming the DEL phenotype expression of these alleles. Both alleles are commonly interpreted as having an RhD negative expression. The *RHD*01N.03* allele has a structure of *RHD*D-CE (2-9)-D*, which suggests it should typically behave as a null allele. Although *RHD*DEL44* is actually catalogued as the DEL allele by ISBT, its DEL phenotype was confirmed only originally in China and just recently in Croatia.

Summary/Conclusions: Our results demonstrate the need for molecular clarification of RhD-negative phenotype in C+ and/or E+ donors. Especially for hybrid alleles it is imperative to determine whether they express the RhD antigen and not to assume its RhD negative phenotype by default, considering the possibility of alloimmunisation in the RhD negative recipients.

P342 | Abstract withdrawn

P343 | Blood group genotyping using an amplicon-based NGS analysis, proof of principle

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Background: Molecular mechanisms behind most blood group systems are known today. There are different commercial kits available for genomic blood group typing but most of them are limited by the number of blood groups and/or alleles that can be analysed simultaneously. With the NGS technology, many more blood groups can be analysed and with the possibility to sequence whole genes, rare or novel alleles can be detected

Aims: The aim of this project was to set up a simple and cost-effective NGS method using an amplicon-based sequencing as contrast to whole genome/gene sequencing.

Methods: Oligos were designed for informative exons in different blood group genes (some shown in table below).

In addition to blood group genes, we also targeted 28 different human platelet antigens (HPA) and 5 human neutrophil antigens (HNA). A first PCR, amplification of amplicons (exons), was followed by a second PCR where index-sequences were attached. PCR-products from different patients were then pooled, cleaned, and run on MiniSeq instrument (Illumina).

DNA samples were analysed from patients that were previously typed with serology (n= 40) or FluoGene system (Inno-train) (n= 109).

P343 – Table 1

Blood group	Gene	Blood group	Gene
ABO	ABO	Lutheran	BCAM
RH	RHD	Diego	SLC4A1
RH	RHCE	Cartwright	ACHE
KEL	KEL	Colton	AQP1
Kidd	SLC14A1	LW	ICAM4
Duffy	ACKR1	Cromer	CD55
MNS	GYPA	Indian	CD44
MNS	GYPB	OK	BSG
Dombrock	ART4	Vel	SMIM1

Results: In all 40 samples, previously typed serologically for different antigens, NGS results showed concordant results. For samples analysed with the Fluogene system, there were some discrepancies regarding the RHD typing. In 26 samples, the Fluogene system either gave a false result (n= 10) or indicated the absence of variants (n= 16) when the NGS method identified different variants. With the NGS method, 7 novel RHD-negative variants were found. Also, using copy number variation (CNV) analysis, RHD-RHCE hybrids could be identified.

No discrepancies were found for other blood groups eligible for comparison.

Summary/Conclusions: With the amplicon-based NGS method, a range of different blood groups could be analysed in the same run. The method is easy to perform with only 45 minutes of hands-on time and up to 96 samples can be analysed in one run.

P344 | Known variants in new combinations result in two novel B alleles present in individuals with aberrant ABO expression

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Background: Many ABO subgroups have been explained at the genetic level, although unresolved samples are still encountered in clinical practice. We report the result of flow cytometric and genetic investigations of two samples that showed weak B antigen expression.

Aims: To identify the genetic background of weak B phenotype in two unrelated individuals.

Methods: Serological testing was performed according to standard blood bank practice. *ABO* genotyping including analysis of the upstream CBF/NF-Y enhancer was performed on genomic DNA using routine in-house PCR assays. *ABO* exons 1 to 7, proximal promoter and parts of intron 1 were sequenced and analysed. Red blood cells (RBCs) were characterised by flow cytometry.

Results: RBCs from proband 1 showed weak reactions with anti-B and unexpected plasma reactions with B RBCs, 1+ and 2+ depending

on method used. RBCs from proband 2 showed varied expression, from negative to 2+ or 4+ depending on what clone of anti-B was used but had the expected reactions for a group B individual in the reverse typing.

Initial routine *ABO* genotyping revealed *ABO*B.01/O.01.01* and *ABO*A2.01/B.01*, respectively. However, in both cases DNA sequencing of the *B* allele revealed two alterations compared to a consensus *B*-allele.

Proband 1: A single nucleotide variant (SNV) c.705C>G, p.235Arg (no rs-number available) affecting one of the A- vs. B-determining amino acids (p.Gly235Ser) was found. This change has previously been found in a B weak individual (Hult et al., Vox.Sang. 2012) but here it was found in combination with c.680C>T, p.Pro227Leu (rs781897939). When tested by sensitive flow cytometry this sample displayed low expression of B antigen, even lower than the genetically defined B_{weak} control RBCs (*ABO*BW.03*).

Proband 2: c.926A>G, p.Tyr309Cys (rs56346931) was found in a *B* allele without one of the seven expected SNVs defining a *B* allele, c.930G>A (rs8176749). By flow cytometry a slightly higher expression than the included B_{weak} (*BW.03*) control was detected.

H levels were normal in both cases.

Summary/Conclusions: The SNV c.705C >G (p.235Arg) in proband 1 has previously been reported to cause weak B expression, however B levels were higher than in the case presented here. The addition of c.680C>T (p.Pro227Leu) seems to further cripple the B glycosyltransferase and give rise to a severely lowered B expression. This individual also has a weak but detectable anti-B present in plasma.

In proband 2 the SNV found in the *B* allele, c.926A>G (p.Tyr309Cys) has previously only been reported on *ABO*O.01.28* where its impact cannot be assessed by phenotyping. Evaluating the SNV with the SIFT and PolyPhen softwares available online, it is deemed to have a significant influence on the enzyme, namely "deleterious" and "probably damaging", respectively. The effect of c.930G is probably minor since it does not cause an amino acid change. One can speculate that this is a hybrid formation of the common *B* allele and *ABO*O.01.28* since c.926A>G and c.930G are closely situated. The variation in B expression strength with different clones of anti-B was unusual and striking. Interestingly, neither p.227 nor p.309 are evolutionarily conserved but are adjacent to invariant/conserved regions of the enzyme.

In conclusion, two novel *B* subgroup alleles, both with dual changes compared to consensus *B.01*, were genetically clarified and B antigen expression was semi-quantified by flow cytometry.

P345 | A compound heterozygote of three novel *FUT1* mutations causing a para-Bombay phenotype and computational prediction on mutation effect

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Background: Human red blood group H antigen is served as the precursor structure for A and B blood group-specific glycosyltransferases.The antigens of ABO blood group could be directly affect by the change of substance H, which is encoded by a-(l,2)-fucosyltransferases genes *FUT1* and *FUT2*. Mutations that negatively affect the a2-fucosyltransferase(α2FucT1) activity (encoded by *FUT1*) will result in reduced or absent H production on red blood cells, while the fucosyltransferasea a2FucT2 encoded by FUT2 determines H antigen expression in secretions. The rare para-Bombay phenotype is usually characterised by the reduced or absence of ABH antigens on red blood cells but the presence of corresponding antigens in saliva.

Aims: Here, the underlying molecular mechanism of a para-Bombay AB phenotype combined three novel mutations of FUT1 was investigated.

Methods: ABH antigen and antibodies in serum were detected using standard serological methods. *ABO*, *FUT1* and *FUT2* genes were directly sequenced by PCR-SBT to determine the genotypes. The haploid type of novel FUT1 alleles were analysed by TA clone sequencing. The 3D structural of wild-type and mutant fucosyltransferases were simulated and analysed by Phyre2 and Pymol software. The effect of mission substitutions on the function of fucosyltransferase was predicted by Polymorphism Phenotyping algorithm (PolyPhen-2) and MutationTaster.

Results: The proband was found to be absence of ABH antigen on the surface of red blood cells by routine serological tests.The ABO genotype was *ABO*A1.02/ABO*B.01*. The results of *FUT2* gene sequencing were Se/Se2. Three novel mutations, including two missense mutations (c.289G>A and c.575G>C)and one synonym mutation c.840G>A were identified in *FUT1* gene. 3D homology modeling showed that amino acid substitution caused by missense mutations changed partial spatial structure of the a-helices where residue 97 and 298 were located. Both missense mutations were defined as "probably damaging" with a specif sore of 1.000.

Summary/Conclusions: Three novel FUT1 mutation were identified in a Chinese individual with para-Bombay AB phenotype, which expanded our knowledge of the underlying molecular mechanism of para-Bombay phenotype and contributed to the improvement of clinical transfusion safety.

P346 | Abstract withdrawn

P347 | Identifying *RHCE* variants in Flanders (Belgium) and implications on regional transfusion policy

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Background: People originating from sub-Saharan Africa (SS-Africa) are known to have more variant red blood cell (RBC) alleles such as FYGATA, UVARs and variants of the Rhesus system. The complexity of the latter is often underexplored in patients as well as donors. In patients, this may result in an increased alloimmunisation risk, due to lack of information on the absence of high prevalence antigens and the presence of low prevalence antigens in SS-Africa donors. This results in a challenging transfusion practice in patients with haemoglobinopathies originating from SS-Africa with a chronic need for blood transfusion.

Aims: This work aims to improve RBC concentrate (RCC) selection for patients with haemoglobinopathies originating from SS-Africa by genotyping patients and donors (proxy FYGATA) for those SNPs that allow us to discriminate between the most relevant RHCE alleles in regard to blood selection based on the presence and/or absence of low prevalence and high prevalence antigens.

Methods: Based on literature and the ISBT table for RHCE, we identified SNPs that allow us to discriminate between the most frequent and clinically relevant RHCE alleles. Both the "LinkSēq™ RBC with RH" kit from Thermo Fisher Scientific and the "CDE eXtend" kit from inno-train meet these requirements and were used to genotype our cohort ($n = 34$).

Results: As seen in other haemoglobinopathy cohorts we found a high frequency of RHCE variant alleles (In total 58,8% of all RHCE alleles are variant alleles, 52,9% of the samples have 1 RHCE variant, 32,4% have a combination of 2 RHCE variants). *RHCE*01.20.01*, *RHCE*01.20.03* and *RHCE*01.20.09* comprise 1/3rd of the total number of RHCE alleles identified and are near to 60% of the total number of variants found in our cohort. These alleles encode for the extra low prevalence V (RH:10) and/or VS (RH:20) antigens, important to identify in donors and a weak to negative high prevalence hrB (RH:31) antigen, which could cause alloimmunisation in patients. These data illustrate the need to genotype both donors and patients originating from the SS-Africa region to enable us to evaluate this risk and build a 'best practice transfusion compatibility matrix' for these patients in our population. Besides RHCE, also other RBC systems have a high prevalence of variants in our cohort, including U+^{var} *GYPB*03 P2*, *NY*, weak *JK*01* and *KEL*02* variants confirming the benefit of extensive RBC genotyping for the SS African population.

Summary/Conclusions: In Flanders, the prevalence of RHCE variants, as well as variants of other blood group systems is high in patients and donors originating from SS-Africa (proxy FYGATA). The most frequent RHCE variant alleles found have different implications when present in a donor or in a patient, emphasising the need of RHCE genotyping to realize the best donor-patient RHCE match for RCC transfusion in an increasingly multicultural population.

P348 | Validation of high-throughput blood antigen genotyping using MALDI-TOF MS technology

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Background: Provision of compatible blood units for patients with multiple alloantibodies is a complex and time-consuming procedure often requiring multiple cross-matchings. The process may, however, be simplified by the access to a donor registry with determined extended blood group phenotypes. In 2021, the Polish Ministry of Health initiated a health policy program entitled "Ensuring the self-sufficiency of the Republic of Poland in blood and blood components for the years 2021-2026". As a part of the program, the establishment of a donor registry to secure/provide antigen-negative compatible blood for alloimmunised patients with multiple alloantibodies has been planned. The donor database will include blood donors with homozygous phenotypes in Rh(C,c,E,e), K/k, Jk(a,b), Fy(a,b) or MNS(M,N,S,s) systems confirmed by genotyping and enriched by molecular methods with the determination of the high/low-frequency antigens, clinically relevant for the Polish population.

Aims: The aim was to validate high-throughput blood antigen genotyping of blood donors using matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS) as a first step to establish the above-mentioned donor registry.

Methods: DNA from 16 Instand EQA no. 235 samples (31-34/2019, 61-64/2020-2022) and 4 WHO Reference Reagents (RBC1, 4, 5, 12 NIBSC), 21 blood donors, 20 RBC reagent panel donors, and 18 control samples with rare genotypes identified in Poland were tested by MALDI-TOF MS technology using Haemo ID™ Donor Quick Screen (DQS) Panel on MassARRAY Dx Analyser 4 with Chip Prep Module (*Agena Bioscience*). Each antigen was tested using (if available), homozygous and heterozygous samples in three different runs; 21 repetitions were performed. The results were analysed with the Typer software according to the producer manual and the Haemo ID™ Panel Reports were compared to known blood group antigen genotypes established using RBC FluoGene RBC-CDE, -vERYfy, -RARE (*Inno-Train);* ID CORE XT (*Grifols*); *in-house* qPCR tests or Sanger sequencing.

Results: The results of 100 tested samples obtained using MALDI-TOF MS technology were focused on 42 antigens from 12 blood group systems, but there was no control for Di(b-), U-, Jo(a-) and Hy- samples in the tested group. The results for 16 Instand EQA and 4 WHO Reference samples were in agreement with genotypes

reported by the producers for Rh(C,c, E,e), K/k, Jk^a/Jk^b, Fy^a/Fy^b and M,N,S,s antigens. The results from the Haemo ID™ Panel Reports for 21 blood donors and 20 RBC reagent panel donors showed complete concordance for all antigens included in Haemo ID™ DQS Panel to the already known blood group antigen phenotypes/genotypes. The exception were four MAR- samples (*RHCE*CeCW/RHCE*CeCW*; with homozygous c.122G change in *RHCE* confirmed by sequencing) that were determined as heterozygous c.122A/G. Rare blood group antigens were correctly identified in all 18 control samples with known rare phenotypes/genotypes such as Kp(a+b-), Yt(a-b+), Co(a-b+), LW(a-b+), Di(a+b+), Lu(a+b-), Sc:-1,2; V, VS, Fy_{null}, Fy^X or with HbS and HbE haemoglobins.

Summary/Conclusions: Blood antigen genotyping with MALDI-TOF MS technology was successfully validated for all antigens included in Haemo ID™ DQS Panel except for Di(b-), U-, Jo(a-), Hy- phenotypes which still require validation and MAR- phenotype which requires manual analysis.

P349 | Use of microarrays as agglutinates sorting for reverse typing innovative approach

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Background: Since 20 years, academic laboratories and compagnies are involved in the multiplexing of blood grouping tests . Literature shows numbers of examples that antigens detection is feasible but antibodies detection is the most challenging. Most of the time, antibody detection is based on EIA (Enzyme ImmunoAssays) tests while existing reverse typing is based on agglutination. EIA tests are widely used in the IVD industry and showed good performances in various applications.

Anyway, even if EIA enable the detection of various type of antibodies, they probably cannot decipher agglutinating antibodies from others. This lead to discrepancies between EIA and agglutination tests.

Based on this assumption, we developed a multiplex agglutination test combined with microarray in order to sort out agglutinates from free cells.

Aims: We developed a multiplex reverse typing assay to improve the throughput, reduce reagents, consumables, waste and build a fully integrated multiplex solution for blood grouping.

Methods: Here we describe an innovative patented multiplex assay from whole blood samples using haemagglutination principles to detect regular antibodies. The developed method allows the detection of both regular antibodies A and B inside one well of a standard 96 well plate. The process will require few microliters of sample with a time to results below 10min.

Results: Our different tests on donors and receivers samples showed very good performances with 99,7% of concordance with existing agglutination tests. The few missing antibodies are under investigation but seems to be due to soluble antigens interference.

Summary/Conclusions: Miniaturization and multiplexing of reverse typing tests were identified as the most challenging part to get a full multiplex solution dedicated to blood grouping. By combining multiplex agglutination and sorting microarrays, we identified the key to challenge current practices and to improve easily the amount of information offered per sample.

P350 | A seven-year experience of external quality assessment program for *RHD* fetal genotyping

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Background: Non-invasive fetal *RHD* genotyping helps the practitioners to improve the monitoring of RhD (RH1) negative pregnant women. In a context of anti-D (anti-RH1) allo-immunization, a positive *RHD* fetal genotyping allows the diagnosis of feto-maternal incompatibility. For non-immunised women, a negative test prevents prophylactic injections of anti-D immunoglobulin (Rhlg) during pregnancy. Since fetal *RHD* genotyping became a key element to monitor RH1 negative pregnant women, an increasing number of laboratories has implemented this test routinely. In 2010, it appeared essential for the French National Reference Center in Perinatal Haemobiology (CNRHP), as part of its missions, to propose an external quality assessment, based on external quality controls (EQC). The CNRHP can rely on more than twenty years' experience in fetal *RHD* genotyping from maternal blood to establish such controls. The laboratory is accredited according to EN ISO 15189 for this test since 2012. In 2015, the CNRHP transferred its EQC program to a certified EQA organism: ASQUALAB.

Aims: The aim of this presentation is to review the results of the EQC program seven years after its launch by ASQUALAB.

Methods: Positive control specimen were prepared from RH1 negative plasma donors spiked with various concentration of RH1 positive plasma in order to reflect RH1 positive fetuses at different gestational ages. Negative control specimen, made also from RH1 negative plasma donors, remained unspiked. After the initial CNRHP analysis, the samples were conveyed to the participating laboratories with a feedback form where they had to state 1) the material and methods used and 2) the results and the clinical biological interpretation in the context of a clinical case. The control samples were sent twice a year.

Results: 14 assessments were conducted since 2015 with an increasing number of laboratories from 7 to 16 in 2022. Each year, we achieved a 100 % response rate. EQC results were most of the time conform to those expected although the laboratories use different extraction and amplification protocols. Some laboratories made erroneous clinical interpretations despite right analytical results.

Summary/Conclusions: The presented EQC meets the criteria required to evaluate the practices of laboratories performing non-invasive fetal *RHD* genotyping. The extension of the field from

analytical to post-analytical process, including results interpretation and biological advices for physicians, was important to improve national harmonization of the results of this specialised examination, and to highlight the importance of giving clinical advices to help prevention of fetal and/or neonatal anaemia.

P351 | Blood group genotyping in donors of African descent

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Background: Patients of African descent requiring chronic transfusion are at particular high risk of alloimmunization in countries where blood donors are predominantly Caucasians. In Switzerland, there is an increasing number of patients of African descent with sickle cell disease or other diseases who might require regular transfusions. Therefore, a targeted donor recruitment and appropriate typing strategies are important to identify blood donors with red blood cell antigens matching these patients. Molecular red cell genotyping represents a powerful tool to find donors with rare blood groups.

Aims: Extended blood group genotyping in donors of supposed African descent based on the presence of the allele *FY*02N.01*, in order to identify rare alleles typical of the African population.

Methods: Samples of potential rare donors were obtained from two blood donation centers. Donors were selected based on the presence of the allele *FY*02N.01* (homo- or heterozygous), which had been identified in a previous routine donor screening for 24 clinically relevant blood group alleles (Lejon Crottet, VoxSanguinis, 2012). Genomic DNA was extracted from EDTA-blood using the QIAsymphony DSP DNA Mini Kit (QIAGEN AG). The in-house genotyping module included four multiplex primer mixes with four to six primer pairs per reaction mix, targeting a total of 17 different single nucleotide variants (SNVs, see tables). Each mix contained primers for both high frequency antigens and low frequency antigens or an internal amplification control. Amplicons were analysed by capillary gel electrophoresis using the QIAxcel DNA Screening Kit (QIAGEN AG). Samples positive for rare alleles were further tested for zygosity.

P351 - Table 1: Genotyping Results (Antigens)

Antigen	Targeted SNV	Number (Total 286)	Frequency [%]
CROM1	c.679G	286	100.0
CROM2	c.155G	286	100.0
CROM3	c.155G>T	1	0.4
DO4	c.323G	286	100.0
DO5	c.350C	286	100.0
KEL6	c.1790T>C	16	5.6
KEL7	c.1790T	286	100.0

P351 - Table 2: Genotyping Results (Alleles)

Allele	Targeted SNV	Number (Total 286)	Frequency [%]
*FY*02N.01* (homozygous)	c.-67T>C	53	18.5
*GYPB*03N.01* (NY)	c.230C>T	0	0.0
*GYPB*03N.03* (P2)	c.270+5G>T	8	2.8
*RHCE*01.04* (ceAR)	c.916A>G	0	0.0
*RHCE*01.06* (ceAG)	c.254C>G	6	2.1
*RHCE*01.07* (ceMO)	c.667G>T	3	1.1
*RHCE*01.20* (ceVS)	c.733G>C	42	14.7
*RHCE*01.20.03* (ceS)	c.1006C>G	1	0.4
*RHCE*02.10* (CeRN)	c.514T>A	2	0.7

Results: Over a period of five years (2018-2022) 286 samples harbouring the allele *FY*02N.01* could be identified. The genotyping results of these 286 samples are summarised in tables 1 and 2. Almost one-fifth (18.5%) of the samples were homozygous for *FY*02N.01*. All samples encoded the high frequency antigens CROM1, CROM2, DO4, DO5 and KEL7. One sample (0.4%) was heterozygous positive for CROM3. Several samples had a *RHCE* or *GYPB* variant, and as expected the *RHCE*01.20* variant allele was detected most frequently (14.7%).

Summary/Conclusions: Here we report results for a blood group genotyping screening targeting SNVs found in rare blood group alleles. The number of donors assumed to be of African descent due to the presence of the allele *FY*02N.01* was limited. Nevertheless, our experience shows that this strategy can increase the number of rare blood donors of African descent in a country with predominant Caucasian population. In conclusion, we have successfully established an extended genotyping assay to detect variant alleles predominantly occurring in people of African descent. Facing the increasing number of patients of non-Caucasian origin living in Switzerland, more effort is needed to recruit donors of different ancestral background, as a source of red blood cells for patients that require rare blood.

P352 | Transfusion management using blood group genotyping of children with sickle cell disease when the pool of Black donors is limited.

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Background: Red blood cell (RBC) transfusion is an effective and beneficial treatment for patients with sickle cell disease (SCD). However, alloimmunization can occur after a single transfusion and can be associated with life-threatening complication, thus complicating further usage of blood transfusion. Prophylactic transfusion matching for D, C, E and K antigens can lower the risk of alloimmunization in SCD patients with altered *RH* alleles. Availability of compatible blood units can be a challenge for blood providers with a limited number of Black donors.

Aims: This study aims to define the frequency of variants in the Rh and Duffy blood groups in a pediatric SCD cohort and to evaluate the likelihood of finding compatible blood donors presenting similar *RH* variants through a genotyped Black donors' database available at our provincial blood center.

Methods: A prospective cohort of 205 pediatric SCD patients was studied for the period of December 2016 to April 2022. Genotype of the *RH* and *FY* genes was performed to evaluate the frequency of variants in these blood groups. Number of transfusions and alloimmunization data were collected. Our capacity to find RhCE matched donors (CcEe, hr^B, Sec and CEAG antigens) was evaluated using a database of 51 000 genotyped donors, which includes 6530 donors from visible minorities or Black donors.

Results: Mean age was 14.2 ± 4.1 years (median: 15.6, IQR: 6.8) at the time of analysis for alloimmunization. Nearly 9.8% ($N = 20$) of patients carried variants causing a partial D and 5.9% ($N = 12$) were D-. Only 45.9% (188/410) of *RHCE* alleles were considered normal, with the majority of variants affecting the RH5 (e) antigen. Most patients (88.8%, $N = 182$) were Fy(a−b−). Among the 205 patients studied, 140 (68.3%) were transfused at least once. A total of 3943 RBC transfusions were given during the study period (range: 1-763, median: 6, IQR: 13). We found an alloimmunization rate of 20.7% and a Rh alloimmunization rate of 7.1 %. Seventy-seven reactions were reported in 39 patients. Most reactions were benign: 61 non-haemolytic febrile reactions (79.2%), 3 minor allergic reactions (3.9%) and 11 attributed to a condition preceding the transfusion (14.3%). Two serious reactions were reported (2.6%): 1 transfusion associated circulatory overload and 1 delayed haemolytic reaction with hyperhaemolysis.

Black donors represented only 1.40% of all donors in our province for the period of the study (4772 of the 343556 unique donors). As such, blood units from D- Caucasian donors were mostly used to provide phenotype matched products. However, compatible blood for patients with rare Rh variants (Sec-, CEAG- or hrb-) were found only in Black donors. Even if a donor with compatible RhCE could be identified for all patients, the blood unit availability remained problematic due to the low number of D- donors or other antigen-negative requirements, including those from KEL and FY systems.

Summary/Conclusions: Based on a single transfusion, we found a compatible RhCE donors for all patients despite the limited number of Black donors. Although Rh-compatible donors were identified, blood units might not be available when needed by the patient and/or the extended phenotype or ABO group might not match the patient. This could be critical for patient needing multiple transfusions. A greater effort has to be made for the recruitment of Black donors to accommodate SCD patients.

P353 | Evaluation of the effect of prophylactic genotypic matching on alloimmunization and autoimmunization in patients with sickle cell disease (SCD)

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Background: : Blood transfusion is one of the mainstays of treatment for patients with SCD but despite of the beneficial effects, transfusion therapy can lead to red blood cell (RBC) alloimmunization with serious complications for the patient including life-threatening delayed haemolytic transfusion reactions and the hyperhaemolysis syndrome. In addition, the presence of alloantibodies, which is often associated with the concomitant presence of autoantibodies, can lead to difficulty in finding compatible units, which can cause transfusion delays. To reduce alloimmunization some strategies have been implemented to provide antigen matched RBC transfusions to patients with SCD who need chronic transfusion support. In this context, phenotypic and genotypic matching protocols have been used.

Aims: Based on this, the aim of this study was to evaluate the effect of prophylactic RBC transfusion genotypic matching on alloimmunization and autoimmunization in patients with SCD.

Methods: Our study included 79 (33 male and 46 female) patients with SCD, homozygous for HbS, on chronic RBC transfusion therapy receiving prophylactic genotypic matching RBC transfusions in the last two years. In this period, patients received RBC units genotypic matched for Rh, K, Fya/Fy/b, Jka/Jkb, S/s, Doa/Dob and Dia antigens. Molecular matching was performed according to previous molecular typing results performed by the HEA BeadChip (Immucor) in patients and donors. The availability of compatible units by performing genotypic matching were compared to serologic matching.

Results: The patients received a range of 5-215 units. And the median age in this group was 39 years old. Of the 79 patients, 17 (21.5%) were alloimmunised before they started receiving genotypic matched RBC transfusions. During this study-period only 2 patients (2.5%) with Rh variants developed alloantibodies (1 anti-C, 1 anti-e) and one patient produced autoantibody. Although the availability of compatible units has decreased when compared to serological matching, it was possible to find compatible units for most patients.

Summary/Conclusions: RBC transfusions with extended genotypic matching had significant effects on autoimmunization and alloimmunization rates in chronically transfused patients with SCD over two years. Alloimmunization rates have shown to decrease from a range of 21.5 to 2.5% in these patients. SCD patients may benefit from receiving prophylactic genotypic matching RBC transfusions as demonstrated by the reduction on the rates of alloimmunization and autoimmunization.

P354 | A patient of Malaysian/Indian origin with the Scianna-null phenotype caused by compound heterozygosity for two novel ERMAP alleles

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Background: The Scianna (SC) blood group system is comprised of nine antigens, seven of high prevalence (Sc1, Sc3, STAR, SCER, SCAN, SCAR, SCAC) and two of low prevalence (Sc2, Rd). All antigens are expressed on the human erythrocyte membrane-associated protein (ERMAP), a 475 amino acid, single pass type I glycoprotein. The gene encoding this protein, *ERMAP*, is located on chromosome 1 and is organised over 12 exons, the first two of which are non-coding. Anti-Sc3 produced by patients with the rare SC_{null} phenotype (SC:-1,-2,-3) has been reported to have caused mild delayed transfusion reactions and mild haemolytic disease of the fetus and newborn. Three molecular bases of the SC_{null} phenotype have been reported: the recognised ISBT alleles c.307_308delGA and c.994C>T (*SC*01N.01* and *SC*01N.02* respectively) and the recently published c.349C>T.

Aims: To present results from the serological and molecular investigations of a patient with anti-Sc3 and to show a novel molecular background of the SC_{null} phenotype.

Methods: Samples were collected from an 82-year-old Malaysian/Indian female, requiring small bowel resection. These were investigated due to an alloantibody in her plasma, reacting with all cells tested except her own. Serological investigations were performed by LISS tube IAT, papain IAT and adsorption and elution techniques were also utilised. Sequencing of targeted blood group panel libraries, prepared using Illumina DNA Prep with Enrichment, was performed on an Illumina MiSeq. Alignments were performed using MiSeq Reporter and *ERMAP* sequence was visualised in Integrative Genome Viewer.

Results: The plasma from the patient reacted moderate strength by LISS IAT with all untreated and papain treated panel cells tested. The patient's cells were found to have the SC:-1,-2,-3 phenotype and anti-Sc3 was identified in her plasma. Alloadsorption with cells matching the patient's common phenotypes successfully removed the antibody from the plasma, and an eluate prepared from the adsorbing cells was also non-reactive with SC:-1,-2,-3 cells. *ERMAP* sequencing revealed two rare heterozygous mutations: c.373C>T in exon 4, encoding p.Arg125Ter (rs772256111; freq. 0.00008) and c.596C>G in exon 7, encoding p.Ser199Ter (not listed in GnomAD database). Both mutations would introduce premature termination of protein translation. Due to the SC:-1, -2, -3 phenotype, we predict that the heterozygous mutations present in this patient are carried *in trans*.

Summary/Conclusions: We report the case of a woman of Malaysian/Indian origin with the rare SC_{null} phenotype and the corresponding anti-Sc3 antibody. The phenotype was shown to arise from compound heterozygosity for two novel null alleles, one carrying c.373C>T in exon 4 and the second carrying c.596C>G in exon 7.

P355 | A novel *RHD* allele in a caucasian blood donor

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Background: The Rh antigens are encoded by two highly homologous genes, *RHD* and *RHCE*. The D antigen is the most clinically significant antigen of the Rh blood group system. Its extreme immunogenicity makes it one of the most important antigen in transfusion medicine. Among European subjects, about 0.2% to 1% carry aberrant *RHD* alleles. Serology and, in some cases, molecular biology cannot discriminate these alleles and mistyping of these individuals may potentially lead to anti-D alloimmunization.

Aims: We reported a case of male caucasian donor with a Ccdee phenotype detected by a regional transfusion centre in north Italy and referred to Lombardy Immunohemathology Reference Laboratory for further serological and DNA investigation to exclude the presence of aberrant *RHD* alleles.

Methods: Serology testing was performed by column agglutination technology (CAT) BioVue (Vision, Ortho Clinical Diagnostic, Raritan, NJ, USA) with anti-D clones D7B8 and RUM1. The Weak D determination was performed in CAT indirect antiglobulin test (IAT) with anti-D monoclonal clones IgM+IgG LDM3/ESD1 and in CAT Reverse with anti-DVI monoclonal clones IgM ESD1M. Genomic DNA was isolated from whole blood using the QIAamp DNA Blood Kit (QIAGEN, Germany) and the *RHD* genotype determined by RHD BeadChip™ kits (Immucor-BioArray Solution, Warren NJ, USA) and PCR- Rh-TYPE SSP kits (BAGene, Germany). Since the phenotype and genotype results were discordant, the sample was further characterised by Sanger sequencing of *RHD* exons 1 to 10 (Grifols Immunohematology Center, S.Marcos, USA)

Results: The donor was Ccdee by serology while the genotype was *Ccee DVI* by both molecular methods. *RHD* zygosity was not performed. DNA sequencing revealed a novel *RHD*D-CE(2-5)-D* allele that has not been previously described in Rhesus site but it is also

possible that this allele could be a deletion of *RHD* exons 2-5. The phenotype is unknown in both instances.

Summary/Conclusions: The sequencing analysis for the resolution of genotype/phenotype discrepancies can lead to identify new variants. It is very important identify donor carrying a D variant in clinical practice. In this case the subject it must consider RhD negative for transfusion needs but as a donor it must be consider RhD positive to avoid alloimmunization or haemolytic transfusion reactions in recipients.

P356 | Characterization of three novel *RHCE* mutations inducing aberrant C expression

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Background: The RH locus is one of the most polymorphic loci of the 43 recognised RBC blood group systems. The RH blood group system is encoding 55 different antigens (31-MAR-2022, ISBT) carried on two proteins (RhD and RhCE) and gene mutations like nucleotide changes or deletions result in variant proteins which can alter the expression of RH antigens. Rare mutations can push the limits of conventional serological or molecular methods. Under these circumstances, a further analysis by Sanger sequencing can help to understand and resolve complex cases.

Aims: In this work, we analysed the molecular background of three novel RHCE mutations, which induced aberrant Rhesus C-expression.

Methods: RH phenotypes were determined by standard blood group serology (BioRad, DiaMed GmbH, Cressier, Schweiz). Genotyping was carried out by PCR-SSP (RBC-Ready Gene RHCE variants and RBC-Ready Gene CDE, Inno-Train, Kronberg). Sanger sequencing on an ABI Prism 310 (Applied Biosystems, Weiterstadt) was requested for further molecular analysis. Custom primers specific for RHCE were used for amplification and sequencing reactions of all ten exons including short flanking intron regions.

Results: Blood samples of the two patients and one blood donor showed differential reactivity with two anti-C clones each. Further analysis with PCR-SSP could not explain the aberrant serological results. The blood donor typed as CcD.Ee, whereas both patients were determined as CcD.ee without indication of any further variation. Sanger sequencing determined an additional heterozygous *RHCE*c.535T>C*, inducing a p.Phe179Leu exchange in the donor. One patient carried a *RHCE*c.482T>G* (p.Phe161Cys) substitution on one allele and lastly in the second patient a heterozygous deletion was detected in exon 9 (*RHCE*c.1190_1191delAT*), which induces a frame shift. Since serological testing revealed a weakened C-expression in all three samples and regarding haplotype frequencies, it can be inferred that the mutations are located on the allele coding for *RHCE*02* (*RHCE*Ce*). According to Wagner et al. (Blood, 1999), the amino acid exchange in the first case should be located within the sixth membrane passage of the RhCE molecule, which could result in a weakened expression. In the second case, the exchange should be located within the third extracellular RhCE loop. Hence, an altered binding of anti-C is possible, explaining the aberrant serological result. For *RHCE*c.1190_1191delAT* the amino acid exchange should be located close to the intracellular carboxy terminus of the RhCE molecule which could result in a divergent expression of C as well.

Summary/Conclusions: All three mutations, the two nucleotide exchanges as well as the deletion, led to the detection of altered C-expressions. Sanger sequencing allowed the identification of these novel mutations and therefore still poses a beneficial tool for the correct analysis of atypical serological results. Neither of the mutations is listed in the ISBT allele tables thus far. The nucleotide sequences of the new mutations have been submitted to the GenBank data base (GenBank accession numbers OQ448881, OQ448882, OQ448883).

P357 | Targeted long-read Nanopore sequencing of the blood group genome by adaptive sampling

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Background: Oxford Nanopore Technologies (ONT) has recently introduced a novel approach to long-read sequencing which eliminates off-target reads, i.e. DNA fragments not covering a region of interest, in real-time during sequencing. This so called 'adaptive sampling' allows selective enrichment of regions of interest, for instance the complete blood group genome. The process is entirely computationally and does not require the development of laborious wet-lab enrichment protocols. Furthermore, it can be adapted to any genomic region in a few minutes, making it exceptionally versatile. Adaptive sampling does not impose restrictions on DNA fragment length, which is particularly advantageous for phasing variants across target loci and for resolving paralogous gene regions encompassing structural variants such as found in the RH and MNS blood group systems.

Aims: The objective of this study was to explore the effectiveness of Nanopore adaptive sampling with regard to sequencing blood group genes. We applied the sequencing strategy to a set of selected samples, focused on complex structural variants.

Methods: Genomic high-molecular weight DNA was used to build Nanopore sequencing libraries. Each sample was run on a separate MinION flow cell (ONT). To increase total sequencing output, flow cells were washed and re-loaded with library several times. The reference FASTA file conveying the genomic regions to enrich, contained all known red cell blood group genes ($n = 39$), the transcription factor genes *GATA* and *KLF1*, as well as the human platelet antigen genes ($n = 7$). We included 50 kb flanking regions for each gene to increase the chance of retrieving long on-target reads. In sum, we targeted ~ 7 Mb. Reads were mapped to the novel human reference genome (T2T-CHM13v2.0) and, in the case

of complex structural variation, also *de-novo* assembled. To assess variant calling accuracy, ONT sequencing results were compared to pre-typed genetic data where applicable.

Results: The mean length of sequenced reads was high with N50 of >30 kb. The maximum read length achieved was over 500 kb. Reads were categorized as either on- or off-target within the first 500 bp (∼1 second of sequencing). From the ∼10% on-target fraction (>100,000 reads per sample), only ∼2% mapped to our regions of interest. This resulted in an expected enrichment of around 5-10x. All target genes were fully covered by at least 5x coverage, with a median coverage of 15x across all genes. Although this was not yet sufficient for reliable single-nucleotide variant calling (∼10% of expected calls below quality threshold), it allowed in combination with very long reads (∼10 reads >100 kb for the RH and MNS locus per sample) to resolve complex structural variants.

Summary/Conclusions: Nanopore adaptive sampling has emerged as a promising tool for cost-effective and straightforward long-read sequencing of all blood group genes on single MinION flow cells. A first evaluation showed still suboptimal coverage for general variant calling, but intriguing potential to resolve complex structural variants in the paralogous RH and MNS regions, a task in which conventional molecular techniques usually fail. Since adaptive sampling is still in the early stages of development, it is anticipated that enrichment efficiency and thus sequencing coverage will further increase.

P358 | Abstract withdrawn

P359 | Genetic basis and ancestry of the Duffy null phenotype in Omani blood donors

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Background: Duffy-negative blood group phenotype was previously reported to be the most common phenotype in the Duffy blood group system among Omanis, similar to what has been reported in many African populations. Homozygosity of the Duffy negative blood group variant (Fy*B^{ES}), which is widespread across sub-Saharan Africa, is thought to confer protection against *Plasmodium vivax* malaria. Oman has struggled with endemic *P. falciparum*, and more recently imported *P. vivax* infections, raising a hypothesis of natural resistance of Omanis to *P. vivax* malaria due to the high frequency of the Fy(a-b-) phenotype. Omanis have had deep historical connections to East African populations through settlements of the Omani Empire along the Eastern African coast, dating back to the 17th century AD. It also has a historical connection with South East Asia through its important roles in Silk Roads exchanges at different points in history. We hypothesize that gene flow from East Africa may explain the high prevalence of the Fy(a-b-) phenotype among the Omanis.

Aims: To test the hypothesis that gene flow from East Africa may explain the high prevalence of the Fy(a-b-) phenotype among the Omani blood donors.

Methods: Blood group phenotyping for the Duffy blood group was performed serologically for 100 Omani blood donors. DNA extraction was performed, and whole genome sequencing was carried out on an Illumina NovaSeq 6000 to 16X coverage. Global ancestry was inferred using ADMIXTURE, and principal component analysis performed with Eigensoft smartpca to compare findings with populations from around the world in the 1000 Genomes dataset. Local ancestry was inferred across the Omani genomes with RFMix v2 using the 1000 Genomes super populations as reference populations.

Results: The frequency of the Fy(a-b-) phenotype was 87%. Using the sequencing data, the genetic basis for the Fy(a-b-) phenotype was found to be due to the Fy*B^{ES} allele in nearly all cases. However, we also discovered three Fy(a-b-) individuals carrying the Fy*B^{x} allele and one carrying a protein-truncating frameshift mutation that has only rarely been found, and we report here for the first time in Oman. We find that the Fy*B^{ES} allele shows much more similar frequency to African populations than to European populations, in contrast to other genetic variants throughout the genome. Global ancestry inferences with ADMIXTURE indicate shared source populations between the Omanis and populations in East (LWK) and West (ESN and YRI) Africa as well as South Asia. Local ancestry inference identifies the Duffy locus as having the strongest excess of African ancestry genome-wide. Admixture dating suggests that AFR admixture occurred between 8.69 and 8.90 generations ago, or ∼240–250 years, corresponding to the time of the settlement of the Omani Empire in East Africa.

Summary/Conclusions: The Fy(a-b-) phenotype is at high frequency in Oman and is caused by three different genetic mutations. The most common is Fy*B^{ES}, which is very common in African populations and rare in Europe. Admixture results suggest the Omanis have African and South East Asian admixture, consistent with historical record of Oman's connections with these regions. The genome-wide evidence for African admixture and high frequency of Fy*B^{ES} and LWK ancestry at the Duffy locus in the Omani population suggests strong positive selection after introduction through admixture and consequently increase to high frequency, possibly under selection driven by *P. vivax* malaria.

P360 | Genotyping of weak D phenotype among blood donors in Southeast Iran

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Background: Antigen D is the most common cause of red blood cell alloimmunization in the Rh blood group system, which is involved in HDFN (haemolytic disease of the fetus and newborn) and HTR (haemolytic transfusion reaction). Genetic alterations and mutations cause quantitative or qualitative changes in the D antigen expression, resulting in a variety of D alleles. These RHD alleles are classified as weak D, partial D and Del. Mutations in the weak D variants lead to decreased RHD gene expression without altering the protein structure. However, Partial D results in alterations of the epitopes in the D antigen structure.

Aims: The aim of this study was to identify common genotypes in serologically weak D phenotype blood donors using molecular analysis.

Methods: DNA samples were isolated from the whole blood of 26 blood donors with weak D phenotype who were present in southeast Iran. The presence of alleles responsible for the D variants was assessed by polymerase chain reaction-sequence specific primers and DNA sequencing using exons 1 to 10 of the RHD gene.

Results: Of the 26 samples with weak D phenotype, 16 partial DLO (61%), 4 partial DBT1 (15.3%), 2 partial DV type 2 (7.7%), 1 weak D type 1, 1 weak D type 4.2.3, 1 weak D type 105 and 1 RHD (S103P) (4%) were determined.

Summary/Conclusions: This study revealed that the most frequent D variant genotype in this area was partial DLO. The present findings suggest that molecular tests should be used in immunohematology reference laboratories to accurately identify RhD type and subsequently increase blood transfusion safety in blood recipients.

P361 | Anomalies below software detection thresholds lead to the detection of a novel *RHCE* allele with c.671A>C change (p.Asn224Thr)

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Background: While a large diversity of *RHD* alleles has been reported, the number of *RHCE* alleles recognised by the ISBT working party on Red Cell Immunogenetics and Blood Group Terminology is comparatively low. Two main reasons explain this difference. One, many countries do not routinely type donors and patients for C, E, c, e antigens and two, *RHCE* alleles are nearly always in heterozygosity and more challenging to characterise.

Aims: We investigated the sample from a pregnant patient with ambiguous typing for RH5 (e) antigen, referred for molecular testing.

Methods: Serologic testing was done by automation with Erythrocyte Magnetized Technology (EMT) (Qwalys EVO, Diagast) and gel card testing (Ortho BioVue, Ortho Clinical Diagnostics). Anti-e reagents were IgM from clones P3GD512+MS63 and MS16+MS21+MS63, respectively. Genomic DNA was extracted from white blood cells and molecular testing was done with RHCE BeadChip (Immucor), real-time PCR assay for detection of c.676G/C (characteristic of e and E, respectively) (Taqman, ThermoFisher Scientific) and Sanger sequencing of *RHCE* exons and flanking intron regions.

Results: The patient's red blood cells (RBC) typed D+C-E+c+ and weaker than expected for e antigen by EMT (score 49, when 99 is expected for conventional e). The RBCs were strongly reactive with anti-e by gel testing (4+). RHCE Beadchip predicted C-E+c+e- with additional c.48G/C and c.733C/G. However, close examination of the raw data chart showed amplification of c.676G, though it was lower than expected for a heterozygous Ee sample and below manufacturer detection threshold, explaining the E/E call. Real-time PCR was in favour of c.676C/C (E/E) but closer examination revealed an atypical profile not consistent with E/E nor E/e. Sanger sequencing confirmed heterozygous c.733C/G, unambiguous heterozygous c.676G/C and identified an additional heterozygous c.671A>C change in exon 5. Unexpectedly, Sanger sequencing found only c.48G/G, suggesting a drop-out. No changes were found in exons 2-4 and 6-10.

Summary/Conclusions: We identified an *RHCE*ce* allele with c.733C>G, a possible c.48G>C and a novel c.671A>C change. The c.671A>C change is predicted to encode p.Asn224Thr, located in a transmembrane position close to the RBC surface. The novel allele is responsible for a weak, probably partial e phenotype. Per national guidelines, e-negative (RH:-5) RBC units would have been selected, but the patient did not require transfusion during delivery. A fresh sample could not be obtained for cDNA analysis but further testing will be necessary to clarify the presence and linkage of c.48G>C. Although the patient's ethnicity was unknown, c.48C would be

expected to be *in cis* with c.733G and the novel change. Per dbsnp database, the c.671A>C change (rs1646339055) is found with 0.00002 allele frequency in the African population (gnomAD – Genomes dataset) and 0.000004 in a dataset of diverse population in the USA (TopMed). The novel c.671A>C change interfered with RHCE Beadchip and real-time PCR detection of E/E polymorphism, due to the proximity between c.676 and c.671. The incorrect calls by both algorithms underlines the importance of critical consideration of interpretation provided by genotyping software, as anomalies below algorithm thresholds may be clues of underlying variants.

P362 | Genotyping of human erythrocyte antigens for safe blood transfusion in Palestinian thalassemia patients

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Background: Management of β-thalassemia is a major challenge, especially in low-resource countries. Blood transfusion is associated with several side effects including haemolytic and allergic reactions, iron overload, and transfusion-transmitted diseases.

Aims: To determine the frequencies of red blood cell alloimmunization, autoimmunization, and genotypes of blood group systems among transfusion-dependent β-thalassemia patients in the West Bank, Palestine.

Methods: Frequently transfused thalassemia patients ($n = 100$) were recruited through Thalassemia Daycare Units in five governmental hospitals. A questionnaire and medical records were used to collect data regarding patient characteristics. Blood samples were collected to measure biochemical, hematological, and hormonal parameters, in addition to screening and identification of antibodies and DNA extraction. DNA samples were genotyped for Rhesus, Kell, Duffy, Kidd, MNS, Dombrock, Colton, Cartwright (Yt), Lutheran, Knops, Deigo, and Vel blood groups. Genotyping for blood groups was performed by sequence-specific primers (SSP)-PCR method.

Results: The mean pre-transfusion haemoglobin level was found to be 7.89 ± 0.99 g/dL and the mean serum ferritin level was 3670.42 ± 3742.71 ng/dL. The results of liver function tests showed that 32%, 42%, and 34% had elevated ALT, ALP, and AST levels, respectively. 10% of the patients had subclinical hypothyroidism and 8% had growth hormone deficiency. 8% of the patients had hypocalcemia and 70% had vitamin D deficiency. Elevated glucose levels were found among 15% of the patients. The most encountered complications were arthropathy (44%), hypogonadism (16%), and hepatic failure and delayed growth (7%). The genotyping results of the *RHD* blood group showed that 88% of the patients were *RHD-positive* whereas 7% were *RHD-negative* and 5% had no clear results. The allele frequencies of *RHCE* alleles were 0.440 and 0.560 for *RHCE*C* and *RHCE*c*, respectively, and 0.165 and 0.835 for *RHCE*E* and *RHCE*e*, respectively. Unexpectedly, for the Duffy blood group system, the null genotype (*FY*02N.01/02N.01*) was observed in 46% of the patients and the allele frequencies of *FY*01* and *FY*02* were 0.195 and 0.345, respectively. Furthermore, the allele frequencies of *GYPA*M* and *GYPA*N* were 0.585 and 0.405, respectively, and those of *GYPB*S* and *GYPB*s* were 0.275 and 0.725, respectively. The *KEL*02*, *KEL*04*, and *KEL*07* allele frequencies were high among the patients in this study (0.920, 0.985, and 0.980, respectively). Furthermore, the allele frequencies of *YT*A*, *LU*02*, *CO*01*, *KN*01*, *DI*B*, *DI*02.04*, and *VEL*01* were 0.940, 0.990, 0.990, 1.000, 0.980, 1.000, and 0.990. In addition, 2% of the patients had the *Vel*01/-0.1* (*Vel/Vel*$_{null}$) genotype. The rate of alloimmunization among patients was 8% and the most common antibodies were anti-E, anti-K and anti-D, and anti-C, respectively. The rate of autoimmunization was 5%.

Summary/Conclusions: The management of thalassemia should be based on internationally established guidelines. Understanding the frequencies of the major blood group systems is essential to provide accurate information regarding the local population's requirements, reduce transfusion-related complications among frequently transfused patients, and facilitate the challenging task of providing antigen-negative blood for patients with multiple antibodies. Phenotyping of patients' RBCs and accurate testing for weak RhD among donors could have prevented the development of alloantibodies against Rh antigens D, C, E, and K. The genotype and the allele frequencies observed among the sample of this study revealed several interesting findings that prompt further research.

P363 | Establishment of a PCR-SBT method for detecting Mur antigen

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Background: Although the Mur antigen was defined as a low frequency antigen, it is highly expressed in Asian population and vary widely in distribution frequency among different populations in China. There were no reports on Mur antigen distribution frequency in Zhejiang Province. Mur antigen can stimulate the body to produce anti-Mur, resulting in acute or delayed haemolytic transfusion reactions and severe neonatal haemolytic disease. However, there are currently no commercially available anti-Mur antibody for the antigen detection. Therefore, it is an effective way to use molecular biological method to identify Mur antigen, which can help to understand the distribution frequency of Mur antigen in the population. It also helps identify anti-Mur, screen and provide compatible blood products to improve the safety of clinical transfusion.

Aims: In this study, a PCR-SBT method for Mur antigen detection was established for detecting the distribution frequency of Mur antigen in blood donors in Zhejiang Province.

Methods: 200 whole blood specimens were collected from healthy blood donors after consents. Genomic DNA were extracted using the commercial kits. Primer pairs were designed according to the

oligonucleotide sequence of *GYP(B-A-B) Mur*(GenBank No: AF090739). PCR amplification was performed using an optimised reaction system and reaction conditions, and sequencing was performed using the Sanger sequencing method. Raw sequencing data were analysed using SeqScape v2.5 software. A 330bp *GYP(B-A-B) Mur* gene containing specific sites was constructed into the pUC57 expression vector as a positive control and named as Mur_pUC57.

Results: Mur_pUC57 was successfully sequenced, which was consistent with the inserted *GYP(B-A-B) Mur* sequence, indicating the feasibility of the established PCR-SBT method for Mur antigen detection. However, no Mur antigen positive was detected in 200 samples collected from blood donors.Therefore, it is predicted that the positive frequency of Mur antigen in blood donors from Zhejiang Province may be lower than 0.5%. In order to obtain more accurate results of the distribution frequency of Mur antigen among blood donors in Zhejiang Province, we need to expand the sample size.

Summary/Conclusions: We have successfully established a PCR-SBT method for detecting Mur antigen with high accuracy, specificity and throughput. The method is beneficial to the establishment of rare blood group database. The frequency of Mur antigen positive in blood donors in Zhejiang Province may be lower than that in other areas of China.

P364 | The study of variant s antigen expression in GP.Mur and sD individuals revealing a novel c.160C>T (p.Arg54Cys) mutation in *GYPB*s* allele associated with partial s phenotype

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Background: Little s antigen is mainly defined by a single nucleotide polymorphism at c.143C (p.Thr48) on the *GYPB* gene. Several mutations on *GYPB* or hybrid genes can alter the expression of s antigen. GP.Mur has an extra 31 amino acids insertion encoded by the active compound exon 3 between exon 2 and exon 4 of *GYPB*, which closely locates on the upstream of p.Thr48 and may also alter s expression.

Aims: To investigate the molecular basis of variant s antigen expression in GP.Mur and s^D individuals.

Methods: A total of 4983 whole blood samples were collected to screen variant s antigen using two monoclonal anti-s (IgM and IgG). Sanger sequencing was conducted to analyse the sequence of *GYPB* exon 4. Flow cytometry analysis was performed to quantify s antigen on RBCs. In vitro expression study was performed to verify the effect of c.173C>G, c.160C>T and several other variants of *GYPB* or *GYP*-Mur* on the expression of s antigen.

Results: We identified four donors and collected five homozygous GP.Mur individuals showing discrepant s typing results. The s antigen of the four donors can be detected by one IgM monoclonal anti-s but failed to react with another IgG monoclonal anti-s, while GP.Mur homozygotes showed the opposite reaction pattern. Sanger sequencing results showed three of donors carried the c.173C>G variant which is specific for s^D antigen, the other one carried a novel *GYPB* (c.160C>T) mutation. Flow cytometry identified a partial expression of s antigen on the RBCs of the four donors and five homozygous GP.Mur individuals. Furthermore, in vitro expression study verified the effect of c.173C>G, c.160C>T and several other variants of *GYPB* or *GYP*Mur* on the expression of s antigen.

Summary/Conclusions: The results demonstrated that in addition to p.Thr48, the four extra amino acids p.Thr44, p.Asn45, p.Arg54 and p.Pro58 are also important for full expression of epitopes of s antigen. Since partial s antigen is at risk for the development of alloanti-s, it is important to select at least two different monoclonal anti-s for correct s typing in homozygous GP.Mur and s^D individuals.

P365 | Molecular epidemiology of *RH* genes in patients with sickle cell disease in Cameroon, Central Africa: fundamental data towards improvement of standard practice

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Background: In the RH blood group system, genetic polymorphism has long been described and known to be highly dependent on the ethnicity/origin of the population of interest. Although molecular complexity in sub-Saharan African populations is well acknowledged, very few studies have addressed that question in specific subsets at a national/regional level. The molecular data that are available thus globally take into account populations at a large scale level independently of their origin, while it is highly suspected that interregional differences occur.

Aims: In order to gain insights into accurate, population-specific molecular data, we sought to investigate *RH* gene polymorphism in individuals originating from a specific geographical area, i.e. Cameroon, Central Africa.

Methods: Blood sample from 109 patients with sickle cell disease was typed by routine serological techniques. After informed consent, genomic DNA was extracted with a commercial kit. Both *RH* (*RHD* and *RHCE*) genes were analysed by a quantitative multiplex PCR of short fluorescent fragments (QMPSF) and direct sequencing for identifying zygosity as well as structural variants (SVs), and single nucleotide variations (SNVs), respectively. Variant frequency was recorded, and phenotype was predicted from the experimental genotyping data using reference databases and compared to the experimental phenotype.

Results: By our means, all patients were genotyped successfully. In *RHD*, in addition to the conventional wild-type allele (allele frequency: 0.505) found in 84 patients, 19 variant alleles were identified. Beside

D-negative alleles (**01N.01*, **03N.01*, **08N01*, and **01N.06*; total: 0.174), the D-positive *DAU0* allele is the most common (0.197), followed by *RHD*weak D type 4.0* and *DAU3*. In line with these results, c.1136C>T (p.Thr379Met), c.667T>G (p.Phe223Val), and c.602C>G (p.Thr201Arg) are the most common SNVs, and 22/109 patients (20.2%) are supposed to be "partial D". In *RHCE*, c.733C>G (p.Leu245Val) is the most frequent rare variant, and a total of 21 alleles were identified, **ce*, **ce.01*, and **ce.VS.01* being the most common (allele frequency: 0.252, 0.225, and 0.202, respectively). Interestingly, 25 patients (22.9%) are assumed to harbor partial C and/or c and/or e antigen(s), which may challenge subsequent transfusion therapy if alloimmunization occurs.

Summary/Conclusions: For the first time, this study provides experimental evidence about the nature and distribution of *RH* gene variants at high resolution in Cameroon, Central Africa. Beyond the fundamental findings, this work is a milestone towards improvement of the standard procedure for RH blood group typing, including selection of serological reagents as well as quality control panels, which is critical for optimizing donor selection and patient management in the country. Also, comparable studies in other sub-Saharan African countries will be valuable to be carried out in order to get a clear overview of *RH* gene variability in Africa.

P366 | A novel missense variant c.1A>T on the *ABO*B.01* allele associated with a Bw phenotype

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Background: Weak B phenotypes are induced by various molecular variants.

Aims: Here we describe a novel missense variant (NM_020469.2: c.1A>T, p.Met1?) on exon 1 of the *ABO*B.01 allele identified in a Korean male individual with a Bw phenotype.

Methods: A blood sample was collected from a healthy Korean male with no history of transfusion or hematologic malignancy. ABO forward typing was performed using the tube method with anti-A, anti-B, and anti-AB (Ortho Clinical Diagnostics), and anti-H (Lorne Laboratories) reagents. ABO reverse typing was conducted using the tube method with A, B and O cells (Ortho Clinical Diagnostics). The strength of agglutination was graded as 0, w+, 1+, 2+, 3+ or 4+. Genomic DNA was isolated from whole blood using a column-based DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega). The isolated genomic DNA was subjected to whole exome sequencing (WES) covering all exons and flanking intronic regions of the *ABO* gene on a NovaSeq platform (Illumina). Target enrichment was performed using custom-designed capture probes (IDT). The exons 6 & 7, the proximal promoter and the erythroid cell-specific regulatory element, called the +5.8kb site, in intron 1 of the *ABO* gene were also amplified and sequenced by capillary sequencing method.

Results: The RBCs of the proband showed weak agglutination (w+) on anti-B and anti-AB reagents. The plasma of the proband exhibited 4+ agglutination with A cells. The eluate displayed 2+ agglutination with B cells in the adsorption and elution test. According to the serologic results, the proband was considered to have a Bw phenotype (Table 1). In the WES study, the mean depth of coverage was 208x, and 98% of the target bases were covered at >10x. WES identified 16 single nucleotide variants in the *ABO* gene, seven of which (c.297G, c.526C>G, c.657C>T, c.703G>A, c.796C>A, c.803G>C, c.930G>A) belonged to *ABO*B.01, and nine of which (c.106G>T, c.188G>A, c.189C>T, c.261delG, c.297A>G, c.646T>A, c.681G>A, c.771C>T, c.826G>A) belonged to *ABO*O.01.02. The remaining one was a novel missense variant (c.1A>T, p.Met?) which is absent in the dbSNP and gnomAD databases and has not been reported previously in the literature.

Summary/Conclusions: The c.1A>T (p.Met?) variant is predicted to reduce the enzymatic activity of glycosyltransferase B, as this novel missense variant occurs at the start codon of the *ABO*B.01. However, we suspect the B antigen was weakly expressed due to another ATG (methionine) codon at exon 2 of the *ABO* gene.

P367 | A novel frameshift mutation in the *XK* gene in a Finnish patient with McLeod syndrome

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Background: McLeod (neuroacanthocytosis) syndrome is an X-linked multisystem disorder characterised by the association of red blood cell acanthocytosis and progressive degeneration of the basal ganglia. Clinical symptoms consist of a choreatic movement disorder, psychiatric manifestations, cognitive decline, and cardiomyopathy. In addition to acanthocytosis, laboratory manifestations include elevated CPK levels, weak or undetectable antigens of the Kell system and the absence of the Kx antigen on red blood cells (RBCs), known as the McLeod phenotype. Expressed in erythropoietic tissues, skeletal muscle, and brain, the Kx molecule functions as a membrane transport protein.

Aims: We report the serological and molecular basis of the McLeod phenotype in a 65-year-old male patient with a clinically suspected diagnosis of McLeod syndrome.

As part of the neurological workup routine, samples were first sent to Blueprint Genetics for genetic testing for neurology and then to the FRC Blood Service in case transfusion support was needed.

Methods: Expression of the k and Kp^b high-prevalence antigens was assessed by routine serological tests (gel method) at the FRC Blood Service laboratory.

Expression of the Ku and Kx antigens was assessed with two sets of in-house reagents (gel method) available at the EFS immunohematology reference laboratory (IRL), Paris, France.

The XK single gene testing (Blueprint Genetics) includes the sequencing analysis and copy number variation analysis of the gene. *XK* sequencing was repeated at the EFS IRL.

Results: The patient's RBCs showed a strong depression of the Kell antigens: non-reactive or very weak for k, non-reactive for Kp^b. The RBC antibody screen was negative.

Ku and Kx antigens were found to be extremely weak and at the limit of detection (<0.5+), consistent with the negative or very weak k and Kp^b reactivity.

The *XK* gene sequencing revealed a novel frameshift mutation, c.304_311del, p.(Ser102Glnfs*15), which deletes eight base pairs in exon3, causing a premature stop codon and a likely non-functional protein. This variant has not been reported to date in control populations.

Summary/Conclusions: The patient was hemizygous for a novel frameshift mutation in the *XK* gene, very likely resulting in a complete absence of the Kx protein and a dramatic decrease in the expression of the Kell antigens. Moreover, the clinical effect of the mutation was severe, leading to the patient´s death soon after the diagnosis. As family studies are yet to be finished, it is not known if the patient inherited the variant from his mother or if it is a de novo mutation.

Because of the extreme rarity of donors of the McLeod type worldwide, standard RBCs (matching the patient´s extended phenotype) are the only feasible option as first-line transfusions, as long as the patient does not become immunised to the Kx antigen. This was even more the case with this patient as he also had the O RhD-negative phenotype.

For patients immunised with anti-Kx, the usual approach of investigating healthy male siblings as possible donors is only possible after thorough genetic counselling with careful attention to psychological support. An international search for compatible donors may be another option. Close collaboration between clinicians, clinical geneticists, hospital blood banks and blood services because of the rarity of McLeod syndrome.

P368 | Introduction of *RH* molecular analysis in donor-patient matching in Cameroon, Central Africa: genotype-phenotype correlation, antigen exposure and risk of alloimmunization

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Background: RH system is the most polymorphic blood group system and the most frequent cause of alloimmunization in patients with sickle cell disease (SCD). While it has been shown that transfusion of ABO/D, C/c, E/e, K-compatible matched units helps significantly to prevent alloimmunization in these patients, it is common practice to restrict matching to ABO/D in low-income countries due to the cost and availability of reagents. Also, the presence of many RH variants in individuals of sub-Saharan African origin may challenge the accuracy of extended serological RH typing. For the past years, genotyping has increasingly become an alternative to identify these variants and to overcome phenotyping ambiguities.

Aims: The objective of the study is to assess, for the first time in a cohort of Cameroonian patients with SCD, how *RH* genotyping may be valuable and complementary to the routine typing procedure to identify RH variants and to gain insights into the risk of alloimmunization associated with RH antigens more accurately

Methods: A cross-sectional study was conducted in two Cameroonian hospitals from September 2020 to November 2022. Ninety-eight patients with SCD, as well as 107 respective donors whose red blood cells (RBCs) were transfused to the patients, were selected for the study. Gel filtration technique was used to perform RH phenotyping (D, C, c, E, and e) on RBC samples. Sanger sequencing and quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF) were carried out to identify RH variants in both *RH* genes. Phenotype was deduced from genotype by following the ISBT Red Cell Immunogenetics and Blood Group Terminology Working Party guidelines. A specific attention was paid to the expression of low-frequency antigens (LFAs) in donors, as well as the expression of partial antigens and deficiency of high-frequency antigens (HFAs) in patients. On the basis of phenotype prediction, antigen exposure, and thus the potential risk of alloimmunization, were assessed.

Results: While a conventional or partial RH5 (e) antigen was predicted to be expressed in all individuals (donors and patients), identification by serological testing was impaired in most samples in our conditions for unknown reasons. Discrepancy in RH2 (C) expression was observed in two donors (routine typing: −; genotyping: +) and two patients (routine typing: +; genotyping: −). Basically, no alloimmunization is thought to occur in 32 patients (32.7%) following transfusion. Alloantibodies that may be produced against major RH antigens are anti-D ($n = 19$), followed by anti-C (18), anti-E (15), anti-e (14), and anti-c (11). Expression of the STEM (RH49) and DAK (RH54) LFAs was respectively predicted in two and three donors whose RBCs were transfused to patients negative for these antigens. Finally, 27 and 25 patients may produce anti-V (RH10) and anti-VS (RH20), respectively

Summary/Conclusions: Extensive knowledge of antigen distribution is critical at the population level in transfusion medicine. For the first time in Cameroon, a molecular study was conducted to investigate comprehensively the expression of RH antigens in donors and patients with SCD matched by routine testing. Our findings provide important clues for optimizing and improving the current practice and resources in Cameroon, notably in diagnostics and selection of reagents for both donor and patient typing as a function of the most expressed, clinically-relevant antigens

P369 | Diagnosis of variant D phenotype (DIII type 7) in a patient with anti-D alloantibody

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Background: We describe the case of a 50-year-old male patient, Afro-American ethnic group O RhD positive, who was admitted due to complicated upper gastrointestinal bleeding that required support transfusional. In the evolution an anti-D mechanism is identified after transfusions with deplasmatized blood ORhD positive this presentation opens the possibility of being a D variant, which later the molecular analysis of the RH locus confirmed that the patient is a carrier of a partial RhD DIII type 7 phenotype, this is a rare variant and we do not have diagnoses in our country.

We highlight the international alliance between Argentine and Uruguayan institutions that allowed the diagnosis.

Aims: Discuss the clinical and diagnostic management of an ORhD-positive patient who develops an anti-D alloantibody

Methods: This is a descriptive observational study, clinical case report type

Results: Genotyping of the RHD gene confirms that the patient is a carrier of a partial D phenotype DIII type 7 (partial D allele).

Summary/Conclusions: The diagnosis of a patient with phenotype D partial DIII type 7 alloimmunised with an anti-D antibody was made, unprecedented in our country, since in our country we do not have the possibility of carrying out the study of the RHD gene, highlighting the importance of co-participation of different institutions in this case the National University of Rosario Argentina to be able to reach the diagnosis of these complex and unusual situations, and facilitating the taking of therapeutic behaviors.

P370 | Two novel alleles harboring a deletion in the KEL and RH blood group systems

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Background: Serologic and genetic results do not always correspond. The increased use of molecular assays such as extended donor blood group genotyping and fetal genotyping is leading to an increasing rate of discrepant findings. This results in further investigations, especially in clinically important systems such as RH and KEL, to determine the correct antigen status and infer transfusion and obstetrics recommendations.

Aims: Investigations of a discrepancy between pheno- and genotype for the blood group antigens KEL2 (donor A) and RH4 (patient B).

Methods: Blood group phenotyping was done by standard serological column agglutination testing (DG Gel ABO/Rh, DG Gel Rh Pheno +Kell, Diagnostic Grifols, S.A.; ID System, Bio-Rad). Adsorption-elution was performed using two different anti-KEL2 antibodies (in-house). Molecular investigation was initially performed by sequence specific primer (SSP)-PCR using commercially available kits (RBC-CDE; RBC-Ready Gene RHCE variants, Inno-Train, RH-Type, BAG Diagnostics) and in-house methods. *KEL* and *RHCE* sequencing was done using DNA or RNA with published and in-house primers for amplification and sequencing. To identify the haplotype of the *RHCE* and *KEL* alleles, allele specific sequencing was performed

Results: Donor A was phenotyped as KEL:1,-2, meanwhile the SSP-PCR identified the single nucleotide variants (SNVs) known for the antigens KEL1 and KEL2. In subsequent absorption-elution testing the KEL2 antigen could not be detected. *KEL* sequencing of all 19 exons including flanking intronic regions confirmed the SNVs for alleles *KEL*01.01/KEL*02* and an additional deletion c.297del in heterozygous state. This deletion leads to a frameshift with an aberrant protein sequence starting from amino acid 100 (p.Cys100Alafs*89). Allele-specific sequencing on cDNA demonstrated that the deletion is located on the *KEL*02* allele, which agrees with the observed phenotype.

Patient B, a pregnant woman presenting with an anti-RH4 allo-immunization during her 9th pregnancy, demonstrated a maternal signal in the fetal *RHCE* genotyping for RH4. She was phenotyped as RH:1,2,-3,-4,5. Genotyping using commercially available kits revealed SNVs known for the antigens RH2, RH3, RH4 and RH5. The *RHCE* sequencing of all 10 exons including flanking intronic regions revealed the heterozygous deletion c.873del in exon 6. This deletion leads to a frameshift with an aberrant protein sequence starting from amino acid 292 (p.-Trp292Glyfs*12). This variant is of demonstrated clinical significance as the patient had active anti-RH4 immunization. The neonate, phenotyped as RH4 positive with a strong positive DAT (titre >1/1024). An anti-RH4 could be eluted from the neonate's erythrocytes. The neonate had a hyperhaemolysis with hyperbilirubinemia necessitating intensive phototherapy. However, no transfusion was needed.

Summary/Conclusions: Here we present two new blood group alleles, which both are due to a single nucleotide deletion. Blood from donor A can safely be used for transfusion of patients with antibodies against the high frequency KEL2 antigen. The second allele, associated with foeto-maternal allo-immunization, was found due to a maternal signal in a fetal *RHCE* genotyping assay. Such variants are probably often missed as *RHCE* fetal genotyping is not performed routinely. The new alleles, *KEL*02.297del* (accession number ON931624) and *RHCE*03.873del* (accession number OP626756), to the best of our knowledge, have not been reported previously. Our results highlight the importance of coexisting phenotyping and genotyping methods.

P371 | *RHD* genotyping of Rh-negative among blood donors in Sistan and Baluchestan Province of Iran

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Background: Antigen D is the most immunogenic antigen of the Rh blood group system, which is important because of its role in causing haemolysis in blood transfusion and haemolytic diseases in infants. The deletion of the RHD gene by the hybrid Rhesus box mechanism causes the Rhnegative phenotype. The presence of this hybrid marker is used to confirm the deletion of the RHD gene and to determine zygocytosis. Clinical applications of rhesus box hybrid determination include resolving inconsistencies in Rh group genotyping, zygocytosis determination in Rhpositive fathers, and RHD determination of Rh-negative fetuses.
Aims: The aim of this study was to investigate the genetic background of RhD-negative phenotype in blood donors in Sistan and Baluchestan province.
Methods: The molecular analysis of the hybrid Rhesus box was performed on the 200 Rhnegative samples using (PCR-SSP) and (PCR-RFLP). The existence of different exons of the RHD gene was investigated using Real-Time PCR.
Results: Of the 200 Rh-negative blood samples, 198 samples were homozygous and lacked the RHD gene (99%), while two samples were heterozygous and had the RHD allele (1%). Heterozygous samples had RHD*01N.73 allele and the RHD*01N.18 allele.
Summary/Conclusions: The results showed that RHD gene deletion is the most common genetic mechanism of the Rh-negative phenotype in Sistan and Baluchestan a province in Southeast Iran.

P372 | Deletion of c.568-570 associated with weak B antigen

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Background: Weak antigen expression or serological discrepancies in the ABO blood group system were studied thoroughly for its important clinical significance. There are novel variations discovered to enrich the database including more than 200 *ABO* blood group alleles. A novel deletion mutation located in exon 7 of *ABO* gene was investigated.
Aims: To elucidate the genetic background of weak phenotypes in the ABO blood group system by Sanger sequencing.
Methods: ABO forward and reverse typing was performed by gel card and tube method. Genomic DNA was extracted from fresh EDTA-anticoagulated whole blood samples. Sanger sequencing was used to detect the exons 1 to 7 of *ABO* gene. The targeted parts of exon 7 were sequenced additionally after cloning in a bacterial plasmid vector.
Results: A 58-year-old male with neurovascular disease took a pre-transfusion test. The proband had no history of blood transfusion before. His red blood cells were 2+ reactive with monoclonal anti-B by gel card. Reverse typing demonstrated 4+ agglutination. The weakened B antigen expression was then confirmed by tube method. To understand the molecular basis, Sanger sequencing was conducted to examine the exons 1 to 7 of *ABO* gene. In Exon 7, heterozygous c.568-570del was found based on *ABO*B.01*. The deduced genotypes are *ABO-B-ZJ85/ABO*O.01.02*. The new allele with variations is noted as *ABO-B-ZJ85* according to the sample number. The deletion mutation in Exon 7 was subsequently confirmed by sequencing after TA cloning of PCR products. The deletion of three bases GAG in c.568-570 affected the protein and led to p.Glu190del by analysing the transcripts. Thus, the B transferase lost a glutamic acid encoded by GAG in its original sequence and had lower catalytic activity to produce B antigen. This is the possible reason to have weak B antigen expression for this case.
Summary/Conclusions: The novel variation of c.568-570del was discovered in ABO blood group system. The deletion mutation was identified to be based on the *ABO*B.01* allele and associated with weak B antigen. The sequence is subjected to a pedigree analysis when available. The relation with weak B phenotype and mechanism needs to be further studied with more samples and cases.

P373 | Molecular RBC genotyping as an aid in pretransfusion testing: Case report

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Background: Transfusion dependent patients present a challenge in pretransfusion testing for several reasons. They are often immunised against multiple red blood cell (RBC) antigens, and an antibody (Ab) titre changes over time depending upon antigenic stimulation. The simultaneous presence of recipient and multiple donor RBC populations makes RBC phenotyping difficult. Certain phenotypes cannot be reliably distinguished by serological methods.
Aims: To present a case in which molecular genotyping was used to confirm and explain serological findings unusual in the Serbian population and make decisions on future transfusions.
Methods: Ab screening and identification were performed by column agglutination method using Coombs Anti-IgG gel cards with ID-DiaCell I-II-III and ID-DiaPanel test cells and NaCl, Enzyme Test and

Cold Agglutinins gel cards with ID-DiaPanel-P test cells (Bio-Rad Laboratories, Inc. Cressier FR, Switzerland). RBC phenotyping was done by standard tube agglutination method with commercial test reagents (CE-Immundiagnostika GmbH Neckargemuend Germany).

DNA was extracted using a QIAamp DNA Blood Mini Kit (QIAGEN Hilden, Germany). Genotyping was performed by FluoGene method, modified SSP PCR with endpoint fluorescence detection by the FluoVista analyser with RBC-FluoGene vERYfy, RBC-FluoGene vERYfy eXtend and RBC-FluoGene CDE kits (Inno-train Diagnostik GmbH Kronberg Germany).

Results: A sample of 7 yrs. old child, of African origin, diagnosed with sickle cell anaemia was sent from the Institute for Health Care of Mother and Child "Dr. Vukan Čupić" on December 14th 2021 with a request for an Ab identification. The patient, typed as O RhD-positive, received transfusions in July and September of the same year, and was previously transfused in another institution. A search of the IBTS information system established that anti-E, anti-C and suspected anti-K were previously identified.

Ab identification showed the presence of anti-E and anti-Fya. RBCs were phenotyped as ccDee Fy(a-,b-). DNA was isolated from the new sample and genotyping tests were performed. The following alleles of clinically significant systems were detected: RHD D, RHCE c, e, KEL-02, JK-01, JK-02, FY-02N.01, GYPA-01, GYPA-02, GYPB-03, GYPB-03N.03. Homozygosity for the Duffy null allele, which is characteristic for individuals of African origin, was proven.

The use of E-negative, C-negative, K-negative and Fya-negative RBCs was recommended for transfusion. Patient has been transfused on multiple occasions since using O RhD-negative ccddee Fy(a-,b+) RBC. Since March 25th 2022 anti-Fya could not be detected in plasma any more.

Summary/Conclusions: By applying molecular genotyping, an unusual serological finding was explained and insight into the present alleles of other clinically significant blood group systems was gained. Extended genotyping allows safe selection of compatible blood for patients on a chronic transfusion program.

P374 | Nanopore sequencing to resolve Lutheran blood group discrepancies

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Background: The Lutheran (LU) blood group system comprises 25 antigens encoded by the *BCAM* gene. There are four pairs of antithetical antigens, including LU1/LU2. The others represent independently expressed high frequency antigens. The Lutheran null phenotype commonly arises either from recessive inactivating mutations in the *BCAM* gene (LU_{null}) or from dominantly inherited loss-of-function mutations in the transcriptional activator gene *KLF1* (In (Lu) phenotype). Since 2015, Blood Transfusion Service Zurich has routinely been genotyping blood donors for 46 blood group antigens including LU1 and LU2 using MALDI-TOF mass spectrometry (MS).

Aims: We evaluated genotype-phenotype concordance of Lutheran-typing. Rare discrepancies were resolved by third-generation Oxford Nanopore sequencing as well as standard Sanger sequencing for inter-performance comparison.

Methods: Phenotyping of donors was performed using standard and extended serological techniques. MALDI-TOF MS based genotyping of *LU*01/02* relied on SNV-detection of c.230G>A. In case of genotype-phenotype discrepancies, once confirmed with commercial PCR-SSP kits (sequence-specific priming; inno-train GmbH, Germany), the entire coding region as well as intronic and flanking regions of *BCAM* and *KFL1* were amplified in specific long-range PCRs (~13.5 and 11.0 kb, respectively). Both amplicons of all samples were sequenced using Nanopore sequencing, which allowed allele haplotype generation along the entire genes. Sanger sequencing of gene regions of interest were used to confirm the results.

Results: Among ~15,000 donors for whom both serology and MALDI-TOF MS data for the Lutheran system were available, we identified six discrepant cases. Our sequencing approach on one *LU*01/02* heterozygous and three *LU*02* homozygous samples revealed one new (c.874A>G; p.Lys292Glu) and three rare known *KLF1* alleles (*KLF1*BGM21*,**BGM62*, **BGM66*), all leading to the dominant In(Lu) phenotype. The other two discrepant cases, both genotyped as *LU*01/02*, were linked to a novel *LU*02* null allele (c.1427G>A; p.Arg476His) and a rare *LU*02.-12.1* (c.100_105del; p.Arg34_Leu35del) allele, respectively. The latter is phenotypically characterised by the loss of the high frequency antigen LU12 (LU:-12), accompanied by strong weakening of LU2 expression. Genetic findings were confirmed by Sanger sequencing and further serologically refined by adsorption/elution techniques.

Summary/Conclusions: Using third-generation Nanopore sequencing of the *BCAM* and *KLF1* genes, both critical for Lutheran phenotype expression on red blood cells, we resolved all LU genotype-phenotype discordances of the last seven years of donor screening at our centre. Four cases were linked to *KLF1* alleles, one of which was novel, leading to In(Lu) phenotype. The remaining two discrepancies were based on a new *BCAM* allele and a very rare allele lacking LU12 expression. In summary, our long-read sequencing strategy appeared well-suited for elucidating genotype-phenotype discrepancies in relation to the respective allelic *LU* background.

P375 | Genetic characterization of panel reagent donors for blood group typing using NGS

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Background: Identification of antibodies to red blood cell (RBC) antigens and phenotyping during pre-transfusion diagnostics of patients

and pregnant women is often challenging. Well-genotyped RBC reagents obtained from panel donors which are used alongside commercial CE reagents strongly facilitate serological diagnostics. Here we used custom targeted exon sequencing (ES) panel to determine full blood group antigen (BGA) genotype of the RBC/DNA reagents obtained from panel donors using.

Aims: Here we used custom targeted exon sequencing (ES) panel to determine full blood group antigen (BGA) genotype of the RBC/DNA reagents obtained from panel donors using.

Methods: Six panel donors of Polish origin whose donations are used as RBC reagents and DNA controls, characterised serologically for the clinically significant antigens, and previously tested with commercial SureSelectXT Human All Exonv5 (*Agilent*), ID CORE XT *(Grifols*) and *in-house* allelic discrimination tests, were sequenced using Hematologypanel-2_1x Twist Custom Panel (Twist Design ID:TE-93158493, *Twist Bioscience*) covering exons of 43 blood group systems and *KLF* and *GATA* on Illumina NovaSeq 6000 platform. The data were processed using Trimmomatic, hg38 BWA (*Burrows-Wheeler Aligner*), HaplotypeCaller (GATK), IGV and *in-house* GeneBeam base. Blood group alleles were identified manually considering rs numbers and nucleotide changes listed in the blood group allele tables provided by ISBT website. Predicted BGA genotype was compared with known basic antigen phenotypes for nine BG systems (from 001 to 009) and basic antigen genotypes for 16 BG systems (from 001-016 except 003, 007, 012; and 032-034).

Results: ES data analysis of BGA genes allowed to determine complete BG genotypes of RBC/DNA donors with mean coverage = 281, number of reads per sample =1144631.286, ge10/ge20 99.71/99.62. Comparison of the genotypes from the ES panel to the already known BGA phenotypes/genotypes showed full concordance, however, a very low number of ES reads for *GYPA*01* (MN antigens) was observed (mean = 14). Among 24 previously untested BG systems, ES identified only a reference allele encoding a common BGA in all cases with the exception of 5 BG systems: 012 XG, 028GLOB, 031FORS, 038SID, 040PEL with detected allelic variants. We observed 4 variants with weak BGA expression but in heterozygous status: *JK*01W.06/JK*02* or *JK*01/JK*02W.03* but serologically Jk(a+b+); *JK*02/JK*02N.17* but Jk(a-b+); *RHD*01W.45/RHD*01* but RhD+ and *ABCG2*01/ABCG2*01W.01* but Jr(a+). In addition, alleles encoding additional specificity: Au(b+) or lack of some antigens: Yk(a-), KDAS+, but not tested routinely: *LU*02.19* ($n = 4$); *KN*01.–05* ($n = 3$), *KN*01.–10* ($n = 1$), respectively.

Summary/Conclusions: Panel ES analysis confirmed BG phenotype/genotype of the RBC/DNA reagent donors used in our reference laboratory but it also added valuable genetic information on the background of 24 previously untested BG systems and on known BGAs such as the presence of hidden rare and weak alleles. Further investigation is required to see if such variants have any impact on BGA expression on the RBC surface.

P376 | Abstract withdrawn

P377 | Genotyping of ABO variants using WES and panel exon sequencing

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Background: ABO blood group typing may be challenging due to discrepancies between the results of forward and reverse ABO grouping or weak agglutination. *ABO* genotyping using Sanger sequencing helps to explain the discrepancies, however in complex cases both exon and intron sequencing of *ABO* is required and sequencing of other related genes may be necessary making the procedure costly and time-cosuming. Exon sequencing panels for all blood group systems avoid the limitation through identification of molecular basis of the discrepancies in a single assay.

Aims: Evaluate the utility of whole exome sequencing (WES) and a custom exon sequencing (ES) panel for *ABO* genotyping in two cases of blood donors with difficulties in ABO blood group typing.

Methods: Two blood donors with *ABO* variants were tested retrospectively (donor#1 with weak A agglutination in standard serological tests and novel *ABO*A c.dup543_563* (#ERP119421) */ABO*B.01* genotype identified by exon Sanger sequencing, published by *Guz K. et al. 2020*; donor#2 with identified O_h (Bombay) phenotype with strongly reactive anti-A, anti-B and anti-H in the serum agglutinated cells of O group cord blood and *ABO*O.01.01/ABO*O.01.01*, *FUT1*01W.27*, *FUT2*01N.02* genotype also found by Sanger sequencing, published by *Michalewska B et al. 2017*). For WES and ES, libraries were prepared using SureSelectXT Human All Exonv5 (*Agilent*) and Hematologypanel-2_1x Twist Custom Panel (Twist Design ID:TE-93158493, *Twist Bioscience*), respectively, and sequenced on Illumina NovaSeq 6000 platform. The ES panel is designed to sequence all known blood group exons and additionally +5.8kb site of the *ABO intron 1* with *GATA and RUNX1* motifs. The WES data were analysed according to Broad Institute recommendations. The ES data were processed using Trimmomatic, hg38 BWA (*Burrows-Wheeler Aligner*), HaplotypeCaller (GATK), IGV, in-house GeneBeam base. Blood group alleles were predicted manually according to the blood group allele ISBT tables.

Results: Both WES and ES analysis revealed the presence of *ABO*A c.dup543_563* variant in donor#1 confirming the duplication site described by classical sequencing (mean coverage for *ABO* was 60 in WES and 256 in ES experiment). In donor#2 WES and ES complex analysis of *ABO*, *FUT1* and *FUT2* genes showed full concordance with *ABO* and *FUT* genotype known from Sanger sequencing explaining rare O_h phenotype (mean coverage for *ABO*, *FUT1* and *FUT2* was 80 in WES and 270 in ES).

Summary/Conclusions: The *ABO*-related gene results justify the use of WES and ES panel for genotyping *ABO* variants in case of difficulties in ABO blood group typing. Complex sequencing of *ABO*-related genes in one experiment facilitates the identification of molecular

basis of the ABO discrepancies. However, we recommend targeted sequencing due to easier bioinformatic analysis of ES panel data, deeper coverage, low cost as well as higher number of tested samples in a single ES experiment.

P378 | Evaluation of Tm-shift assay for screening Asian-type DEL in Thai blood donor

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Background: DEL is one of D variants that express very low amount of D antigens. Routine serological testing has limitation to discriminate DEL from true D-negative therefore DEL blood is transfused to D-negative patient and stimulate anti-D production. Asian countries have a high prevalence of DEL, particularly Thailand, where 15.6% of DEL observed from D-negative. Tm-shift assay was introduced for screening Asian-type DEL (*RHD*01EL.01* allele or c.1227G>A) before implement to routine testing in Thai blood donor.

Aims: To assess the effectiveness of Tm-shift assay for screening Asian-type DEL (*RHD*01EL.01* allele or c.1227G>A) in Thai blood donors

Methods: Tm-shift assay (previously designed by Fichou, Blood Transfus, 2022) was applied to target c.1227 nucleotide position at exon 9 of *RHD* gene with specific primer sets. Total of 60 samples were obtained for performance evaluation. All 30 of known negative samples include 20 samples carrying *RHD* deletion (*RHD*01N.01)* and 10 samples carrying *RHD-CE(3-9)-D* allele. The remaining 30 of known positive samples compose of 16 samples carrying Asian-type DEL (c.1227G>A), 11 samples carrying wild-type (c.1227G), and three samples carrying heterozygous (c.1227G/A). Tm-shift results were verified by quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF) and direct sequencing of *RHD* exon 9.

Results: Asian-type DEL (c.1227G>A) samples were distinguished from wild-type (c.1227G) according to their melting temperature (Tm). All 16 samples carrying hemizygous Asian-type DEL (c.1227G>A) displayed single peak with Tm (°C) values of 78.02 ± 0.10°C, while 11 samples carrying hemizygous wild-type (c.1227G) showed Tm (°C) values of 80.03 ± 0.24°C. Three samples carrying heterozygous (c.1227G/A) demonstrated two peaks with different Tm. For negative samples both *RHD* deletion (*RHD*01N.01)* and *RHD-CE(3-9)-D* allele, amplification of *RHD* exon 9 was not observed (no peak). Tm-shift results of all positive and negative samples correlated with the results from quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF) and direct sequencing of *RHD* exon 9 with sensitivity, specificity, and accuracy were 100%.

Summary/Conclusions: Tm-shift assay showed highly efficient to distinguished Asian-type DEL (c.1227G>A) from true D-negative. Furthermore, it can initially identified samples carrying common *RHD* exon 9 which can be further differentiated the allele types by another molecular method. This assay can be used for screening Asian-type DEL (c.1227G>A) in Thai blood donors.

P379 | Three novel *B3GALNT1* null alleles detected in two patients with the Pk phenotype

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Background: The Globoside histo-blood group system (GLOB) was established in 2002, and now comprises three antigens; P (GLOB1), PX2 (GLOB4) and ExtB (GLOB5). The ß-1,3-N-acetylgalactosaminyltransferase 1 enzyme, encoded by the *B3GALNT1* gene, is responsible for transfer of N-acetylgalactosamine (GalNAc) to the terminal galactose of the P^k antigen, the final step in the synthesis of the P antigen. The same enzyme is also responsible for synthesis of PX2 from its paragloboside precursor, and ExtB from the B antigen. The P1, P^k and NOR antigens of the closely related P1PK system are produced by a different glycosyltransferase, 4-α-galactosyltransferase, encoded by the *A4GALT* gene. Homozygosity (or compound heterozygosity) for rare inactivating mutations in *B3GALNT1* results in a lack of synthesis of P and PX2 antigens, and consequent strong expression of P^k antigen. All P^k individuals have naturally occurring anti-P in their serum, which has been associated with severe haemolytic transfusion reactions.

Aims: We report case studies of two female patients, each presenting with an unidentified alloantibody to a high prevalence antigen in their plasma. We present results from serological and molecular investigations, providing evidence for different novel genetic backgrounds of the P^k phenotype in each patient.

Methods: Samples from each patient were investigated due to the presence of a strong pan-reactive alloantibody. Serological investigations were performed by standard LISS tube IAT and direct agglutination techniques. Genomic DNA was extracted, and sequencing was carried out for both *A4GALT* and *B3GALNT1* genes.

Results: Both patients were found to have the P^k phenotype (P−, PX2−, P^k+), and anti-P and anti-PX2 were identified in their plasma. Patient 2 was also shown to have weakly reacting anti-P1. Sequencing of *A4GALT* demonstrated an *A4GALT*01/01.02* (c.109A>G, p.Met37Val) genotype for Patient 1, predicting a P1+, P^k+ (P_1) phenotype. Patient 2 showed homozygosity for the *A4GALT* intron 1 polymorphism (rs5751348; NM_017436.7:c.-188+3010G>T) associated with the *A4GALT*02* allele, predicting a P1−, P^k+ (P_2) phenotype in this case. *B3GALNT1* sequencing revealed compound heterozygosity for two novel mutations in Patient 1: c.794T>G (rs772158348, freq. 0.000004), encoding p.Phe265Cys; and c.341delC, encoding p.Thr114AsnfsTer5. Patient 2 was shown to be homozygous for a different novel missense mutation; c.725G>A (rs1480042758, freq. 0.000007), encoding p.Gly242Asp. None of

these mutations appear in the ISBT list of GLOB null alleles, and all appear to be extremely rare (c.341del mutation was not listed in the GnomAD database). It is presumed that none of these alleles encode functional transferase enzymes, resulting in the observed P_1^k phenotype in Patient 1 and P_2^k phenotype in Patient 2.

Summary/Conclusions: We have identified three novel *B3GALNT1* null alleles in two patients with the rare P^k phenotype. One patient shows compound heterozygosity for two alleles; one carrying a single nucleotide deletion, resulting in a frameshift and premature truncation of the protein product, the other carrying a missense mutation. The second patient carries a single homozygous missense mutation. These changes are predicted to result in inactivation of the enzyme produced by *B3GALNT1*, adding to the catalogue of known null alleles of the Globoside system.

P380 | Discovery and phasing of a novel null allele in a *FY*A/FY*B* individual with Nanopore sequencing

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Background: In the field of blood group genetics, direct determination of novel variants to their respective allelic background can enhance the accuracy of blood group phenotype prediction. In fact, haplotype-resolved sequences may be essential to interpret the functional consequences of identified variants, particularly when phenotypic data are unavailable. For example, it is important to know on which allelic background a novel silencing variant lies for deducing its effect on the blood group phenotype. Phasing variants, however, is not possible with the majority of standard techniques in molecular diagnostics, such as Sanger sequencing, PCR-SSP or other genotyping approaches.

Aims: We present here a case study illustrating the advantages of long-read Oxford Nanopore Technologies (ONT) sequencing for resolving a novel null allele in the Duffy blood group as complete gene haplotype.

Methods: The presented sample was initially identified as a genotype-phenotype discrepancy for the Duffy blood group in course of the routine donor typing at Blood Transfusion Service Zurich. Phenotyping was conducted via established serological methodologies. Genotyping was performed via MALDI-TOF mass spectrometry (MS) targeting the *FY*A/FY*B* defining single-nucleotide variant (c.125G>A), as well as the most common weak (c.265C>T) and null (c.-67T>C) defining variants. To elucidate the cause of the discrepancy, the entire length of the *ACKR1* gene, including flanking regions, was amplified (~2.1 kb) and sequenced using an ONT MinION flow cell. The results were further confirmed by Sanger sequencing of both *ACKR1* exons.

Results: The sample was identified as having a Fy(a-b+) phenotype. MALDI-TOF MS analysis, however, revealed heterozygosity for *FY*A/FY*B* and did not detect the presence of c.265C>T or c.-67T>C variants. This was the only genotype-phenotype discrepancy observed among ~15,000 donors for which both serological and genotyping data was available. Subsequent Nanopore sequencing of the discrepant sample uncovered a novel 1-bp deletion (c.655delG) and an adjacent c.657C>G SNV (rs748896745) in the second *ACKR1* exon of the *FY*A* allele. Both variants were verified by Sanger sequencing.

Summary/Conclusions: Nanopore long-read sequencing was found to be effective and accurate for resolving incongruities between Duffy blood group genotype and phenotype. It proved to be clinically beneficial by facilitating direct phasing of a newly discovered frameshift mutation to the corresponding *FY*A* allelic background. This study serves as an illustrative example of the potential of functionally characterizing blood group alleles by sequencing them as complete gene haplotypes, which could become the emerging standard.

P381 | Two novel varient alleles in the Kell blood group system: a novel K0 allele described in a patient with anti-Ku and a variant allele apparently abolishing expression of Jsb in a blood donor

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Background: The Kell blood group system contains 37 antigens carried on a type II transmembrane glycoprotein, a zinc-dependent Kell metallo-endopeptidase. The glycoprotein is encoded by the *KEL* gene, comprising 19 exons and located on 7q34. The rare phenotypes Kell-null (K_0) and K_{mod} are encoded by mutations in *KEL* and are characterised by either complete absence or weak expression of Kell antigens, respectively. K_0 individuals are able to make anti-Ku when exposed to Kell protein, which has been associated with both HDFN and HTR. Although these are very rare, ISBT lists 70 unique K^0 alleles, mostly resulting from either single point (nonsense or missense) or splice site mutations and a further 25 unique K_{mod} alleles, usually resulting in homozygosity or heterozygosity for missense mutations or heterozygosity for nonsense mutations.

Aims: We report the serological and genetic investigations of two individuals: a female patient (PT1) of Syrian origin, with history of pregnancies and transfusion, presenting with a K_0 phenotype and a possible anti-Ku; and a female blood donor (D1) showing discrepancy between Js^b phenotype and genotype results, suggesting the presence of a variant allele.

Methods: Serological investigation was performed by LISS tube IAT with untreated and papain treated cells. Alloadsorption/elution studies were carried out using standard techniques. Genomic DNA was isolated from whole blood of PT1 and D1. Genotyping for D1 was performed by the IBGRL Molecular Diagnostics department using real-time PCR-based

allelic discrimination assays. For PT1 and D1, all 19 exons of the *KEL* gene were amplified by PCR and bidirectionally Sanger sequenced.

Results: PT1 was confirmed to have the K_0 phenotype [cells were found to be K− k− Kp(a−b−) Js(b−) Ku− Kx+] and anti-Ku was present in her plasma, reacting with moderate strength with both untreated and papain treated cells by LISS IAT. Two examples of K_0 cells were found to be compatible with PT1 plasma and no additional antibodies were detected. D1 cells appeared to have normal k expression, but did not react with monoclonal anti-Js^b (MIMA-8) nor with two examples of human derived anti-Js^b, although genotyping showed the donor to be *JS*A/B*. D1 cells were confirmed to be Js(a+) with one example of human anti-Js^a. *KEL* sequencing confirmed both samples to be homozygous *KEL*02* and D1 to be heterozygous *KEL*02.06/07* (*JS*A/B*). PT1 was homozygous for a silent mutation, c.1680A>C, in exon 15 and a novel mutation, c.400+1g>a, in intron 4. The latter is a rare variant (not recorded in the gnomAD database) located in the donor splice site of intron 4 and thus, likely to result in aberrant splicing of the *KEL* gene and abolished expression of the Kell glycoprotein. D1 was found to have a heterozygous silent mutation c.1899A>G in exon 17, encoding p.Leu633 and a heterozygous novel mutation, c.811C>A, in exon 8, encoding p.His271Asn. The c.811C>A is a very rare variant, also not recorded in the gnomAD database, that is affecting the expression of Js^b antigen.

Summary/Conclusions: Here we have described two novel variant *KEL* alleles identified in two case studies. The K^0 allele in PT1, with a novel mutation c.400+1g>a in intron 4, is associated with aberrant splicing of *KEL* and the variant allele in D1, with a novel missense mutation c.811C>A, p.His271Asn, is associated with abolished expression of Js^b antigen.

Immunohaematology

Rare donors

P382 | Successful liver transplantation in a -D- patient with anti-Rh17 antibody

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Background: Liver transplantation typically requires large amounts of blood transfusions and is a challenging case in transfusion medicine, especially for patients with rare blood groups or antibodies to highly prevalent antigens. In this case report, we aim to share our experiences in providing transfusion support to a patient with -D- blood type and anti-Rh17 antibodies undergoing liver transplantation surgery.

Aims: Our aim is to share our experiences in providing transfusion support to a patient with -D- blood type undergoing liver transplantation.

Methods: This report is based on a case study.

Results: A 62-year-old man was admitted for liver transplantation due to liver cirrhosis arising from hepatitis B virus infection. Blood grouping tests showed that his blood type was A, RhD+. Antibody screening tests were positive, and his serum showed panreactivity to all panel cells in antibody identification tests. RhCcEe phenotyping tests were performed using commercial antisera (anti-C, anti-c, anti-E, and anti-e) to determine that he did not have any of these four antigens. Based on these findings, we concluded that his blood type was -D- and he had anti-Rh17 alloantibodies. The Korean Red Cross Blood Services recruited eight -D- donors and we found that one of his sisters also had a -D- blood group. Liver transplantation was successfully performed with the transfusion of four units of red blood cells. During the postoperative period, 4 units of -D- red cells were additionally transfused. The patient was discharged 1 month after transplantation.

Summary/Conclusions: This case report highlights the successful provision of transfusion support for a liver transplant patient with a rare blood type and antibodies. It is essential to expand rare donor registries to recruit more donors and cover other rare blood groups.

P383 | Abstract withdrawn

P384 | Abstract withdrawn

Immunohaematology

Platelet immunology

P385 | Abstract withdrawn

P386 | Newborn characteristics from the French cohort of pregnant women having a non-invasive prenatal testing for fetal platelet genotyping

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Background: Fetal platelet genotyping is essential for the diagnosis of feto-maternal platelet incompatibilities and the management of fetal

and neonatal alloimmunization thrombocytopenia (FNAIT) due mainly to Human Platelet Antigen (HPA) -1 but also to HPA-3, -5, and -15. Non-invasive prenatal testing (NIPT) to determine fetal platelet genotype in these four main HPA systems was performed on droplet digital PCR (QX200 Droplet PCR System, BioRad).

Aims: The aim of this retrospective study was to describe the newborns characteristics regarding the introduction or not of maternal antenatal treatment by intravenous immunoglobulin (IVIG), the HPA fetal status and the maternal alloantibodies.

Methods: From 10/2020 to 06/2022, 101 cases of pregnant women needed NIPT based on clinical decisions were collected and newborn information at birth followed. This study focused on 90 NIPT cases from 89 pregnant women where all information on fetus/newborns were available at birth.

Results: Pregnant women were immunised in 55.5%. Fetuses were compatible with their mother in 33.3% and incompatible in 65.5%, mainly in one HPA system (71.2%). In one case, fetal genotyping failed.

Ninety children were born between 25 GW +3 D and 41 GW +5 D by vaginal delivery (52.2%) and by C-section (47.8%). Male represented 48.9%, female 51.1%; their weight varying from 775 g to 4550 g. In 88.9%, platelet count (PC) was performed ranging from 6 to 459 G/L. Thirteen newborns were thrombocytopenic at birth (16.25%). Thrombocytopenia was observed in both compatible ($n = 3$) and incompatible fetuses ($n = 10$), but with a 3-fold enrichment in incompatible fetuses (PC mean 97 vs 95.7 G/L respectively, $p = 0.811$). Only 2 mothers immunised with an anti-HPA-5b alloantibody and having incompatible fetus had IVIG antenatal treatment. Newborn platelet counts were 73 and 83 G/L at birth.

Indeed, HPA-5 incompatibility alone or associated was the most frequent in immunised pregnant women, with an anti-HPA-5b alloantibody in 66% of cases. However, the only one newborn with as severe thrombocytopenia was related to an anti-HPA-1a alloantibody without maternal antenatal treatment.

Regarding non thrombocytopenic newborns and taking account HPA fetal status and maternal antenatal treatment, platelet count is lower in incompatible newborns with treated mothers ($p = 0.0010$) whatever the anti-HPA alloantibodies present. However, concerning anti-HPA-5b alloimmunization, there is no difference in platelet count at birth focusing on NIPT indications (FNAIT history vs incidental discovery of alloantibody during pregnancy), HPA fetal status and maternal antenatal treatment.

Summary/Conclusions: This is the first study describing newborn characteristics after fetal platelet genotyping on maternal plasma. These results show that 16% of newborns are thrombocytopenic but severe thrombocytopenia is rare (only one case). In our cohort, pregnant women are mainly immunised with an anti-HPA-5b alloantibody and this study highlights that in HPA-5b alloimmunization, there is no significant difference in newborn platelet count at birth when taking into account the indications for fetal platelet genotyping, the compatibility of the fetus with its mother and the maternal antenatal IVIG treatment.

Given these initial results, the follow-up of this cohort should be consolidated due to the small number of newborns in some groups for instance in thrombocytopenic incompatible fetuses with or without maternal treatment. This cohort needs to be further enriched to strengthen these results in order to improve practices particularly in case of HPA-5b alloimmunization and revisit recommendations in NIPT indications.

P387 | Abstract withdrawn

P388 | Platelet activation Index in a flow cytometric functional assay for the diagnosis of heparin-induced thrombocytopenia

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Background: Serological assays for the diagnosis of heparin-induced thrombocytopenia (HIT) detect both platelet-activating and platelet-non-activating anti-heparin/platelet factor 4 (PF4) antibodies and have therefore a limited positive predictive value. Functional assays confirm the presence of platelet-activating antibodies but require platelets from healthy donors, whose response to patient serum differs. In the Slovenian institute for transfusion medicine, platelet-activating antibodies are detected with a functional flow cytometric assay. The assay is based on detecting two platelet markers: CD61 (all platelets), and CD62P (activated platelets). The test is performed with platelets from four randomly selected blood donors and with a low and a high heparin concentration in parallel. HIT is confirmed when at least two of the four donor platelets are activated after incubation with the patient's serum.

Aims: Our aim was to assess the strength of donor platelet activation and to quantify the results of flow cytometry (in addition to the classical reporting of a positive or negative result), similar to immunoassays, where the optical density value corresponds to the concentration of anti-heparin/PF4 antibodies. To develop a prediction tool, we designed an Index of platelet activation, which additionally reduces the variability of platelet response and standardises the results.

Methods: A retrograde cohort of 308 HIT-suspected patients with a positive immunoassay, who had been previously tested with flow cytometric assay according to local protocols, was included in the evaluation of the proposed Index formula. Especially due to the specificities in control samples used in our in-house test, we had to adjust the formula to our routine testing conditions. As positive controls, we test known HIT-positive sera with different antibody titres and platelet activation capacity. As negative controls, we test sera from HIT-negative patients.

Results: We designed the Index of donor platelet activation, which is adjusted to our testing conditions and is defined as follows: (%A H0.2 - %A H200) / 5.4%. %A H0.2 is the mean donor platelet activation (average of 4) with patient serum in the low (0.2 IU/mL) heparin step. %A H200 is the mean donor platelet activation (average of 4) with

patient serum in the high (200 IU/mL) heparin step. The results are standardised by putting a mean value of multiple negative control platelet activation in the denominator. 5.4% is the mean negative control platelet activation percentage in the low heparin step and is calculated based on 538 negative control reactions with 136 different sera. We statistically tested the impact of the Index on the functional test result when the result is interpreted according to the standard operative procedures. As a functional test result predictor, using the threshold value of 1.27, Index sensitivity is 97%, specificity 98%, and overall accuracy is 97%.

Summary/Conclusions: Calculating the Index of platelet activation reduces the impact of donor platelet variability to HIT antibodies. The Index has a statistically significant influence ($p < 0.05$) on the functional test result and could help to predict it. Furthermore, the Index also shows the degree of donor platelet activation and the strength of the platelet response to HIT antibodies, which are related to the severity of the clinical picture in HIT and could provide important information to the clinician treating the patient.

P389 | Specific distribution of platelet antibodies in patients with immune-mediated platelet transfusion refractoriness

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Background: Antibodies (Abs) against human platelet antigens, including specific HLA-I, HPA, CD36 allo/isoantibodies and nonspecific platelet glycoprotein autoantibodies, are the main cause that leads to immune-mediated platelet transfusion refractoriness (iPTR). The analysis of specific antibodies in iPTR patients and the selection of platelet units with corresponding antigen negative for transfusion are effective methods to solve iPTR. However, the distribution data of platelet antibodies in Chinese population is rarely reported.

Aims: To elucidate the specific distribution of human platelet antibodies in Chinese iPTR patients.

Methods: A total of 1062 patients with clinically suspected iPTR were sent to our laboratory for platelet cross-matching from Jan 2021 to Oct 2022. Platelet matching was performed for each patient with an average of 5.1 donors using a solid-phase RBC adherence technique (Capture-P). Serum from patients with a positive matching with at least one donor was further identified for platelet antibodies using a luminex-based assay (PakLx).

Results: 525 patients with frequency of 49.4% (525/1062) were positive with at least one donor in platelet matching. Various platelet antibodies were detected in 319 patients with frequency of 60.8% (319/525). A total of 367 antibodies were determined in 319 patients, averaging 1.15 antibodies per patient (367/319). Of all antibodies, the constituent ratio of alloantibodies (HLA-I and HPA), autoantibodies (GPIIbIIIa, GPIaIIa and GPIbIX) and isoantibody (CD36) were 83.7%, 16.1% and 0.3%, respectively. The ranking of antibodies prevalence was HPA-I > GPIIbIIIa > GPIaIIa > HPA-5b > GPIbIX > HPA-3a > HPA-1b and 2b > HPA-3b, 4b and CD36. The antibodies against HPA-1a, 2a, 4a and 5a had not been detected in all patients (table 1).

Summary/Conclusions: The specific distribution of platelet antibodies in Chinese iPTR patients was determined. In addition to HLA-I antibodies, autoantibodies against platelet glycoproteins also play an important role in iPTR patients. In the field of HPA antibody, unlike Caucasians, HPA-5b rather than HPA-1a may be dominant in Chinese.

P389 - TABLE 1. Specific distribution of platelet antibodies in 319 iPTR patients

Antibodies against	Abs sum (single Abs, combined Abs)	Constituent ratio (%)	Abs / patients (%)	Ranking of prevalence
alloantigens:	288 (257, 31)	78.47	90.28	1
HLA-I	3 (0, 3)	0.82	0.94	7
HPA-1b	3 (0, 3)	0.82	0.94	7
HPA-2b	4 (1, 3)	1.09	1.25	6
HPA-3a	1 (0, 1)	0.27	0.31	9
HPA-3b	1 (0, 1)	0.27	0.31	9
HPA-4b	7 (1, 6)	1.91	2.19	4
HPA-5b				
isoantigen: CD36	1 (1, 0)	0.27	0.31	9
autoantigens:	38 (16, 22)	10.35	11.91	2
GPIIbIIIa	15 (2, 13)	4.09	4.70	3
GPIaIIa	6 (2, 4)	1.63	1.88	5
GPIbIX				
Total	367 (280, 87)	100	115	/

P390 | Anti-HPA-5b antibodies much less frequently induce in vitro platelet phagocytosis than anti-HPA-4b antibodies

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Background: Fetal and neonatal autoimmune thrombocytopenia (FNAIT) is caused by maternal IgG antibodies against human platelet antigen (HPA). In Japan, anti-HPA-5b alloantibodies were frequently detected in pregnant women, but it rarely caused FNAIT. In contrast, anti-HPA-4b antibodies were detected in more than half of Japanese FNAIT cases (Tomiyama et al, 2018).

Aims: In this study, we investigated the potential clinical significance of anti-HPA-5b antibodies by in vitro platelet phagocytosis assay.

Methods: Peripheral blood mononuclear cells (PBMCs) and platelets were obtained from HPA-4 and -5 typed donors. Platelet phagocytosis was performed as previously described (*Takahashi et al, 2017*). In

brief, pHrodo fluorescence labelled platelets were incubated with either monoclonal antibodies (mAbs) against GPIIIa (SZ21) or GPIa (Gi9 or Gi14) or sera containing anti-HPA-4a ($n = 4$), -5a ($n = 3$), or -5b ($n = 5$). Antibody-sensitized platelets were incubated with adherent PBMCs, and the phagocytosis was analysed by flow cytometry. PI (Phagocytosis Index) was determined as the ratio of monocytes that engulfed with antibody-sensitized platelet to those with buffer-sensitized platelet. PI > 8.5 was defined as positive phagocytosis. Bound antibodies to platelets were assessed by flow cytometry (PIFT: platelet immunofluorescence test) and PIFT index, a ratio of mean fluorescence intensity of antibodies compared to controls was calculated. The expression levels of CD62P, Annexin V, and CD47 on platelets were measured by flow cytometry.

Results: In comparison to anti-GPIIIa (SZ21), mAbs against GPIa (Gi9 and Gi14) did not induce platelet phagocytosis (PI-SZ21: 37.8 ± 14.2 versus PI-Gi9: 0.71 ± 0.3 and PI-Gi14: 0.73 ± 0.1, $p < 0.01$, respectively). Similarly, anti-HPA-5a (PI-HPA-5a: 5.3 ± 3.4) and anti-HPA-5b (HPA-5b: 8.0 ± 7.3) did not cause platelet phagocytosis when tested with HPA-5ab typed platelets. In contrast, strong phagocytosis was found with anti-HPA-4a antibodies (PI-HPA-4a: 61.4 ± 38.0). Interestingly, increased anti-HPA-5b mediated platelets phagocytosis was observed with platelet expressing higher GPIa/IIa (TT805 typed platelets) compared to lower GPIa/IIa (CC805 typed platelets) surface density. Furthermore, we found that anti-HPA-5 antibodies did not cause significant changes in the expression levels of P-selectin, CD47, or Annexin V.

Summary/Conclusions: Our result suggested that in contrast to anti-HPA-4 against GPIIIa, antibodies specific for HPA-5a or -5b did not cause platelet phagocytosis, activation and apoptosis, most probably due to the lower surface expression of GPIa/IIa (2.000-6.000 molecules) compared to GPIIb/IIIa (50.000–100.000 molecules) on platelets. This result supports recent finding showing that the neonatal platelet counts between suspected FNAIT cases with and without anti-HPA-5b were not significantly different (*Alm et al., 2022*). However, the expression of GPIa/IIa can vary by several fold in healthy individuals; currently explained by single nucleotide polymorphism (SNPs) that alter the transcription rate by altering the affinity for its transcription factors (GATA and estrogen) (*Adorno-Cruz & Liu, 2019*). The question whether anti-HPA-5 could induce occasionally thrombocytopenia in patients with increased GPIa/IIa density is intriguing.

P391 | Abstract withdrawn

P392 | Transfusion of type II CD36 deficient platelets to leukemia patient with type I CD36 deficiency

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Background: Type I CD36 deficient individuals experienced isoimmune could developed CD36 antibodies to induce platelet transfusion refractoriness (PTR). However, the frequency of platelet (PLT) CD36 deficiency in Chinese populations was less than 5%, especially for type I CD36 deficiency (<0.8%).So it is limited to available type I CD36 deficient PLT products in time for clinical usage. The efficacy of transfusion of type II CD36 deficient PLTs to patient with type I CD36 deficiency is rare report.

Aims: To analyse and identify the CD36 deficient in both the patient and donors, as well as the CD36 antibody，and to evaluate the efficacy of type II CD36 deficiency and ABO-matched PLTs transfusion.

Methods: A 4-year-old male (blood group O) failed to respond to the first three ABO-matched PLT transfusions during acute lymphoblastic leukemia induction therapy. Antibodies against PLT were screened by Capture-P kit and identified by PakLx assay. The CD36 antigen expression on PLT and monocyte were analysed by flow cytometry (FCM).Donors were recruited from the pre-established CD36 deficient registry of Zhejiang Blood Center and CD36 deficient PLTs were collected. The 24-hour post-transfusion corrected count increment (24h-CCI) was calculated to evaluate the effect of PLTs transfusion. A 24h-CCI greater than 4.5 was considered a successful response.

Results: Antibody investigation using the Capture-P demonstrated that the patient's serum was all positive with 20 random group O PLTs. The antibody identification was negative for HLA Class I antibodies and PLT-specific GP, except for antibody against CD36(GPIV). FCM result show that the patient was type I CD36 deficiency(both PLTs and monocytes CD36 antigen negative).A total of 1 donor with type I CD36 defect and 17 donors with type II CD36 defect was candidate in 347 group O apheresis donors from CD36 deficient registry. Four donors were recruited successively all with type II CD36

P392 - Table 1. Response of type I CD36 deficiency patients to transfuse of type II deficiency PLTs.

Date of PLTs transfusion	PLT units(u)	PLTs (10^9/L) Pre/Post transfusion	24h-CCI	Fever/ infection
2021.12	9	13/140	33.9	Yes/Yes
2022.4	10	4/59	13.5	Yes/Yes
2022.8	7	3/26	8.9	No/No
2022.9	10	2/56	14.6	No/No

deficiency. The patient showed significantly improved in PLT counts and 24h-CCI after transfused with type II CD36 deficiency PLTs (table.1) The CD36 antibody of patient remained positive at the last transfusion of type II CD36 deficient PLT.(This work was supported by the Science Research Foundation of Zhejiang Province with LTGY23H080004 and 2023KY088)

Summary/Conclusions: We established a donor registry to supply CD36-negative PLTs for patients in need. The patient with type I CD36 deficiency received satisfactory response after transfusion of type II CD36 deficiency PLTs. However, it is unavoidable to stimulate the patients to continuously produce CD36 antibody by a small amount of mononuclear cells in the collected PLTs.

P393 | Abstract withdrawn

P394 | Abstract withdrawn

P395 | In vitro generated megakaryocytes for the detection of human platelet antigen-specific alloantibodies

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Background: Serologic detection and characterization of anti-human platelet antigen (HPA) alloantibodies are essential for proper diagnosis and treatment in fetal neonatal alloimmune thrombocytopenia (FNAIT). The monoclonal antibody immobilization of platelet antigens (MAIPA) assay is the standard assay for the detection of anti-HPA antibodies. For this assay, fresh platelets from HPA-genotyped donors are required, which is a limitation for laboratories with no direct access to blood donors. HPAs are expressed on glycoproteins on the surface of platelets as well as megakaryocytes.

Aims: In the current study, we developed a monoclonal antibody immobilization of megakaryocyte antigens (MAIMA) assay for the detection of anti-HPA antibodies.

Methods: CD34+ cells were isolated from buffy coats of blood donors and differentiated for 28 days in vitro into megakaryocytes. Megakaryocyte cell lines were characterised by flow cytometry using CD41, CD42a and CD42b expression and DNA content. The performance of MAIMA was assessed using WHO reference reagents (HPA-1a, HPA-3a and HPA-5b) and patients' sera with well characterised anti-HPA antibodies.

Results: The WHO anti-HPA-1a reference reagent showed similar binding to megakaryocytes and platelets in MAIMA and MAIPA, respectively. On the other hand, OD values for the WHO anti-HPA-3a reference reagent were lower in MAIMA than in MAIPA. Anti-HPA-5b antibodies were not detectable in MAIMA. Patient's sera containing anti-HPA-1a antibodies were successfully detected in MAIMA in all clinical samples ($n = 7$). OD values in MAIPA and MAIMA showed high correlation ($r = 0.96$, $p < 0.001$). MAIMA was reactive for samples with anti-HPA-3a as well as anti-HPA-3b, however, OD values were 33-50% lower compared to MAIPA. Interestingly, all patient samples with anti-HPA-5b antibodies tested negative in MAIMA ($n = 4$).

Summary/Conclusions: In vitro generated megakaryocytes can be used to detect anti-HPA-1a alloantibodies. However, despite this potential, they may be less suitable for the detection of alloantibodies against other HPAs such as HPA-5b.

P395 – Table 1

Gene	Exon	Amplicon size	Chrom	System
HLA-A	exons 1-8	4942 bp	6	HLA Class I
HLA-B	exons 1-8	3906 bp	6	HLA Class I
ITGB3	exons 3-4	2057 bp	17	HPA-1, HPA-10, HPA-4
ITGB3	exon 10	700 bp	17	HPA-6, HPA-7
ITGA2B	exon 26	1111 bp	17	HPA-3, HPA-9
GP1BA	exon 2	966 bp	17	HPA-2
ITGA2	exon 13	1726 bp	5	HPA-5
CD109	exons 18-19	2260 bp	6	HPA-15

P396 | A novel high-throughput NGS method for simultaneous HPA and HLA genotyping of platelet apheresis donors

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Background: Platelet transfusion refractoriness (PTR) is a common complication of patients receiving multiple platelet transfusions. Alloantibodies against HLA Class I and/or HPA antigens are involved in about 60% of PTR cases. The provision of compatible platelets for these patients depends on the availability of a large cohort of previously typed platelet apheresis donors. Although great efforts have been made to build a database of HLA and HPA genotyped donors, there is a continuous need to expand this group. Firstly, because of the high degree of polymorphism, especially in the HLA system and, secondly, because it is a dynamic group with ins and outs, as any donor population.

Aims: Taking advantage of a previously developed in-house HLA genotyping method by NGS, we aimed to expand this development by setting-up a new NGS-based tool for the extensive genotyping of newly recruited platelet apheresis donors, simultaneously covering the HLA-A and HLA-B loci as well as the clinically most relevant HPA systems.

Methods: We designed a multiplex PCR to coamplify with the SequalPrep system the following loci of interest:

Library preparation was performed by enzymatic fragmentation of PCR products and double indexing using the NGSgo kit (GenDx). The pooled library was sequenced using a paired end (2x150) sequencing protocol on the MiSeq system (Illumina) with a standard v2 Reagent Kit.

The HLA class I genotype was determined with NGSengine 2.28.1 (GenDx) using the IMGT 3.50.0 reference database. HPA reads were mapped to the human genome reference sequence hg19 (GRCh37) using CLC Genomics Workbench 23.0.1, masking the reference to only include HLA and HPA amplicon coordinates. For variant analysis, we used the Fixed Ploidy (ploidy = 2) Variant Detection tool (CLC Genomics Workbench).

Results: A pre-validation study was carried out with 8 genotyped (HLA+HPA) samples, selected to represent different HPA genotypes (heterozygous and homozygous), as well as the most frequent HLA haplotypes in our population. Some coverage variability was found among the different amplicons, although it was corrected by adjusting primer concentrations. All loci could be accurately genotyped. Notably, heterozygous positions were detected at frequencies of 50 ± 3, indicating an adequate allele balance. For the clinical validation, a total of 192 DNA samples, processed in two independent 96-sample runs have been included. These samples correspond to platelet donors previously typed for HPA (ID HPA XT, Progenika-Grifols) and HLA (NGS HLA Typing, GenDx). In the first run analysed so far, the mean value of the reading depth in all exon regions was 1870 ± 1030 and the results have been 100% concordant with the known typing.

Summary/Conclusions: Here we report an NGS-based high-throughput method for the simultaneous determination of both HLA Class I and HPA donor typing. Preliminary results of the validation study show 100% accuracy. In addition, the new method can be adapted to automated processing, using the infrastructure already in place for HLA typing. The availability of this method will contribute to significantly expand our current platelet apheresis donor database.

P397 | Platelet transfusion refractoriness: 20 years of experience diagnosing and treating patients

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Background: Platelet transfusion (PT) is essential to prevent and to treat bleeding complications. Platelet Refractoriness (PR) occurs when platelets do not increase as expected after transfusion. The cause of PR can be immune, with the presence of alloantibodies against platelets, or non-immune, caused by peripheral consumption. When dealing with immune PR, it is essential to select compatible platelets to ensure adequate platelet increment.

Aims: The aim of this study is to share the 20-year experience of a tertiary hospital with the diagnose and management of immune PR.

Methods: All patients transfused with platelets in our hospital have their post-transfusion platelet increments since 2002. If the corrected count increment (CCI) is less than 2,500 after 24 hours, another 1hour-CCI is calculated after at least 2 consecutive PT (ABO compatible platelets with less than 72 hours of storage). If the 1-hour CCI is less than 5,000, the cause is more probable to be immune. In these cases, APA testing is performed using PIFT (immunofluorescence test by flow cytometry) and MAIPA (to search for anti-HLA and HPAs antibodies). HLA and HPA genotyped platelets from our bank of frozen platelets were used to execute APA tests.

Results: A total of 195 patients were referred for APA testing, 124 from our Hospital (63.5%), 70 from other hospitals (36%) and 1 from another state (0.5%). APA tests were positive in 110 patients (56.4%) and negative in 85 (43.6%). Of the 110 patients with positive APA, antibody identification was not performed in 6 patients and one patient had drug-dependent antibody, resulting in 103 patients with identified antibodies. Of these 103, 68 had HLA antibodies (65.9%), 19 HPA antibodies (18.9%) and 16 glycoprotein antibodies of undetermined specificity (15.2%). The most frequent HPA antibodies were: anti-HPA-1b (30.69%), anti-HPA-5b (26.98%), anti-HPA-1a (15.34%), anti-HPA-2b (11.64%), anti-HPA-3b (11.64%), anti-HPA-5a (3.7%). A total of 94 patients with positive APA (85.45%) were submitted to the program for selecting compatible donor, with 14,370 crossmatches performed by PIFT, finding 2357 compatible donors (16.4%). For 71 of the 94 patients, 2,390 crossmatches were also performed by MAIPA, resulting in 53% compatibility.

Summary/Conclusions: Most patients with a 1-hour CCI below 5,000 had a positive APA test (56.4%), indicating immune PR. These patients showed significant improvement in CCI after receiving compatible platelets, with 1-hour CCI going from 0 to over 10,000 in some cases. Despite its prevalence, PR is still neglected by many health professionals. Randomly transfusing is not the solution, since patients will not show a satisfactory increase in platelets counts with incompatible platelets, triggering possible catastrophic bleeding complications. PR is a challenging clinical condition and should be promptly diagnosed and managed in order to ensure patient safety.

Immunohaematology

Granulocyte immunology

P398 | Abstract withdrawn

P399 | A bead-based screening method for antibody diagnostics in autoimmune neutropenia: Performance compared to the granulocyte immunoflouresence test (GIFT)

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Background:
Autoimmune neutropenia (AIN) involves the peripheral neutrophil destruction by autoantibodies targeting various glycoproteins on the cell surface, in particular the neutrophil-specific Fc gamma receptor CD16b. Detection assays for autoantibodies in AIN depend on access to neutrophils from donor blood. These assays are time consuming, require trained staff and offer variable sensitivity depending on test cell antigen expression levels.

Beads-based antibody detection platforms offer a high-throughput screening for granulocyte antibodies, requiring little patient material and eliminating the requirement for trained staff and donor blood. The Labscreen-Multi (LSM) platform was developed for diagnostics of antibodies against human neutrophil antigen (HNA) allotypes with potential to cause transfusion related acute lung injury. Specific neutrophil antigens are conjugated to the beads allowing determination of antigen specificity. The granulocyte immunofluorescence test (GIFT) remains the gold standard for anti-neutrophil autoantibody testing in AIN but generally cannot determine autoantibody specificity. The usefulness of the LSM in diagnostics of AIN autoantibodies has not been evaluated.

Aims: To evaluate the performance of the LSM in comparison with the granulocyte immunofluorescence test (GIFT).

Methods: Three hundred sixty-eight plasma samples from children aged 0-17 years, tested during an 18 month's period (2020-2021), were identified. For the GIFT, neutrophils both from donors homozygous for HNA-1a or HNA-1b were tested. Plated cells were incubated with patient plasma for 30 minutes at 37 degrees Celsius. Cells were subsequently stained with a cocktail of anti-human IgG (FITC) anti-human IgM (PE) secondary, anti-human CD66b and a viability dye (7-AAD) before FACS analysis. The LSM was performed as per the manufacturer's recommendations.

Results: LSM detected 59 positive and 309 negative patient plasma samples (**16%**). When retested with the GIFT assay we found 84 positive and 282 negative samples (**23%**). The specificities of the autoantibodies detected by LSM were nearly always CD16 with the majority reacting against both major allotypes. Five percent of the samples reacted with beads expressing the HNA-3a allele of CTL-2. No samples reacted against HNA-2, 4 or 5 allotypes.

Summary/Conclusions: The LSM assay identified fewer positive samples compared to GIFT and no antibodies against CD11a, CD11b or CD177 were seen. Compared to GIFT, LSM showed a bias towards reactivity against the whole CD16 glycoprotein rather than a particular allele. Reactions against HNA 3 have also been observed in another group of adult patients (not shown) and further investigation will rule out non-specific reactivity, as autoantibodies toward CTL-2 are not reported to any major extent in AIN. Further analysis will focus on antibody strength and potential links with patient characteristics.

P400 | Characterization of macrophage phagocytosis of neutrophils triggered by autoimmune neutropenia patient plasma

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Background: Anti-neutrophil factors in the plasma of primary autoimmune neutropenia (AIN) patients are thought to mediate neutrophil clearance. Humoral factors such as autoantibodies may trigger neutrophil clearance via macrophage phagocytosis potentially in the bone marrow, liver, or spleen. However, AIN plasma-triggered macrophage phagocytosis of neutrophils has been scarcely studied directly, and the potential factors and mechanisms involved are not fully clear.

Aims: Our objectives were to i) evaluate the capacity of plasmas from patients with autoimmune neutropenia to trigger macrophage uptake

of neutrophils, and ii) relate the magnitude of neutrophil uptake with patient factors such as autoantibody presence.

Methods: Anti-neutrophil antibodies from a variety of sources were assessed for the capacity to trigger macrophage uptake of neutrophils. Five select plasmas from pediatric patients with suspected autoimmune neutropenia were evaluated: one plasma negative for autoantibodies, and four plasmas positive for autoantibodies via the granulocyte immunofluorescence test (GIFT) or a commercial Luminex platform. Phagocytosis assays were performed blinded to the identity of autoimmune neutropenia specimens. Plasmas from adults positive for alloantibodies to HNA-1b, HNA-3a, or HNA-4a, and a monoclonal rat IgG2b anti-CD11b antibody were also assessed. Plasmas from two healthy normal adults or phosphate buffered saline were used as negative controls. Neutrophils from normal adult donors were isolated and fluorescently labelled with PKH67 before incubation with sources of human anti-neutrophil plasmas or controls. Neutrophils were then incubated with macrophages derived from THP-1-CD16A cells, which express multiple major activating FcγRs (FcγRI, FcγRIIA, and FcγRIIIA). Uptake of neutrophils was evaluated via microscopy. Quantification of uptake was performed via ImageJ.

Results: Macrophage uptake of neutrophils was substantially increased by the monoclonal anti-CD11b antibody relative to neutrophils incubated with phosphate buffered saline only. Plasmas positive for anti-HNA-3a or anti-HNA-4a alloantibodies also triggered increased uptake of neutrophils. The suspected AIN plasma negative for autoantibodies failed to trigger phagocytosis beyond the normal donor plasma level. However, a plasma positive for autoantibodies by both the GIFT and Luminex tests triggered substantially increased uptake of neutrophils. Two AIN plasmas positive by only one method (GIFT or Luminex) triggered a modest increase in macrophage uptake of neutrophils. Curiously, neutrophil uptake was associated with the presence of punctate fluorescent particles within macrophages possibly originating from a phagocytic "nibbling" process or potential trogocytosis.

Summary/Conclusions: The capacity of some plasmas from primary AIN patients to trigger uptake of neutrophils is demonstrated directly. Uptake triggered by AIN plasma was associated with the presence of detectable autoantibody. However, our data also suggests that the presence of detectable anti-neutrophil antibodies is not necessarily sufficient to trigger neutrophil uptake, hinting at the importance of other factors or autoimmune processes which explain neutropenia. Exploration of neutrophil uptake triggered by plasmas from a wider cohort of AIN patients as well as correlates between neutrophil uptake and patient characteristics, autoantibody test result magnitude, and antigen specificity are being explored.

Immunohaematology

Fetal-maternal immunology

P401 | The importance of maternal and paternal blood test in prenatal test

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Background: The ABO blood group is an important blood group in blood transfusions because ABO group antigens are the most immunogenic antigens. In addition to blood transfusions, ABO blood type is also important for the health of the mother and fetus. The mother can form antibodies when the baby has a different blood type than the mother. The baby's blood type is also influenced by the blood group gene inherited from the father. The risk that occurs if the mother forms antibodies and these antibodies cross the placenta can cause the fetus to have Haemolytic Disease of the Fetus and Newborn (HDFN).

Aims: To study the possibility of the baby's blood type and the possibility of the mother forming antibodies, especially the IgG subtype. In addition for preliminary data for future study.

Methods: Study was carried out in 30 patients (group of mother, father and baby) at RSUPN Dr. Cipto Mangunkusumo - Jakarta and RSIA Budi Kemuliaan - Jakarta. Patients' medical record were used for individual data information. Mother, father and baby's blood group was tested using the tube and slide method. The mother's IgG subtype was tested using the ELISA method.

Results: All 30 patients (group of mother, father and baby) were all RhD positive, but for the ABO blood group, the mothers blood type were A (22%), B (25%) and O (53%), the fathers blood type were A (31%), B (22%), O (22%) and AB (25%), the babies blood type were A (25%), B (39%), O (33%) and AB (3%) respectively. 22 mothers of them developed IgG, IgG1, IgG3 and IgG4 anti-A and B had a significant increase on mothers blood type ($p = 0.181$, 0.359, 0.028, 0.004, 0.182 and 0.050).

Summary/Conclusions: This study re-emphasized the importance of testing blood group especially for Indonesian married couple who will be parents to reduce risk of haemolytic disease of fetus and newborn. Applying blood group test for maternal and paternal, also subtype IgG anti-A and anti-B are important. When mother have IgG won't always have a risk for the fetus, like IgG4. IgG doesn't have receptors to activate complement system. But when mother have IgG 1 and/or IgG3, the fetus will have higher risk to developed Haemolytic Disease of Fetus and Newborn.

P402 | A National comparison of the uncertainty of measurement in different D positive red cell volumes by flow cytometry

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Background: Feto-maternal haemorrhage (FMH) can occur at any point during pregnancy. It has the potential to cause D sensitisation of D negative woman carrying a D positive fetus. Anti-D is a well-documented cause of haemolytic disease of fetus and newborn in pregnancy, with consequences that range from anaemia to stillbirth. Administration of the correct dose of prophylactic anti-D (PAD) after an FMH event prevents sensitisation to the D antigen. Even though it is routine practice to administer PAD to D negative pregnant women, sensitisation still occurs in a small percentage of pregnancies. The uncertainty of measurement (UoM) in the test is the quantification of the doubt associated with any measurement result. For accurate PAD dosing there must be an understanding of the UoM related to FMH testing and its inclusion in test results. This is also a requirement to meet the ISO15189 standards for accreditation by the United Kingdom Accreditation Service for quantitative testing. Lack of a robust validated process for calculation and assessment of the UoM value, or its incorrect application, is recognised as potential risk for D sensitisation in D negative pregnancies. The Red Cell Immunohaematology (RCI) department carries out an annual exercise at 4 mL for the assessment of the UoM in its flow cytometry (FC) based FMH testing. It was unknown if the UoM for the test varies across different FMH volumes. There was a perceived risk that the current method for PAD calculation did not consider this variation.

Aims: The aim of this study was to influence best practice for the determination of the UoM in FMH investigations by FC by:
Understanding if the UoM is dependent on the volume of D positive red blood cells (RBCs).
Establishing the best FMH volume to be used for the assessment of the UoM.

Methods: Samples simulating FMH volumes: 4 mL; 8 mL; 12 mL; 16 mL; 20 mL and 24 mL were produced using donor A1rr and OR1r cells. Four RCI laboratories tested each sample a minimum of ten times over a seven-day period. Beckman Coulter Navios flow cytometers were used with BRAD3-FITC (anti-D), AEVZ5.3-FITC (negative control) and BIRMA17C-PE (for granulocyte exclusion). The Mollison formula was used to calculate the FMH volume. The UoM (%) was calculated as follows: 1.96 x ((Standard Deviation/mean) x 100).

Results: The calculated range of UoM across the four RCI laboratories was:

Summary/Conclusions: The results demonstrate that the UoM associated with FC based FMH investigation in RCI is dependent on the FMH volume. The UoM (%) decreased as the D positive RBC volume in the sample increased. To prevent D sensitisation in D negative pregnant women, sufficient PAD dose must be administered. Inclusion of the UoM in the PAD calculation is critical for the administration of accurate PAD dose. The findings of this study demonstrate that a 4 mL FMH volume is associated with a greater UoM. This supports current practice in RCI, and the wider transfusion community, for the use of a 4 mL FMH volume to establish the UoM in FC investigation.

P402 – Table 1

UoM exercise (mL)	Minimum UoM value calculated (% ; mL)	Maximum UoM value calculated (% ; mL)	National Average (% ; mL)	Number of investigations
4	8 ; 0.33	12 ; 0.47	10 ; 0.40	40
8	2 ; 0.12	6 ; 0.44	4 ; 0.32	40
12	3 ; 0.30	5 ; 0.66	4 ; 0.44	40
16	3 ; 0.54	4 ; 0.69	4 ; 0.64	44
20	2 ; 0.30	3 ; 0.65	3 ; 0.53	40
24	2 ; 0.43	3 ; 0.71	3 ; 0.58	39

P403 | Non RH1 alloimunization in pregnancy – case series from a tertiary hospital

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Background: Haemolytic disease of the fetus and newborn occurs when maternal IgG alloantibodies cross the placenta and destroy fetal erythroid cells carrying the corresponding antigen. While RHD alloimunization was the most prevalent and often the most severe form, routine prophylaxis with anti-D immunoglobulin to antigen negative mothers shifted the prevalence of alloimunization to other erythroid antigens. The risk to develop severe disease with other antibodies, with a few notable exceptions, is much lower.

Aims: To review a series of consecutive pregnancies with non RHD alloimunization and their clinical management and outcomes.

Methods: Clinical and laboratory electronic records of pregnancies resulting in live births occurring from January 2021 to December 2022 were searched for alloimunization. In cases with positive antibody screening other than RHD, titre levels, mother and newborn ABO, newborn direct antiglobulin test and development of haemolytic disease of the fetus and newborn were recorded. In cases with titre above 1/16 (or in any anti-K titre), Doppler ultrasound to assess the fetal middle cerebral artery peak systolic velocity (MCA-PSV) records were reviewed, as a surrogate for fetal anaemia.

P403 - Table 1. Pregnancies at risk of Haemolytic Disease of The Fetus and Newborn

Antibody specificity	Previous Gestations/ Deliveries	Highest titre	Newborn Direct Coombs Test	Haemolytic Disease of The Fetus and Newborn
M	G0D0	1:64	0	No
M	G5D4	1:32	0	Mild
E	G8D3	1:32	0	No
c	G2D1	1:128	+3	No
S	G2D0	1:128	0	No
E	G2D2	1:32	0	No
S	G3D2	1:128	+4	No
S	G2D1	1:512	+4	No
K	G3D2	1:2	0	No

Results: A total of 5831 pregnancies occurred during the period of this study, with 28 women developing non RHD alloimunization, resulting in an incidence of 0.48%. The most common antibody specificities were in the MNS and RH blood groups: 7 anti-M, 6 anti-E, 4 anti-S, 3 anti-C, 2 anti-C^w, 1 anti C+e and 1 anti-c. Four patients developed antibodies in the Kell and Kidd blood groups: 2 anti-K, 1 anti-Jk^a and 1 anti-Jk^b. All gestations were carried to term.

Nine of these cases were first-time pregnancies: 6 anti-M, 1 anti-E, 1 anti-C and 1 anti-C^w. With the exception of one case with anti-M with a 1:64 titre, all patients presented with titres of 1:2, negative Coombs test on the newborn and no previous alloimunization stimuli were identified. As such, these antibodies likely represented naturally occurring antibodies of the IgM isotype.

A total of 10 patients were considered at risk during follow-up, due to a titre level >1/16 or presence of antibodies in the Kell group. One patient who presented with an anti-K of 1:1024 had been previously sensitized by erythrocyte transfusion. The father was evaluated and was found to be K-negative. Details of the remaining 9 at risk pregancies are presented on table 1. The other women in this series had low titre antibodies (1:2), with no elevation during follow-up. All cases at risk of haemolytic disease of the fetus and newborn were assessed by Doppler scanning of the fetal MCA-PSV, with no evidence of fetal anaemia. The only newborn with evidence for disease was submitted to phototherapy with no severe complications during follow-up.

Summary/Conclusions: Non-RHD alloimunization is an uncommon event during pregnancy and it more often occurs in multiparous women. Mothers should be assured of the low rate of complications to prevent unnecessary anxiety. Antibody screening and appropriate follow up is essential to provide optimal care during the pregnancy and perinatal periods.

P404 | HDFN ABO incompatibility is caused by the ABO IgG subtype in pregnant women with blood group O who birth infants with non-O blood groups.

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Background: Immune and non-immune factors can induce HDFN; nevertheless, incompatibility of blood groups such as IgG ABO is one of the most common reasons. Haemolytic disease of the fetus and newborn (HDFN) IgG ABO causes anaemia in the fetus or infant. ABO incompatibilities arise when the mother and child have different blood groups, causing the mother's immune system to recognise the fetus' red blood cells as foreign and produce specific antibodies. These antibodies infiltrate the fetal body and lyse red blood cells, resulting in anaemia, jaundice, and hyperbilirubinemia, and in extreme cases, brain damage that is fatal. Variations in antibody IgG subtypes are responsible for the varying clinical symptoms observed in HDFN.

Aims: To determine whether subtype of ABO IgG antibody is responsible for hyperbilirubinemia and jaundice in infants born from women with blood type O

Methods: The sample includes the blood of pregnant women with blood type O who are married to men with blood types other than O, as well as the umbilical cord blood of thirty newborns with different blood groups than their mothers. ELISA was utilised to analyse ABO IgG subclasses, whereas the tube method was used for titration analysis. We utilised control for infants without jaundice.

Results: Subtype of IgG1, IgG3 anti-A and B had a significant effect on hyperbilirubinemia in infants ($p = 0.032, 0.018, 0.027, 0.029$) and IgG4 anti-A and B had a nonsignificant effect on hyperbilirubinemia in infants ($p = 0.63, 0.54$)

Summary/Conclusions: IgG1 and IgG3 play a higher part in triggering clinical issues than other subtypes of IgG. IgG4 has a function in inhibiting IgG activation so that infant red blood cells are not destroyed.

P405 | Parity and gravidity are risk factors for anti-HPA-5b immunisation and not for anti-HPA-1a immunisation

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Background: Fetal and neonatal thrombocytopenia (FNAIT) is most commonly caused by maternal Human Platelet Antigen (HPA) type 1a antibodies that can be transported to the fetal circulation, destruct fetal platelets and cause thrombocytopenia and bleeding complications in an antigen-positive fetus or newborn. In our recent case series, anti-HPA-1a was found in 85% of suspected FNAIT cases and led to clinically relevant disease in first pregnancies (Vos, BJH, 2021), followed by anti-HPA-5b, found in 15% of suspected FNAIT cases, often after a previous pregnancy.

Aims: To compare, in a prospective study of pregnant women, the occurrence of HPA-1a antibodies and HPA-5b antibodies in first and later pregnancy.

Methods: Data obtained with the HPA-screening In Pregnancy (HIP) study was used. Between 1-3-2017 and 1-5-2020, women that participated in the routine red cell antibody screening at the 27th week of pregnancy were asked for consent to be typed for HPA-1a, platelet antibodies and clinical data collection in ProMISe. For every HPA-1a negative woman, 3 HPA-1a positive women were selected at random as control group. HPA-1a negative women were screened for anti-HPA-1a by PAKLx (Lifecodes, WI). The control group was screened for anti-HPA-5b, irrespective of their HPA-5b type, using the monoclonal antibody based immobilization of platelet antigens assay (MAIPA) and positive reactions were specified by PAKLx assay.

Results: This study included 3659 pregnancies from 913 HPA-1a negative and 2746 HPA-1a positive women. Anti-HPA-1a was detected in 85 pregnancies (85/914; 9.3%) and anti-HPA-5b in 63 of 3672 women, one woman showed both type of antibodies. Since 84% is HPA-5b negative in the Dutch population, the frequency of anti-HPA-5b in HPA-5b-negative pregnant women was calculated to be 2%. As the frequency of HPA-5b positive fetuses is more than 10-fold lower than the frequency of HPA-1a positive fetuses, HPA-5b is more immunogenic than HPA-1a.

The percentage of women in their first pregnancy (primigravid) with anti-HPA-1a was 32% (26/81) and not significantly different from the frequency of first pregnancies in the non-immunised group: 1236/3587; 34% ($P = 0.458$). The percentage of primigravidae was significantly lower in HPA-5b immunised women: 4/63; 6% versus 1232/3524; 35% (P<0.001). Similarly, no significant difference in nulli-parity was seen between the HPA-1a immunised and non-immunised pregnancies: 41% vs. 44%, $P = 0.645$, whereas there was a significant difference in nulli-parity between the HPA-5b immunised and non-immunised pregnancies: 16% vs. 44%, P<0.001.

Summary/Conclusions: Our results show a striking significant difference in both gravidity and parity for HPA-1a versus HPA-5b immunised pregnancies. Unlike in HPA-1a immunised pregnancies, HPA-5b immunised pregnancies are more often multigravidae and multipara pregnancies compared to non-immunised pregnancies. Because the samples were taken at the 27th week of pregnancy these findings suggest that HPA-1a immunization can already actively occur during pregnancy, whereas HPA-5b immunisation is occurring mostly after the 27th week of gestation, possibly during delivery. Integrin beta-3, the carrier of HPA-1a/b has high expression on platelets and trophoblast cells, whereas alpha-2, the carrier of the HPA-5a/b system, is expressed on platelets and only on a subset of trophoblast cells. Therefore, the maternal immune system is possibly less exposed to HPA-5b than HPA-1a during pregnancy.

P406 | Anti-U in pregnancy and haemolytic disease of the fetus and newborn: a case series and literature review

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Background: Anti-U is a clinically significant red cell antibody against the high prevalence U antigen. Management of anti-U in pregnancy or haemolytic disease of the fetus and newborn (HDFN) is therefore challenging as U antigen negative (U-) blood is rare. Anti-U in pregnancy is infrequently reported with only case reports and one case series published to date. Anti-U causes a variable degree of HDFN, ranging from no symptoms to significant anaemia requiring transfusion. The literature has not identified a definitive anti-U titre that is associated with earlier onset of HDFN in the antenatal period or need for neonatal transfusion.

Aims: By analysing a small case series and performing a corresponding literature review, we aim to identify if there is a definitive anti-U titre that may predict risk of HDFN in the antenatal period and need for neonatal transfusion. The transfusion approaches adopted in HDFN secondary to anti-U will also be outlined to aid clinicians managing cases with this rare red cell requirement.

Methods: Four cases of anti-U in pregnancy were identified from Australian Red Cross Lifeblood (Lifeblood). A literature review using PubMed and MEDLINE identified 12 case reports and 1 case series between 1976-2021. Pearson correlation coefficient was used to assess for a correlation between higher anti-U titre and antenatal onset of HDFN or need for neonatal transfusion.

Results: Higher anti-U titres appeared to be associated with earlier onset of HDFN in the antenatal period, however only a small correlation of 0.129 ($p = 0.61$) was observed. Higher anti-U titres were moderately correlated with need for neonatal transfusion with correlation coefficient of 0.316 ($p = 0.187$). Antenatal HDFN and intrauterine transfusion (IUT) could occur with anti-U titres >1:128. Fresh or

cryopreserved U- red cells or maternal directed donations were safely used for neonatal transfusion in HDFN from anti-U.

Summary/Conclusions: Pregnancies with anti-U titres >1:128 should be closely observed due to potential for antenatal HDFN or neonatal transfusion. Maternal directed donations or cryopreserved deglycerolised red cells, which are traditionally not preferred for neonatal transfusion, may be safely used in cases of HDFN secondary to anti-U when fresh units are unavailable.

P407 | Poor fetal and neonatal outcomes due to inadequate immuno-hematology management of pregnancies: a French national survey

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Background: Currently in France, the management of immuno-hematology (IH) testing during pregnancy follows guidelines detailing the tests to perform (blood group and Rh Kell phenotyping, antibody screening tests, and, in case of screening test positivity: antibody identification and quantification (titration) tests) and when to perform them. However, there are no national recommendations based on the results to guide the monitoring of pregnancies and assessment of the risk of haemolytic disease of the fetus and newborn (IH follow-up and ultrasound monitoring by measurements of the peak systolic velocity in the fetal middle cerebral artery (MCA-PSV)). A working group created in 2018 within the Société Francophone de Transfusion Sanguine (French-speaking Society of Blood Transfusion (SFTS)) aims to propose such guidelines, thanks to a review of current practices and the literature.

Aims: We conducted a retrospective, national survey to identify and document cases where the management of IH testing during pregnancy failed significantly between 2010 and 2020, to provide an overview of the main errors and pitfalls to avoid.

Methods: A questionnaire containing the main points of the IH monitoring from which the inadequate management could originate, was drawn up by the members of the working group. The survey was circulated to their networks by major blood banks (Etablissement français du sang network (EFS)), haemovigilance coordinators (from hospitals and regional government bodies, the Agences Régionales de Santé (ARS)) and the Centre National de Référence en Hémobiologie Périnatale (French National Reference Center in Perinatal Haemobiology (CNRHP)). For each case, the questionnaire was anonymized and filled out online by a member of the working group together with the person (physician, haemovigilance coordinator, or medical biologist) who reported the case of IH management failure.

Results: Over the 10-year period, 26 cases were reported. Anti-D (anti-RH1) (54%) and anti-c (anti-RH4) (31%) were the main allo-antibodies involved, with fetus or newborn morbidity (hearing loss or neurological sequelae) or mortality in 23% of cases. In 46% of cases, the antibody was detected too late due to non-compliance of the patient with the recommended antibody screening schedule, or identified too late due to delayed positivity of antibody screens for anti-c or misinterpretation of allo-immune anti-D as prophylactic anti-D by the laboratory. Other common causes were inappropriate medical advice provided with the IH results (31%) and failure to communicate pathological results to obstetricians or pediatricians (31%). In 92% of cases, the causes of poor management were multifactorial, also involving poor patient compliance and/or poor clinical surveillance.

Summary/Conclusions: Although probably not exhaustive, this survey highlights major risks and identifies areas for improvement, on which future national recommendations for the IH management of pregnancy should focus.

P408 | Haemolytic disease of the newborn (HDN) caused by anti-P1 passed from mother: A case report

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Background: Here, we report a rare haemolytic disease of the newborn (HDN) case caused by anti-P1 antibody passed from mother.

Aims: The aim of this presentation is to share our experience with experts worldwide.

Methods: A female baby whose gestational age took 36+1(weeks) was born in a local clinic. At birth, her body weight and Apgar score were 2.55kg and 9(of 10), respectively. However, her total bilirubin concentration in capillary blood increased up to 23.1(mg/dl), so be transferred to our institution. On initial laboratory findings in our institution, her complete blood cell count was 12.48($\times 10^3$/μl)-14.8(g/dl)-239($\times 10^3$/μl), and total/direct bilirubin concentrations were 19.3(mg/dl) and 1.1 (mg/dl), respectively. Moreover, her direct/indirect coomb's test were 3+/2+, respectively. Following antibody screening/identification test

reveals anti-P1 in her plasma, even though accompanying antigen profiling test reveals P1 antigen on her red blood cell. In a clinical impression that this antibody could originate from her mother's blood, subsequent antibody screening/identification test with her mother's blood reveals also anti-P1 in her mother's plasma.

Results: On hospital day 1 (HD 1), A baby began phototherapy without any transfusion. Until HD 3, her total bilirubin concentration had declined to 9.1(mg/dl), so further phototherapy stopped. And until HD 6, her total bilirubin concentration had sustained below 10.0 (mg/dl) although her direct/indirect coomb's test had sustained at 3+/2+, respectively. With clinical judgement that it would not get much worse, she was tolerably discharged on HD 15 and started outpatient department follow-up.

Afterward, she had visited our institution per one-to-two weeks and showed no significant problem. Currently, about 88-days has gone since she was born, her total/direct bilirubin concentrations were 0.29 (mg/dl) and 1.1(mg/dl), respectively. Moreover, her direct/indirect coomb's test had declined to 1+/±, respectively. It suggested that the antibody in fetus passively passed from mother get diminished especially when there was no additional exposure.

Summary/Conclusions: Usually, anti-P1 is known as naturally-occuring IgM antibody which does not pass placenta. Moreover, P1 expression could be very weak in fetal RBC, anti-P1 may not react with those. Through this case, we could figure out the existence of anti-P1 in both newborn and mother's plasma. Furthermore, we could observe that the baby became improved even without additional specific therapy. HDN caused by Anti-P1 passed from mother seems not to be serious if adequate initial intervention precedes.

P409 | Follow-up with titration in pregnancy for suspected allo-anti-HPA5b FNAIT: Case study

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Background: FNAIT is mainly caused by the platelet-specific antigens (Ag) HPA1a but other Ag such as HPA5b may also be involved. It is known that allo-Ab titration is performed during pregnancy, mainly for anti-HPA1a in order to monitor any increase and quickly activate appropriate therapy for the prevention of fetal damage such as ICH. Below we describe a case of a patient studied in two successive pregnancies with anti-HPA5b Ab titre evaluation

Aims: The aim of this study was to monitor and titrate the anti-HPA5b allo-Ab found in a woman in her second pregnancy with a previous abortion for ICH.

Methods: Investigations performed: Screening (ST) with and without chloroquine (SPRCA Capture-P Ready Screen Immucor). Identification of specificity (IT) (Pak plus Immucor ELISA). Identification of anti-HLAI Ab (Luminex technology). Molecular typing of HPA Ag (HPA BeadChip array technology). Titration of anti-HPA5b Ab (SPRCA Capture-P Ready Screen Immucor). Platelet cross-match (CM) (Capture-P Immucor).

Results: In May 2021 comes to our attention for suspected allo-immunization in pregnant woman with fetal abnormality. No allo-Ab nor HPA nor HLA I was detected. CM test between maternal serum and paternal PLT was compatible. However, molecular typing of the spouses was performed which results in: mother: HPA1a/1a, 2a/2a, 3a/3a, 4a/4a, **5a/5a**, 6a/6a, 9a/9a, 15a/15b, father: HPA1a/1a, 2a/2a, 3a/3a, 4a/4a, **5a/5b**, 6a/6a, 9a/9a, 15a/15b. The risk of anti-HPA5b immunization in subsequent pregnancies was highlighted. In April 2022 the woman was pregnant at 12 w. Having found positive for allo-Ab anti-HPA5b, it was decided to monitor her with ultrasound and titration every 3 weeks. Ultrasound scans were always negative. Tests showed: platelet-adherent Ab: neg, ST pos, IT pos anti-HPA5b, anti-HLAI Ab: anti-HLA A2 (MFI 5273), B51 (MFI 1602), B52 (MFI 1088) and B63 (MFI 1085). CM mother serum vs father PLT: pos, mother serum vs son PLT: pos, mother serum vs 6 random donors: 04/06 COMPATIBLE. Titration of anti-HPA5b Ab: 19/04/2022: **1:64**; 10/05/2022: **1:64**; 31/05/2022: **1:128**; 21/06/2022: **1:128**; 12/07/2022: **1:128**; 02/08/2022: **1:128**; 23/08/2022: **1:128**; 12/09/2022: **1:128**. Molecular typing of son's Ag HPAs: HPA1a/1a, 2a/2a, 3a/3a, 4a/4a, **5a/5a**, 6a/6a, 7a/a, 8a7a, 9a/9a, 11a7a, 15a/15th. Platelet count of the newborn at T0 358,000 PLT/µl and at 72h old 354,000 PLT/µl.

Summary/Conclusions: No allo-immunization was evidenced in the woman's serum at the first pregnancy, and it was only through molecular typing that we were able to alert clinicians to perform close monitoring of woman in case of new pregnancy. At the second pregnancy, the appearance of anti-PLT antibodies anti-HPA5b was detected with titre that, over time remained at 1:128. The presence of anti-HLAI Ab did not interfere in titration tests for a titre below the threshold. At the baby's birth considering the ultrasound findings, his molecular typing and platelet count, we were able to rule out a FNAIT. Although FNAITs from HPA5b are not considered the most severe, this monitoring, in close collaboration with clinicians, allowed us to avoid a hypothetical FNAIT

P410 | Diagnosis of neonatal alloimmune thrombocytopenia and identification of causative antibodies in Korea

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Background: Neonatal alloimmune thrombocytopenia (NAIT) is a major cause of severe thrombocytopenia in the fetus and neonate, and is caused by maternal antibodies against fetal platelet antigens, such as human leukocyte antigen (HLA) and human platelet antigen (HPA). In Caucasian populations, the frequency of the HPA-1a negative phenotype is approximately 2.5% and anti-HPA-1a is the most common cause of NAIT. However, the HPA-1a-negative phenotype

frequency in Korean population is less than 1%, and common causative antibodies of NAIT in Korea have not been investigated.

Aims: This study was conducted to diagnose NAIT in neonates with thrombocytopenia, investigate the causative antibodies, and propose efficient diagnostic tools of NAIT in Korea.

Methods: As a part of the Korean Rare Blood Program, samples from neonates suspected of having NAIT and their parents were sent to the laboratory of Seoul National University Bundang Hospital for evaluation. Anti-HLA antibodies were identified by single antigen bead-based multiplex antibody assay. To determine whether the identified anti-HLA antibodies correspond to neonatal HLA antigens, HLA genotyping was performed by bead-based reverse polymerase chain reaction-sequence specific oligonucleotide. To identify anti-HPA antibodies, firstly, HPA-1 to HPA-17 genotyping was performed by sequencing. Then the specificity of anti-HPA antibodies suspected to have caused the NAIT were predicted by comparing the genotypes of neonates, mothers, and fathers. The presence of anti-HPA antibodies in maternal serum were confirmed by platelet crossmatching based on solid phase technology using platelet rich plasma from neonates and/or several numbers of random platelet concentrates.

Results: Among 24 cases available for analysis from July 2013 to January 2023, there were 8 cases of anti-HLA and 7 cases of anti-HPA. Identified anti-HLA included anti-A2, anti-A29, anti-B8, anti-B44, anti-B51, anti-B61, anti-B62, and anti-A24 with anti-B39. Identified anti-HPA included anti-HPA-3a, anti-HPA-3b, anti-HPA-15a (3 cases), and anti-HPA-15b (2 cases). Notably, in a case with anti-HPA-15b, crossmatching using five random platelet concentrates and maternal serum showed positive in 80% (4 out of 5 platelet concentrates); the frequency was similar to the known HPA-15b positive phenotype frequency in Koreans (76.2%). Of the remaining 9 cases, anti-HPA was suspected in 2 cases but inconclusive, one case was suspected of anti-CD36, and 6 cases had no evidence of maternal antibodies against neonatal platelet antigens.

Summary/Conclusions: A total of 15 cases with NAIT due to anti-HLA or anti-HPA were diagnosed and causative antibodies of NAIT were identified using HLA/HPA genotyping, the bead-based antibody assay, and platelet crossmatching. The specificities of frequently identified antibodies were different from in Koreans and in Caucasians, which should be taken into account when establishing a screening algorithm for NAIT.

P411 | Prevalence of unexpected red blood cell antibodies in pregnant women and follow-up of pregnancy outcome in pregnant women treated with Intra-uterine Transfusion (IUT)

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Background: For the management of haemolytic disease of the fetus and newborn (HDFN), it is important to detect unexpected red cell antibodies in pregnant women. We assessed the prevalence of unexpected red cell antibodies in consecutive pregnant women attending antenatal clinics (ANC). More importantly, cases with unexpected antibodies causing severe anaemia were followed-up for intervention (Intra-uterine transfusion {IUT}) and outcome of pregnancy (still-birth/live-healthy).

Aims: Primary: To find the prevalence of unexpected RBC antibodies in pregnant women

Secondary: To find out the specificity of unexpected antibodies and to do the follow-up for IUT and outcome of pregnancy (still-birth, live-birth) in antibody-positive women

Methods: This was a prospective study from January 2021 to May 2022 at two tertiary care centers. All antenatal samples received by the laboratory were screened for unexpected red cell antibodies. Whenever the antibody screen was positive, antibody identification was performed. Patients, who positive for unexpected antibodies and anaemia were followed up for any transfusion-based intervention and outcome of pregnancy.

Results: A total of 539 consecutive samples were worked up and among these, 10 samples (1.85%) were found to be antibody positive. The antibodies identified were Anti-D ($n = 6$), anti-Le^b ($n = 1$), anti-M ($n = 1$), anti-C ($n = 1$), and anti-E ($n = 1$). The prevalence of unexpected antibodies in Rh-positive and Rh-negative pregnant women was 0.83% and 10.9% respectively. Follow-up was done for all 10 cases with unexpected antibodies and anaemia was monitored by MCA PSV (middle cerebral artery peak systolic velocity). Two women developed severe anaemia thus requiring a single intrauterine transfusion (at 26 weeks and 28 weeks respectively) each, for correction of anaemia. In both these cases, a healthy male child was delivered. At the 3-month follow-up, both children were alive and healthy.

Summary/Conclusions: The study found the prevalence of unexpected RBC antibodies in pregnant women as 1.85 %. The study also underlined the importance of transfusion-based interventions contributing to a successful outcome in a couple of cases with severe anaemia.

P412 | Quantitative feto-maternal haemorrhage assesment with the use of cytometry and microscopy after cesarean section for twins

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Background: Quantification of feto-maternal (FMH) is very important for determination of an accurate dose of anti-RhD Ig immunoprophylaxis of haemolytic disease of foetus and newborn. In various countries, doses of anti-D Ig vary from 100 µg to 300 µg. There are different regulations concerning FMH analysis, and opinions about different tests (flow cytometry, Kleihauer -Betke, serological test) are inconclusive. Findings of authors, concerning fetal red cells quantification after cesarean delivery of twins were different.

Aims: Evaluation of fetal erythrocytes in maternal blood with the use of cytometry and microscopy methods after cesarean delivery of twins.

Methods: 15 blood donors and 15 cord blood samples were collected to preapre mixtures imitating FMH (0.1%, 1%). Blood samples from 30 woman were tested (15-after cesarean delivery of twins, 15-single pregnancy). Two flow cytometry tests with anti-HBF, anti-HbF + CA, anti-GPA antibodies, and modifications of microscopic Kleihauer-Betke (supplemented DAPI staining) test were used.

Results: Results obtained by cytometric (anti-HbF, anti-HbF + CA, anti-GPA) and microscopic (with DAPI staining) in samples from mothers didn't differ significantly. Percentage of fetal red cells was < 0.1% and ranged from 0.01 to 0.08% in mother samples after cesarean delivery of twins. Percentage of fetal red cells was similar after single pregnancy.

Summary/Conclusions: Methods with the use of flow cytometry and anti-HbF, anti-HbF + anti-CA antibodies as well as microscopic modified Kleihauer-Betke test detecting fetal red cells, have similar satisfying sensitivity and specifity. Using these methods, we obtained an objective results for analysis of FMH. The application of anti-GPA specific to erythrocytes and DAPI specific to nucleu s allowed the clear separation of erythrocytes and more accurate and the precise qualitative analysis.

P413 | Incidence of anti-G antibodies mimicking anti-C+D antibodies in alloimmunised pregnancies in the Regional Blood Center Poznan in 2017-2022

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Background: The G antigen which was first described in 1958 by Allen and Tippet is one of the components of Rh blood group system. It is produced by RHD gene and RHCE gene and appears on D-positive and/or C-positive red blood cells. It is highly immunogenic. The discovery of the G antigen helped explain the presence of anti-D and anti-C antibodies in sera of pregnant women immunised during pregnancy despite the absence of antigen C on RBCs of newborns. The identification of anti-G antibodies is difficult because it follows the same pattern as anti-D+C and sometimes coexists with either anti-D or anti-C.

Aims: Retrospective frequency analysis of anti-G antibodies mimicking anti-C+D identified in sera from alloimmunised pregnant women in the context of appropriate management of haemolytic disease of the fetus and newborn (HDFN).

Methods: In the Regional Blood Centre Poznan the identification of antibodies is performed routinely for women with positive screening for red blood cell antibodies. This analysis includes studies of immunised women for whom the identification was done from Jan. 2017 to Dec. 2022. Sera from alloimmunised women, initially identified as containing anti-C+D antibodies, were analysed by adsorption/elution studies by both D+C-G+ and D-C+G+ red blood cells. The identification of alloantibodies was performed using a commercial identification panel combined with gel-microtubes. Sera were tested in: Indirect Antiglobulin Test (IAT) and Enzyme Test (using papainized cell reagents). Eluates were tested in IAT only.

Results: In the analysed period, alloantibodies were identified in 573 pregnant women, 228 of which were RhD-negative (40%). In 74 cases out of 228 RhD-negative immunised women anti-D antibodies were identified and 38 initially identified as containing anti-C+D antibodies. Anti-G antibodies were identified in 6 out of 38 samples analysed by adsorption/elution studies. Apart from anti-C+D (32 samples), anti-G+C (4 samples), anti-G+D (1 sample), and anti-G+D+E (1 sample) were identified. 3 out of 4 women with anti-G+C received anti-D immune globulin during pregnancy and postpartum.

Summary/Conclusions: For routine transfusion the differentiation is not needed. But it is crucial to distinguish anti-G from anti-D+C during antibody identification among pregnant women, female children and women of maternal age. The right identification of anti-G (with exclusiuon of anti-D antibodies) gives the opportunity for patients to receive anti-D immune globulin prophylaxis to prevent RhD HDFN.

Anti-D and anti-C are regarded as ones causing more serious HDFN than anti-G.

Incorrect antibody identification of anti-D+C in a RhD-negative couple (a woman and her partner) may have serious consequences in the course of the HDFN. Unfortunately the number of reported cases of anti-G and antiG+C immunization is quite low. More data may help understand in a better way the clinical relevance of these antibodies.

A large percentage of RhD-negative women (inconsistent with the population distribution) with identified antibodies shows clearly more frequent screening for RBC antibody in RhD-negative than RhD-positive women.

Relatively high immunization with the RhD antigen in RhD-negative women (32% of immunised women), despite widespread anti-D immune globulin prophylaxis, indicates the necessity of analysis of the reasons for the failure of administration anti-D immune globulin and the failure of prophylaxis.

P414 | Study of various clinical, serological parameters on the early prediction and diagnosis of haemolytic disease of newborns due to ABO incompatibility

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Background: Only a few cases of ABO incompatible neonates are diagnosed as having significant ABO haemolytic disease of Newborn (ABO-HDN). Rapidly developing hyperbilirubinemia is common, but severe anaemia is rare. Early identification of the clinical features may aid in early diagnosis when combined with laboratory parameters.

Aims: The primary objective was to analyse the laboratory and clinical parameters to determine the neonatal outcome in neonates born to O blood type mothers with ABO feto-maternal incompatibility.

Methods: This prospective observational study was conducted in a tertiary care centre for one year and included all O blood type mothers and their neonates. The ABO RhD typing, IgG anti-A/B titration and antibody screening were done for all mothers. The non-O group neonates were followed up. The ABO RhD typing, Direct antiglobulin test (DAT) were performed on the neonates' samples. Heat elution was performed on DAT-positive samples. Records of clinical management, Complete Blood Count, Total Bilirubin (TB) and any other relevant investigations were evaluated. ABO- HDN was defined based on different criteria.

Results: The neonates born to O blood type mothers ($n = 213$) were divided into various groups and analysed for laboratory parameters like Haemoglobin (Hb), TB and clinical parameters like anaemia, hyperbilirubinemia, phototherapy as in table 1. A positive correlation was noted between TB in newborns and maternal IgG Anti-A/B titres in ABO incompatible newborns. For 1-unit increase in TB, IgG Anti-A increased by 12.68 units and IgG Anti-B increased by 15.76 units. There was a significant association between DAT positivity and TB ($p = 0.002$). 35.2% of ABO-incompatible neonates received phototherapy. The ABO-HDN cases received more duration of phototherapy (p value $= 0.013$).

Summary/Conclusions:

ABO incompatible babies were at a higher risk of anaemia. Mild anaemia was present in 69% of ABO-HDN cases, but there were no cases of severe anaemia. ABO-incompatible neonates had higher mean TB, which has been confirmed by similar studies. A linear association between maternal IgG titres and TB was observed in ABO-incompatible neonates. Prophylactic phototherapy in these ABO-incompatible neonates was associated with a significant reduction in TB. The combination of both laboratory and clinical parameters has improved diagnostic accuracy in disease prediction. As there is no single test that may predict the severity or outcome in ABO-HDN, the approach of performing a combination of multiple tests may be beneficial.

P414 - Table 1: Description of laboratory/clinical parameters in study population

Parameters	Group 1 ABO compatible neonates ($n = 91$)	Group 2 (i) ABO incompatible neonates without hyperbilirubinemia ($n = 75$)	Group 2(ii) ABO incompatible neonates with hyperbilirubinemia but no HDN ($n = 34$)	Group 2(iii) ABO incompatible neonates with hyperbilirubinemia and ABO-HDN ($n = 13$)
Hb (g/dl) Mean(±SD)	16.3 ± 2.93	16.58 ± 2.83	16.05 ± 4.04	15.08 ± 4.15
% Neonates with anaemia	16.4%	29.3%	35.1%	69.2%
TB (mg/dl) Mean (±SD)	7.188 ± 3.249	6.89± 2.734	12.22 ± 4.04	12.986 ± 3.422
% Neonates with Hyperbilirubinemia	17.5%	36.8%		
% Neonates that received Phototherapy	14.2%	12%	82.%	69.2%
Phototherapy duration(in hours)	28 ± 16.4	-	30 ± 18.2	22.6± 14.01

P415 | Non-invasive *KEL*01.01* genotyping with estimation of fetal fraction using multicolor digital PCR

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Background: During pregnancy K-negative women may produce anti-K antibodies against fetal K antigen from Kell blood group system which leads to haemolytic disease of fetus and newborn (HDFN). In this setting, non-invasive prenatal testing (NIPT) of the fetal *KEL*01.01* from maternal plasma is a valuable tool to identify high-risk pregnancies. A confirmation of the fetal *KEL*01.01* negative results is extremely important in the diagnostics of immunised women.

Aims: To establish a multicolor droplet digital PCR (ddPCR) protocol for fetal *KEL*01.01* genotyping together with fetal and total cfDNA fraction.

Methods: DNA from plasma samples of 7 pregnant women with anti-K antibodies (16 to 26 week of gestation, anti-K titre 1:8-1024) and 3 donors (K/k, k/k, 5%K+ /SRY+ spike-in control) was extracted using easyMag (Biomerieux) and used to determine *KEL*01.01*, *KEL *02* alleles and *SRY* gene in triplex assay (with FAM, VIC and Cy5- labelled probes) using Naica 6-color ddPCR (Stilla). The results were compared to fetal/neonatal *KEL*01.01* genotypes and sex.

Results: The ddPCR results of *KEL*01.01/*02* and *SRY* genotyping were concordant with the genotype of donors, pregnant women, and their children. Total cfDNA fraction in 7 pregnant women was 368-1428 *KEL*02* positive events (corresponding to 855-3422 copies/ml of plasma). In 3 cases of pregnant women carrying K-positive fetuses the fetal *KEL*01.01* fraction was 7-73 positive events (corresponding to 16-169 copies/ml of plasma). In 4 cases carrying K-negative fetuses no *KEL*01.01* positive events were observed. In 6 cases with male fetuses 9-41 *SRY*-positive partitions (corresponding to 21-97 copies/ml of plasma) were detected whereas in 1 case carrying female fetus no *SRY*-positive partitions were detected.

Summary/Conclusions: The multicolor ddPCR triplex assay determines fetal *KEL*01.01* genotype as well as fetal (*SRY*) and total (*KEL*02*) cfDNA fractions which allow to confirm *KEL*01.01* negative status for male fetuses during the same experiment. However, for female fetuses NIPT requires further standardization of multicolor ddPCR assays for ins/del polymorphisms.

P416 | Abstract withdrawn

P417 | Intravenous immunoglobulin treatment in a woman with high anti-B titre and a history of previous anaemia of the newborn

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Background: Haemolytic disease of the fetus and newborn (HDFN) is caused by transplacental passage of maternal immunoglobulin G (IgG) antibodies, which bind to paternally inherited antigens present on fetal red blood cells (RBCs). ABO incompatibility between the mother and fetus is present in about 20% of pregnancies, yet only 1-4% develop HDFN, which rarely causes severe fetal anaemia.

Aims: To present a case with a high titre maternal anti-B treated with intra venous immune globulin (IVIG).

Methods: Antibody titres were performed manually or on the Vision (Ortho™) using commercial B cells.

Paternal ABO genotyping was done using Inno-train Diagnostik GmbH kits at Magen David Adom National Blood Services. Fetal ABO by cell-free fetal DNA (cffDNA) was determined at the department of Clinical Immunology, Copenhagen University Hospital, Denmark.

Peak systolic velocity in the middle cerebral artery (PSV-MCA) was performed using ultrasound doppler.

Results: A 33 year-old, gravida 4 para 2 woman presented for preconception counseling. Her blood type was O RhD positive and the father's blood type was B RhD positive, genotype *BO*. Her first child had neonatal anaemia (Hb 12.3 g/dL) and hyperbilirubinemia (9 mg/dL) for which he was treated with phototherapy for 8 days. Her second pregnancy ended in spontaneous abortion at 7 weeks of gestation. Her third pregnancy required induction of labor at 37 weeks. Neonatal haemoglobin was 11.8 g/dL and phototherapy, IVIG administration and exchange transfusion were required. Maternal anti-B titre was 16,000 at 34 weeks in her third pregnancy, 64,000 postpartum and 32,000 prior to her current pregnancy.

Pre-implantation genetic diagnosis was offered but the patient elected to conceive spontaneously.

Fetal blood type was *BO* by cffDNA. Maternal anti-B titre increased to 128,000 at 12 weeks of gestation. Because of the high anti-B titre early in pregnancy, weekly IVIG 1gr/kg was initiated at week 15. Following IVIG, anti-B titre decreased and remained stable until 35 weeks. PSV-MCA measurements showed mild anaemia at 16, 17 and 24 weeks. At 33+5 weeks Doppler assessment of the umbilical artery and MCA pulsatility index were normal, but PSV-MCA was 66-71 cm/second (1.46-1.52 MOMs). Corticosteroids were given. Ultrasound assessment of the fetal heart was normal and there were no signs of hydrops. Two further

Doppler assessments demonstrated normal PSV-MCA. At 35-+1 weeks the anti-B titre increased to 32,000. At 35+5 weeks severe fetal anaemia was suspected (PSV-MCA 95 cm/second, 1.88 MOMs), without hydrops. Measurements on 2 consecutive days revealed PSV-MCA of 81 cm/second (1.5 MOMs, moderate anaemia) and at 36+1 weeks PSV-MCA was normal (61 cm/second, 1.17 MOMs). Labor was induced and at 37+3 weeks of gestation a boy weighing 2,830 gram, was delivered vaginally. Apgar score was 9 and 10 at 1st and 5th minutes. Neonatal haemoglobin at birth was 12.9 g/dL. The mother and her baby experienced no complications and were discharged at day 5 post-partum.

Summary/Conclusions: Little is known regarding the appropriate management of pregnant women with extremely elevated anti-B titres without evidence of severe anaemia in previous pregnancies. In our patient, weekly IVIG resulted in a decrease in anti-B titre which remained stable until week 32. At 37th week of gestation, the baby was delivered and no further therapy was required. The role of IVIG in delaying or mitigating ABO moderate/severe HDFN requires further investigation.

P418 | Abstract withdrawn

Clinical transfusion

Neonatal and paediatric transfusion

P419 | Using unexpected antibody titration to predict the haemolytic disease of the fetus and newborn

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Background: Haemolytic Disease of the Fetus and Newborn(HDFN) is life-threatening and even causes severe morbidity. identifying the maternal specificity of antibodies can find matching blood for intra-uterine or post-natal blood transfusion and exchange. However, there are still challenges to the antigen-negative blood supply in pregnant women with high-frequency antibodies. Effective and reliable risk prediction can contribute to assessment.

Aims: 1. To demonstrate different outcomes in 3 pregnant cases with high-frequency antibodies (anti-Jk3, anti-Tja, and anti-Dib)

2. To compare the results obtained by conventional tube technique (CTT) with CAT and solid phase red cell adherence (SPRCA)

3. To Compare the performance of titration between husband and panocell heterozygous antigen

Methods: We review the antibody titres of the three pregnant women who were admitted to the hospital before delivery and the health status of the newborn. The target antigen red blood cell uses the panocell and husband to titrate the titres respectively. In the CTT method, the plasma of pregnant women was treated with DTT and heat; SPRACA was conducted according to the operating instructions of Capture R select (Immucor. Inc. US), and the samples were not processed by DTT and heat.

Results: The first case: newborns born to pregnant women with anti-Jk3 received severe HDFN and were given 50 ml of Leukocyte poor RBC from the pregnant women's brother 16 hours after birth. The titre results showed that the CTT titre was 1:512 both in the husband and panocell; the SPRACA titre results showed 1:2048 in the husband and 1:1024 in the panocell. The second pregnant woman with anti-Tja born and a baby without HDFN. However, The CTT titre showed 1:128 in the husband and 1: 64 in panocell, compare to the SPRACA titre results showed 1: 16 both in the husband and panocell. The pregnant woman with anti-Dib was born with a baby with silent HDFN. The CTT titre showed 1:64 in the husband(Di(a-b+)) and 1: 16 in panocell(Di(a+b+)), compared to the SPRACA titre results showed 1: 8 both in the husband and 1:4 in panocell.

Summary/Conclusions: Both CAT and SPRCA have the same performance in serious HDFN risk prediction of high-frequency antibodies, whether using a husband or panocell heterozygous red blood cell. However, CAT has a higher dilution titre in newborns without HDFN, which may have the risk of a false positive. It still needs further analysis to evaluate the HDFN risk prediction performance of SPRCA for high-frequency antibodies

P420 | Abstract withdrawn

P421 | Abstract withdrawn

P422 | Transfusion of Amotosalen-UVA pathogen reduced platelet components to mature and premature neonatal infants

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Background: Platelet components (PC) prepared with amotosalen-UVA (A-UVA) pathogen reduction (PR) are indicated to reduce the risk of transfusion-transmitted infections (TTI) and transfusion-associated graft versus host disease (TA-GVHD). PRPC (INTERCEPT™, Blood System, Cerus BV, Amersfoort, Netherlands) were mandatorily implemented in Switzerland in 2011 for all patients. Clinical data on neonates are sparse.

Aims: We reviewed the use of PRPC for treatment and prophylaxis of bleeding in neonates before and after implementation of PRPC with a focus on concurrent PC transfusion and phototherapy for neonatal jaundice.

Methods: A retrospective review of hospital records for premature and term neonates (0-28 days old) transfused with PC was conducted for two cohorts: patients receiving conventional PC (CPC) in 72 months before (2005-2010) and PRPC in 57 months after (2011-2015) PRPC implementation. The standard transfusion thresholds for premature and term non-bleeding neonates were < 50 x 10^9/L and < 30 x 10^9/L, respectively. CPC were stored for up to 5 days and irradiated prior to transfusion. PRPC were not gamma irradiated and stored up to 7 days. Medical records were audited for: gestational age, birth weight, phototherapy, indication for PC transfusion, PC transfusions, pre-transfusion platelet count, post-transfusion platelet count, count increment, phototherapy treatment, and adverse events related to transfusions associated with phototherapy. P-values for the treatment difference are based on Fisher's Exact test and a 1-way ANOVA model, respectively, for categorical and continuous variables.

Results: 100 neonates received 234 PRPC and 91 received 171 CPC. In both cohorts, patients were dosed with 5 mL/kg of PC. PC platelet content was not measured at transfusion, but pre and post transfusion (1-4 hour) patient platelet counts were measured. Similar proportions of patients in each cohort had bleeding (central nervous system 18% vs 19%, lung 3% vs 3.3%) as the indication for PC transfusion. All other PC transfusions were prophylactic. The average gestational ages and birth weights were similar between cohorts. There were no substantial differences in the numbers of PC or FFP transfusions, but more RBC transfusions were reported in the CPC cohort. Similar proportions of patients required phototherapy in both periods (51%). Including the number phototherapy treatments as a covariate did not impact the observed difference in RBC transfusions between cohorts. Platelet count increments were within therapeutic ranges in both cohorts. No differences in adverse events related to PC transfusion and concurrent phototherapy were reported.

Summary/Conclusions: The data support the efficacy and safety of PRPC in neonates who require concurrent platelet transfusion and phototherapy for jaundice.

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P424 | Abstract withdrawn

P422 – Table 1

n = patients with data: Mean ± SD	PRPC (n = 100)	CPC (n = 91)	P-value
Gestation age (weeks)	32.8 ±5.3 n = 93	32.5 ± 5.0 n = 84	0.699
Birth weight (Kg)	1.8 ± 1.1 n = 100	1.8 ± 1.1 n = 91	0.649
Age at phototherapy initiation (days)	2.2 ± 2.9 n = 49	2.1 ± 2.6 n = 41	0.950
Number of phototherapies	3.8 ± 3.0 n = 51	2.3 ± 1.3 n = 46	0.002
Bleeding prior to first PC transfusion (%)	21%	22%	0.901
Number of PC transfusions	2.3 ± 2.9 n = 100	1.9 ± 1.2 n= 91	0.162
Pre transfusion platelet count (10^9/L)	53.2 ± 48.9 n = 100	42.5 ± 30.1 n = 91	0.074
Post transfusion platelet count (10^9/L)	130.4 ± 45.5 n = 100	137.8 ± 58.8 n = 91	0.330
Platelet count increment (10^9/L)	82.5 ± 45.0 n = 100	96.4 ±53.0 n = 91	0.052

P425 | CRP-response to predict granulocyte transfusion success

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Background: Granulocyte transfusion (GT) is favourable for some, but not for all patients. The production is demanding for both donors and the donation and service staff, and GT is a load for the patient despite its vital requirement for drug-refractory neutropenic sepsis.

Aims: We wanted to retrospectively identify patients that benefit most from GT.

Methods: Data on GT were collected from 17 adult and 46 paediatric patients who received 99 and 295 granulocyte concentrates in 2011/12 and 2012–19, respectively. Patients were investigated for CRP and blood counts before and during GT series.

Granulocyte concentrates were prepared with hydroxylethyl starch (adult patients) and with modified fluid gelatin (paediatric patients).

Results: Granulocyte contentrates for adult patients contained 5.1 × 10^10 granulocytes per unit (mean; range: 1.3–12.6 × 10^10) and were administered at a dose of 7.4 × 10^8/kg (mean; range: 1.5–18.4 × 10^8). Nine of the adult patients survived (53%) and had CRP-values of 89%, 75%, and 56% on day 2, 3, and 5 after first GT, respectively, relative to pretransfusion values. Patients, who did not survive, had CRP-values of 107%, 89%, and 100% on day 2, 3, and 5, respectively.

The paediatric patients received 3.4 × 10^10 granulocyes per unit (mean; range: 0.1–11.6 × 10^10) corresponding to 6.5 × 10^8/kg (mean; range: 0.3–81.8 × 10^8). Overall, 41 of the paediatric patients survived (89%). In them, CRP decreased by 12.0 mg/L per GT, while the decrease in non-survivors was 5.3 mg/L per GT.

Summary/Conclusions: Monitoring of GT through CRP and blood counts helps to identify patients who profit most from GT and those who require more intensive therapy.

P426 | Abstract withdrawn

P427 | Coagulation assays at birth in very preterm infants: lack of agreement with the literature

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Background: Available evidence on reliable reference values for coagulation assays in preterm infants is very limited, especially in those born below 32 weeks of gestation. Additionally, analysers and reagents used in different hospitals and neonatal intensive unit (NICU) may vary from those reported in the literature, which potentially limits the generalizability.

Aims: Our aim was to assess the coagulation test results on the first day of life (<24 hours after birth) in very preterm and extremely preterm infants admitted to our NICU and compare them with the reference intervals reported in the literature.

Methods: We conducted a single-center retrospective study of coagulation test results (PT (protrombin time) and aPTT (activated partial thromboplastin time)) at birth in very preterm and extremely preterm infants admitted to the NICU at the Leiden University Medical Center, Leiden, the Netherlands. All preterm infants with a gestational age (GA) < 32 weeks admitted to our department between January 1st 2004 and December 31st 2019 in which coagulation tests were obtained at birth were included. Coagulation testing at birth were not part of our standard care during this period and were only performed at the discretion of the attending physician. We excluded neonates diagnosed with major intraventricular haemorrhage (IVH ≥ Grade III) during the first 24 hours of life and neonates who received plasma transfusion prior to the coagulation assay. Neonates in whom the coagulation test results were greater than the upper limit of testing were also excluded, in accordance with Neary et al. (2013). Coagulation ranges will be presented using 5th to 95th percentile, stratified GA

P427 - Table 1. Coagulation ranges at birth

	Infants with GA at birth below 28 weeks ($n = 33$)	Infants with GA at birth between 28 and less than 32 weeks ($n = 65$)
PT at birth, median (5th to 95th percentile)	18.0 (11.7 – 33.3) seconds	18.0 (12.3 – 36.8) seconds
aPTT at birth, median (5th to 95th percentile)	43.5 (26.1 – 77.4) seconds	46.3 (30.3 – 74.5) seconds

P427 - Table 2. Reference values from literature

	Neonates with GA at birth below 28 weeks (Neary et al., 2015)	Neonates with GA at birth between 30 to 36 weeks (Andrew et al., 1988)
PT at birth	18.1 (12.9 – 28.5) seconds*	13.0 (10.6 – 16.2) seconds**
aPTT at birth	87.1 (53.7 – 139.3) seconds*	53.6 (27.5 – 79.4) seconds**

*Median, (5th to 95th percentile) **Mean (95% confidence interval)*

at birth: below 28 weeks (extremely preterms) and between 28 and 32 weeks (very preterms).

Results: During the study period, coagulation assays at birth were performed in 144 out of 2577 preterm infants. Following application of the exclusion criteria, the remaining 98 neonates were included in the analysis. The coagulation ranges stratified for extremely and very preterm infants are shown in Table 1. The reference values as currently applied in our NICU are presented in Table 2.

Summary/Conclusions: Our results show similar coagulation ranges between extremely and very preterm infants, suggesting that distinction between the two groups may not be required. Additionally, our coagulation ranges differ strongly from the published normal values, underlining the need for reference ranges tailored to the equipment used per NICU. In the absence of reference values calibrated with the analysers used per NICU, we should be cautious about interpretation and possible treatment based solely on longer coagulation values.

Clinical transfusion

Therapeutic apheresis

P428 | Therapeutic plasma exchange in various clinical settings at a tertiary hospital – a retrospective analysis from a technical point of view

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Background: More than a hundred years ago, Therapeutic plasma exchange (TPE) was first described as an extracorporeal blood purification technique. It plays a key role in the management of various diseases as it can remove pathogenic substances such as auto antibodies, lipoproteins and circulating immune complexes and/or administer substances present in plasma of healthy donors providing immense benefit to the patient. According to ASFA 2019 guidelines, TPE is considered first-line treatment in Guillain-Barré Syndrome, Goodpasture Syndrome, Myasthenia Gravis, Thrombotic Thrombocytopenic Purpura, Acute liver failure etc.

Aims: To analyse the frequency of different clinical indications for performing TPE in a tertiary hospital.

To analyse the variations in calculated plasma volume in different clinical indications.

To review plasma volume exchanged in these patients.

Methods: This is a retrospective analytical study of all the TPE procedures performed in a tertiary care teaching hospital and associated blood centre between 1st January to 31st December 2022. Different clinical indications were clustered into categories and their individual frequency calculated. Patient parameters were enumerated and their plasma volume and exchanged plasma volumes were calculated. Statistical relationship between variables was calculated using IBM SPSS Statistics 26 and the significance was set as p-values <0.01.

Results: Within the study period, a total of 723 TPE procedures were performed on 181 patients. The frequency distribution of different indications presented as major cluster categories is as follows: Neurological (83.4%) – Majority being GBS (62.5%), Transverse myelitis (11%), Neuromyelitis Optica (3.3%); Immunological disease and Vasculitis (14.4%) – Majority being Myasthenia gravis (11%), TTP (1.2%), SLE (0.96%); Other conditions such as Acute liver failure (1.5%) and Mixed Connective tissue disorder (0.69%). The calculated plasma volume was significantly higher in patients with immunological diseases and vasculitis (2728 [2491-3148] ml) compared to patients with neurological disorders (2376 [2078-2872] ml) [p value - <0.01]. This is majorly due to hematocrit value being higher in patients with neurological disorders. Moreover, the mean exchanged plasma volume was lower than recommended in both the strata being 0.92 (0.72-1.1) in neurological diseases and 0.77 (0.69-0.82) in immunological diseases and vasculitis with the latter being significantly lower than the former (p value <0.01). But there was significant clinical improvement in almost all the patients in spite of lower exchange volumes.

Summary/Conclusions: The majority of the patients that received TPE had some sort of neurological conditions, single major indication being Guillain-Barré Syndrome in this study population. Patients with high body weight and lower hematocrit did not receive recommended exchange volume. The exchange volume should be calculated individually for all patients bearing in mind key variables such as body weight and hematocrit.

P429 | Comparison of efficacy of Plasma Exchange vs. Intravenous Immunoglobulin as an add on therapy in acute attacks of Neuromyelitis Optica Spectrum Disorder

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Background: Plasma exchange (PE) is considered a Category II option for the treatment of acute attacks and relapse cases of Neuromyelitis Optica Spectrum Disorder (NMOSD). However, neurologists are also considering Intravenous Immunoglobins (IVIg) as an add-on therapy for this disorder.

Aims: To evaluate the efficacy of PE in acute attacks of NMSOD-diagnosed patients as compared to IVIg treatment, in terms of improvement in Expanded disability status scale (EDSS) and Activities of daily living (ADL) scale score and to evaluate the effect of PE on anti-Aquaporin P4 (AQP4)-antibody in seropositive patients.

Methods: The study was conducted in the Department of Transfusion Medicine in collaboration with the Department of Neurology at a tertiary care center in north India (from July 2020 to December 2021). In this comparative study, we enrolled 43 NMOSD-diagnosed patients in two groups: 29 patients in group 1, who received steroids and plasma exchange therapy, and 14 patients in group 2, who received steroids with intravenous immunoglobulin. The baseline clinical scores (EDSS, ADL) were noted and compared with the change in scores at end of therapy, 4 weeks, and 12 weeks. Also, a change in anti-Aquaporin P4 antibody was determined post-therapy in seropositive patients of both groups.

Results: A total of 129 plasma exchange procedures were performed in 29 patients in group-1 with an average of 4.5 ± 1.5 procedures. In group 2, 14 patients received IVIg (2g/kg for 5 days). We observed an adverse event rate of 3.8% in group 1. The most common adverse event observed was hypovolemia (1.5%), followed by hypocalcemia (0.78%), febrile non-haemolytic transfusion reaction (0.78%), and central line blockage (0.78%). No adverse event was observed in group 2. We observed a significant difference in EDSS ($P = 0.00$) and ADL score ($P = 0.00$) at day 10 and 3 months) in group 1 as well as in group-2 ($P = 0.00$ and $P = 0.05$). However, no significant difference in EDSS, as well as ADL score from baseline ($P = 0.83$; $P = 0.25$) to 3 months ($P = 0.85$; $P = 0.19$), was observed when delta change of score at 3 months (from baseline) was compared across the two groups ($P = 0.39$; $P = 0.52$). We observed a significant decline in AQP4 antibody concentration after plasma exchange therapy (at day 10) in group-1 seropositive patients ($n = 12$) ($P = 0.013$), however, in group-2 seropositive patients ($n = 4$) no significant difference was found in antibody concentration on day 10 after IVIg ($P = 0.715$). EDSS and ADL scores of anti-AQP4 seropositive patients in group-1 were compared separately with seropositive patients of group-2 and it was observed that the scores improved significantly at day 10 ($P = 0.027$; $P = 0.026$) in group-1.

Summary/Conclusions: Plasma Exchange (PE) is more effective as an add-on therapy in seropositive NMOSD patients to bring down the concentration of anti AQP4 antibody concentration with significant improvement in EDSS and ADL scores in these patients as compared to seropositive patients receiving IVIg. Thus, anti-AQP4 antibody testing of the patient needs to be done as a part of the diagnostic workup for NMOSD and PE should be considered as a choice of add-on therapy for seropositive patients.

P430 | Therapeutic plasma exchange efficacy in treating severe Anti-NMDA Receptor Encephalitis: A case report

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Background: Autoimmune encephalitis (AE) is a potentially reversible disorder with a good clinical outcome if diagnosed and treated promptly. However, fulminant cases remain a challenge, and fatal cases are still seen. As an example, 75% of anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis patients may require treatment in the intensive care unit (ICU). Therapeutic plasma exchange (TPE) is a potential first-line therapy for various subtypes of AIE. Here, we present a case of an 9 year old boy diagnosed with autoimmune encephalitis who showed no response to steroid and immunotherapy and was successfully treated with TPE.

Aims: To emphasise the important role of plasma exchange in the treatment of AE

Methods: A 9 year old boy presented to the pediatric emergency of University Hospital Mother Teresa in Tirana with nausea, vomiting, lost of conciseness, apathy, did not react to verbal stimuli, ataxia. CT Brain showed unremarkable findings. Initial cytological examination of her cerebrospinal fluid revealed normal protein and glucose concentration. Immunologic for Herpes simplex virus tested negative, Anti-SARS Cov-2 IgM and IgG tested also negative. Examination for auto antibodies, ANA, Anti ds DNA, ENA and MPO revealed normal levels. Anti-NMDAR antibodies were detected in his serum confirming the diagnosis of Anti-NMDA Receptor Encephalitis. He was treated with immunotherapy included intravenous glucocorticoid therapy (methylprednisolone), antibiotic therapy, antiviral which showed no improvement but on the contrary his clinical course deteriorated as he became nonverbal, did not react to any stimuli, had respiratory problems with low levels of O2 saturation. At this point rituximab was added to therapy but it did not was effective and the patient was transferred to the pediatric intensive care unit with mechanical ventilation. Considering that no other treatment was effective, on the day 22 of the hospitalization he underwent five procedures of TPE. All TPE procedures were performed using the Spectra optia instrument. Citrate was used as the only anticoagulant for all the procedures. He received 1 PV exchange per procedure, using both 5% albumin and 0.9% normal saline as replacement fluid. What needs to be noted is that after the third procedure of TPE his status changed from unconsciousness to conscious and an improvement from ventilator-assisted treatment to removal of the ventilator.

Results: After 5 procedures of TPE the patient was transferred to the neurology department and was discharged from the hospital fully recovered after 1 week.

Summary/Conclusions: According to the recent guidelines published by the American Society for Apheresis, the use of TPE is recommended in patients with anti-NMDAR encephalitis (Category I, grade 1C) and our case proved it has been successful. TPE might be a reasonable option to consider in patients with severe antibody-associated AE with absent or limited improvement after pulse steroids or IVIG after weighing the potential benefits and risks on an individualized basis.

P431 | Abstract withdrawn

P432 | Pediatric case of pertusis with leukemoid reaction treated with therapeutic leukocytapheresis: A tertiary care experience

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Background: Pertusis is caused by Bordetella Pertusis.Malignant pertusis is charcterized by leukocytosis,refractory hypoxemia,pulmonary hypertension and cardiopulmonary compromise.Leukocytosis causes hyperviscosity syndrome and leukostasis which can be complicated by Intracranial haemorrhage and pulmonary hypertension.Leukemoid reaction means total count more than one lakh.

Aims: To evaluate the role of therapeutic leukapheresis in pertusis with leukemoid reaction.

Methods: We report a case of 18 month old female in PICU, case of Pertusis with leukemoid reaction treated by therapeutic leukocytapheresis at our centre. She presented with chief complains of cough and cold from 2 days with one episode of convulsion day before. At the time of admission her general condition was poor. Her temperature was 102^0 F, Heart rate: 167, Respiratory rate was 26/min and Spo2 was 80% on room air.She was intubated and admitted to PICU. Her haemoglobin was 7gm/dl, total WBC 1,13,800/cmm and platelet count was 4,58,000 /cmm. Therapeutic Leukocytapheresis was performed on Spectra Optia cell separator. Procedure lasted for 3 hours. Patient's oxygen saturation, BP, Heart rate as closely monitored. She was also transfused with 80 ml of RCC after the procedure.

Results: Her haemoglobin increased to 8.40 gm/dl her total WBC counts reduced to 40,200/cmm and platelet counts reduced to 74,000/cmm. Patient's clinical condition improved gradually with antibiotic coverage and Leukocytapheresis. She was able to maintain 97% Spo2 on room air after 21 days of ICU stay.

Summary/Conclusions: Hence we conclude that leukocytapheresis is considered as adjunctive treatment (ASFA category 2) to reduce leuocytosis and its adverse consequences like pulmonary hypertension and cardiac compromise. First line treatment was targeted antibiotic therapy with supportive measures.

P433 | Clinical efficacy of low volume therapeutic plasma exchange in yellow phosphorous poisoning leading to acute liver failure in resource limited setting

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Background: It has been estimated that Indian burden of Yellow phosphorous (YP) is 75:1 to paracetamol poisoning and can be considered equivalent to paracetamol induced toxic liver necrosis in the west (Eapen, J Clin Exp Hepatol, 2021). The ingestion of YP can lead to acute liver failure (ALF) resulting in encephalopathy and death following an initial 72 hours of asymptomatic period. Liver transplantation is indeed the curative treatment, but it is difficult to obtain a suitable liver donor in an acute setting. Low volume therapeutic plasma exchange (TPE) replaces the circulating toxic products from the blood with normal plasma components and act as a bridge to liver transplantation.

Aims: To assess the clinical efficacy of low volume TPE in acute liver failure following YP poisoning.

Methods: This was a prospective interventional study for one year on the outcome of ALF following YP poisoning. All consenting patients above 18 years of age with confirmed YP poisoning toxicology report were included. The decision to initiate TPE was taken by a multidisciplinary team of gastroenterologists, critical care and transfusion medicine specialists. The plan was to do 1 – 1.5 total plasma volume every cycle and replace it with 0.9% normal saline, 5% albumin and fresh frozen plasma (FFP). TPE was performed as per the American Society for Apheresis guidelines (ASFA 2019). Patient outcome, length of hospital stay, number of cycles of TPE, TPV processed, changes in Haemoglobin, platelet count, prothrombin time, activated partial thromboplastin time, SGOT, SGPT and total bilirubin were assessed. The primary end point to stop TPE was no signs of encephalopathy, normalization of coagulation times and liver enzymes.

Results: A total of 19 patients (13 males) underwent TPE in YP poisoning in 2022 with a mean age of 34.31 (±15.7). TPE was initiated after a median gap of 3 days (4-11 days) from the ingestion, when the liver functions were deranged. The average length of hospital stay was 10.73 (±7.37) days, and the mortality rate was 31.6%. Median number of TPE performed was 4 (range 1-5) processing an average plasma volume of 3226.3 (±587.9) in each cycle. It was replaced with a combination of 0.9% normal saline, 5% albumin and FFP in each cycle (Table 1) due to the clinical status. There was a significant percentage reduction in coagulation times and liver enzyme levels post TPE (Table 2). The presence of hepatic encephalopathy and a delayed initiation of TPE were associated with poor outcome.

Summary/Conclusions: Low volume TPE was found to be a useful bridging tool in ALF due to YP poisoning in resource limited setting.

P433 - Table 1: Details of TPE performed.

Median number of cycles	Plasma volume exchanged	Normal saline (units)	5% albumin (units)	FFP (bags)
4(1-5)	3226 ± 587.99	3.5 ± 0.82	2.36 ± 0.89	4.79 ± 1.83

P433 - Table 2: Laboratory profile of 19 patients before and after TPE

Laboratory profile (normal range, unit)	Before TPE (Mean ± SD)	After TPE (Mean ± SD)	Percentage change	P
Haemoglobin (12-15 g/dL)	15.14 ± 1.87	12.55 ± 2.87	-17.01	0.006
HCT (36-53 %)	44.05 ± 5.05	37.74 ± 7.27	-14.32	0.003
Platelet count (150-400x10^3/μL)	176.06 ± 85.9	124.83 ± 82.75	-29.09	0.06
Prothrombin Time (10.4-12.2 seconds)	31.8 ± 25.64	18.84 ± 11.07	-40.53	0.002
Activate partial thromboplastin time (26.3-31.3 seconds)	40.21 ± 23.29	33.86 ± 8.63	-15.7	0.27
AST (0-32 IU/L)	632.78 ± 423.4	213 ± 220	-66.29	0.0005
ALT (0-32 IU/L)	526.45 ± 392.15	236.58 ± 221.48	-55.13	0.0081
Total Bilirubin (0.1-1.2 mg/dL)	4.03 ± 2.44	6.14 ± 2.62	50.14	0.014

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P435 | Two cases of post-COVID-19 Guillain-Barré syndrome treated by therapeutic plasma exchange

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Background: Guillain-Barré syndrome (GBS) is currently recognised as a complication of COVID-19 usually treated with IntraVenous ImmunoGlobulins (IVIG). Therapeutic Plasma Exchange (TPE) was rarely reported as a post-COVID-19 GBS treatment.
Aims: We report two cases of post-COVID-19 GBS treated by TPE as a first-line therapy at the Blood Center of Sfax, Tunisia.
Methods: A 38-year-old man and a 44-year-old woman were admitted for tetraparesis and swallowing disorders. Standing and walking were impossible in both patients. The electromyography confirmed GBS showing acute sensory-motor demyelinating polyradiculoneuropathy with axonal loss. Albuminocytorachic dissociation was found in one patient. COVID-19 infection was diagnosed 10 and 21 days earlier (nasal swab). Patients were exchanged every other day with 4% albumin substitution. TPE was performed by means of continuous flow cell separator. Plasma exchanged volumes were set at 1.2 of total plasma volume.
Results: The delay between symptoms onset and TPE was respectively of four and eight days. Each patient underwent a series of 4 TPE sessions via a central venous catheter. Mean exchanged volumes were respectively 2 923 mL (extremes 2 387 – 2 972) mL and 3 108 mL (extremes 2 833 – 3 422) mL. The mean duration was respectively of 88 and 72 minutes. No adverse events were reported. Disease progression stopped after the third and the second TPE session respectively. Immediately after the latest TPE session, the gain in muscle scores was 1 point in both patients. Walking was possible with assistance at week 4 in the first patient and without assistance at week 2 in the second patient.
Summary/Conclusions: Post-COVID-19 GBS has mainly been reported in men after 50 years in its demyelinating form. The axonal form and the TPE treatment were particular features in our patients. In very high-income countries, IVIG are preferred as a first-line treatment because of the TPE invasive risk. In our country, TPE is a less costly, more rapidly accessible, possibly effective, and secure alternative to IVIG.

P436 | A case of high-dose steroids refractory severe acute optic neuritis responding to therapeutic plasma exchange

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Background: Isolated optic neuritis (ON) is rarely reported. The risk is total and permanent blindness. The first-line treatment consists of high-dose intravenous steroids. In case of steroid refractoriness, Therapeutic Plasma Exchanges (TPE) are recommended as a second-line therapy.
Aims: We report and discuss a case of high-dose steroid irresponsive acute isolated bilateral ON responding to TPE
Methods: A 50-year-old woman, with a history of diabetes and hypothyroidism with no previous ophthalmologic history, had a prodromic paroxysmal headache and bilateral tinnitus. On day seven, she was urgently admitted for a bilateral and rapidly progressive sight decrease. She also reported eye pain, photophobia, metamorphopsia, and dyschromatopsia. The initial ophthalmological examination noted a severe, bilateral, and isolated decrease in visual acuity or 1/10 in both eyes. The fundus examination showed bilateral papillary edema (stage I on the right and stage II on the left). Elsewhere, the neurological examination was normal. The cerebrospinal fluid analysis found a slight increase in proteins. The bilateral visual evoked potential was uninterpretable. Cerebral MRI and CT angiography confirmed a bilateral ON. Antibodies anti-MOG were positive, and anti-AQP4, anti-TPO/TG were negative. She immediately received high doses of intravenous Methylprednisolone (1 g per day over 5 days) along with symptomatic treatment resulting in the absence of a total sight recovery. She only was able to light sight. By day 9, she underwent TPE every other day on a continuous flow cell separator via a central catheter with ACDA anticoagulation, and 4% albumin substitution.
Results: The patient totally recovered her sight after the first TPE session. Two additional TPE sessions were performed as maintenance. She was, then, put under Azathioprine to avoid relapse.
Summary/Conclusions: We report a case of high-dose steroid irresponsive acute isolated bilateral ON with profound sight loss, who responded to TPEs avoiding blindness. In fact, TPE is a recognised second-line therapy of ON as long as it is started early in the disease course as in our patient. Reported total TPE sessions varied between 2 to 20. The session number is to be individually adjusted. In our patient, sight recovery occurred from the 1st TPE session, resulting in few maintenance TPE sessions.

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P440 | Nmdar encephalitis responding to a therapeutic plasma exchange as a second-line therapy: A case report

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Background: NMDAR encephalitis is a scarce and acute inflammatory brain disorder including N-méthyl-D-aspartate receptor antibodies and neuropsychiatric symptoms. First-line therapy includes high-dose

steroids, intravenous immunoglobulin (IVIG), and/or Therapeutic Plasma Exchange (TPE) with no broad consensus about the exact order.

Aims: We report a case of a female child with post-herpes NMDAR encephalitis who responded to TPE after refractoriness to high-dose steroids and IVIG.

Methods: A 12-year-old female child had nasal herpes. On day 7, she suffered from confirmed herpes encephalitis with cerebrospinal fluid-positive NAT for HSV1 and favorable MRI features. She had Zovirax, corticosteroids, and anticonvulsants, and was discharged. On day 15, she was readmitted for persistent behavioral disorders, newly diagnosed aphasia, agitation, and coma (Glasgow Score = 10/15). A second MRI showed lesions extension concomitantly to HSV1 NAT negativity and anti-NMDA and anti-recoverin antibodies positivity (IIF). Despite immediately adding high-dose steroids (30 mg/kg of Methyl-Prednisolone over 5 days), followed by IVIG (2 mg/kg over 2 days), no clinical or radiological improvement was noted. A week later, TPE was indicated. Exchange of a plasma volume equivalent to 1.2 of total plasma volume was indicated every other day by means of a continuous flow cell separator via a central catheter with ACDA anticoagulation, and 4% albumin substitution.

Results: The patient underwent eight TPE sessions. The average exchanged plasma volume was 2 765 (range 2 352 – 3 284) mL. Blood priming was not needed. Myoclonus and then aphasia and agitation resolved respectively after TPE sessions 2 and 6. Glasgow's score began to improve (12/15) after TPE session 4. No TPE adverse events were reported. The patient was discharged three weeks later. After one year, she is now in a rehab educational program including normal physical activities and mild impairment in intellectual skills.

Summary/Conclusions: Fifty percent of NMDAR encephalitis resolves within a month after immunomodulation therapy including high-dose steroids, IVIG, and/or TPE in the front line. High-quality evidence studies in TPE indications for NMDAR encephalitis are unavailable due to their scarcity (ASFA, grade 1C) and no broad consensus is reported on the exact order of different immunomodulation therapies. TPE followed by IVIG was reported to give better results. Our case supports the effectiveness of TPE as a salvage therapy after steroids and IVIG refractoriness.

P441 | Therapeutic plasma exchange for SARS CoV-2 vaccination neurological adverse events: a case series

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Background: A variety of neurological complications after SARS-CoV2 vaccination have been reported. The most frequent were: Guillain-Barré Syndrome (GBS) and Acute Disseminated EncephaloMyelitis (ADEM). Therapeutic plasma exchange (TPE) is less often reported as a first-line treatment than IntraVenous ImmunoGlobulin (IVIG).

Aims: We report the clinical and therapeutic features in patients treated with TPE for post-SARS-CoV-2 vaccination's GBS and ADEM.

Methods: A retrospective study was conducted in the Blood Center of Sfax, Tunisie from February 2021 until February 2022. Six patients having post-SARS-CoV-2 vaccination's GBS and two others having post-SARS-CoV-2 vaccination ADEM were treated with TPE between February 2021 and February 2022. For all patient, there was no history of current SARS-CoV-2 infection or autoimmune disease. TPE was performed on a continuous flow cell separator every other day via a central catheter. Albumin (4%) was used as replacement fluid.

Results: Among the patients, 6 were male and 2 were female. The mean age was 54 (range: 38–68) years. Received vaccines were, viral vector vaccine and mRNA vaccine in GBS patients, and viral vector vaccine and inactivated vaccine in ADEM patients. The delay between vaccination and neurological symptoms ranged from 7 to 15 days with an average of 11 days. In GBS patients, the assessment of muscular scores revealed a mean score of 2 in the lower limbs and of 3 in the upper limbs (range 1-3). One patient had facial diplegia. Electromyography showed axonal and demyelinating polyradiculoneuropathy in all patients. The ADEM patients' had flaccid paraparesis rated 2 with difficulty in walking in one case, dysarthria, and dysphagia in the other case. Brain MRI showed respectively confirmed encephalitis and cervical myelitis C3–C7. The mean delay in initiating TPE was 14 days (range 10–17 days) for GBS, and 35 days for ADEM. The patients underwent 1 to 4 TPE sessions. The average treated volume was 2.7 L (extreme 2.1–3L). Immediate muscle score gains were of 1 (range:0-2) in GBS patients. One ADEM patient had partial recovery (walking with assistance) and the other one died.

Summary/Conclusions: In GBS treatment it is proven that TPE is equivalent to IVIG. in post-SARS-CoV-2 vaccination's GBS, TPE was rarely reported as a 1st line therapy. TPE seems to be as efficient as in others GBS aetiologies especially since post-SARS-CoV-2 vaccination GBS are reported to be mild as in our patients. In ADEM, TPE which is a salvage treatment (grade 2C), is started after corticosteroid failure. This causes a long delay in TPE initiation, considered as a poor prognosis factor.

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Clinical transfusion

Evidence based transfusion medicine practice

P444 | Prevalence of cold agglutinin in a teaching hospital

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Background: Cold agglutinin(CA) are antibodies that agglutinate erythrocytes at a temperature of 0-4°C. This antibody is frequently detected in patients with autoimmune haemolytic anaemia, secondary proliferative disorders (e.g., lymphoma, CLL, etc.), and Mycoplasma pneumonia infections. Erythrocytes may even clump together at room temperature in people with severe cold agglutinin syndrome. The pathological cold agglutinin sometimes masks irregular antibody screening test and causes the discrepancy between ABO and the positive result of alloantibodies.

Aims: The aim of this study was to investigate the frequency and incidence of cold agglutinin in transfusion patients at our institution.

Methods: This study is conducted in a 600-bed teaching hospital in central Taiwan. Retrospective data were collected from 2011 to 2021. The manual polybrene (MP method) on antibody screening procedure for transfusion patients. In this procedure, RBC screening cells are incubated with patient sera in a low ionic medium at room temperature for one minute. Polybrene is a quaternary ammonium polymer that causes non-specific aggregation of red blood cells. The test tubes are centrifuged, the cell-free supernatant is decanted, and the polybrene effect on the cells is neutralized by adding a dilute sodium citrate-glucose solution. The hemagglutination results are evaluated macroscopically and microscopically. The entire MP method is completed in less than three minutes. Positive results from this MP screening method are then further using prewarmed technique or AHG test for detecting serum containing cold agglutinins.

Results: In this study, a total of 72,644 patients were screened for the irregular antibody for pretransfusion testing from 2011 to 2021, and 1264 patients were identified as cold agglutinin. The average positive rate for autoantibody was1.4%. A total of 636 male patients (50.3%) and 628 female patients (49.7%) in this CA autoantibody population. The mean age was 67.6 years, and the majority of patients were between 71-80 years old (24.2%). The detection rate of positive CA was not significantly different between men and women in our study.

Summary/Conclusions: Antibody screening test is an important step before blood transfusion. In clinical practice, cold agglutinations interfere with serologic testing. Strongly cold-responsive autoantibodies can mask the presence of clinically significant alloantibodies and also cause ABO discrepancy. It is crucial to transfuse the warmed blood products to prevent a haemolytic reaction in patients with severe cold agglutinins. Despite the fact that several drugs have been used in treating cold agglutinin patients, the outcome of those medication has not been very successful and promising. However, different new therapeutic strategies may be helpful in the future given the improved knowledge and advancement in clinical care. Before that, appropriate safety precautions and keep monitoring patient's response are necessary to prevent transfusion reactions while undergoing a transfusion operation.

P445 | Rethinking and personalising the transfusion pathway in myelodysplastic syndromes (MDS): a novel trial of weekly matched red cell transfusion to improve quality of life

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Background: Anaemia is common in MDS and associated with poorer quality of life (QoL) and physical function. Transfusions are integral to supportive care management. Current standard practice typically involves regular transfusion of multiple red blood cell (RBC) units every few weeks, which can result in peaks and troughs in haemoglobin (Hb) between transfusions. Observational data suggest that maintaining a more stable Hb may improve QoL.

Aims: The REDDS2 (Red Cell Transfusion in MDS) study is investigating the feasibility of a weekly low-dose RBC transfusion schedule, personalised for each patient, in transfusion-dependent MDS patients to maintain more stable Hb, and the effects of this on QoL and physical function.

Methods: In this n-of-1 study, each patient receives 2 transfusion treatment arms, with randomised allocation of treatment sequence: arm A, the patient's usual transfusion schedule and arm B, weekly transfusion. To facilitate timely delivery of weekly transfusion and reduce the burden of hospital visits for patients, extended matched RBCs (D,c,C,E,K, Fya,Jka,Kpa) are provided during arm B. Patients are transfused based on the previous week's Hb and antibody screening/cross-matching results, to eliminate the delays of awaiting contemporaneous cross-matching on each occasion. Transfusion algorithms and Hb targets are individualised per patient. The primary outcome is feasibility of delivering a weekly transfusion schedule, defined by a difference in median time between transfusions of 7 days or more between the 2 arms. Secondary exploratory outcomes are: RBC usage, protocol compliance, QoL measurements (QUALMS-1, EQ-5D-5L, EORTC-QLQ-C30, Community Integration Questionnaire), functional activity measurements (6-minute walk test, handgrip strength, accelerometer outputs), number of adverse events (including alloimmunisation). A qualitative sub-study also explores the experiences of patients and staff members with weekly versus standard transfusion schedule.

Results: The first patient was recruited in 2020, but enrolment was delayed by COVID19 pandemic. Currently, it is open to recruitment at 6 sites in Australia, Netherlands and UK. Differences between countries in the provision of RBCs on trial have been observed (table 1). To date, 8 patients have completed treatment. 4 patient qualitative interviews and 1 staff focus group have been conducted. No serious adverse events have been recorded. The trial is ongoing.

Summary/Conclusions: This randomised control trial is evaluating a novel transfusion approach for MDS patients which includes innovative features such as weekly personalised transfusion protocol (with transfusion treatments, Hb targets, and matched RBCs personalised for each patient), changes in pre-transfusion testing requirements to reduce burden on patients, and exploring the utility of QoL and physical functional tools. The study will inform the acceptability of different transfusion schedules, and the design of further definitive studies.

P445 - Table 1: Inter-country differences in provision of RBCs

	Australia	England	Netherlands
Arm A (standard of care)	ABO Rh(D) compatible	ABO Rh(D) compatible	ABO Rh(D) compatible
Arm B: patient testing to select units	Genotype or serological phenotype (varies depending on local availability and practice)	Genotype or serological phenotype (varies depending on local availability and practice)	Genotype
Arm B: Additional requirements	Donor units additionally require Kpa phenotyping by hospital blood bank as this is not routinely provided by national blood supplier	Donor units additionally require Kpa phenotyping by hospital blood bank as this is not routinely provided by national blood supplier	Nil

P446 | Does treatment of anaemia improve quality of life or physical function for patients with myelodysplastic syndromes (MDS): a systematic review

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Background: Anaemia is universal in MDS and may impact on patients' health-related quality of life (HRQoL) and physical function. Different supportive care interventions have been tested in studies for anaemia including red blood cell (RBC) transfusion and growth factors, but the overall effects on measures of HRQoL or physical function are unclear.

Aims: To perform a systematic review of the literature to (1) Record the range of instruments used to assess HRQoL and physical function outcomes, and (2) Assess whether improvements in anaemia outcomes are reflected in measures of HRQoL and physical function.

Methods: Databases (MEDLINE, Embase, CINAHL, Transfusion Evidence Library, The Cochrane Library, PsycINFO, ClinicalTrials.gov) were searched from inception until 18 July 2022. Non-English language studies were excluded.

Inclusion criteria:

- Study design: Randomised controlled trials (RCTs), controlled clinical trials, cohort studies, case-control studies and cross-sectional studies
- Patients: at least 50% of patients in the study were aged 18 years or over, with a diagnosis of MDS, myelodysplastic/ myeloproliferative neoplasm or chronic myelomonocytic leukaemia
- Intervention: at least 1 supportive care intervention for anaemia. Disease-modifying therapies were excluded due to their potential wider impacts on HRQoL outside of their effect on anaemia
- Outcome: HRQoL and/or physical function as a primary or secondary outcome measures

Risk of bias assessment was done using the Cochrane RoB1 tool for RCTs and the ROBINS-I tool for non-randomised studies.

Results: 23 studies (9 RCTS and 14 observational studies) were identified for inclusion, from a search strategy that identified 6656 citations. The main reasons for exclusion were ineligible study population and no reporting of outcomes on HRQoL/physical function.

Interventions in the studies were growth factors/erythropoiesis-stimulating agents (ESAs) ($n = 11$), RBC transfusion ($n = 9$), erythroid maturation agents (EMAs) ($n = 1$), or a combination of these ($n = 2$). All studies reported HRQoL outcomes using 11 different tools. 4 studies also reported physical function outcomes, using 6 different tools.

Many limitations in study design or reporting were identified, when benchmarked against international standards such as the SPIRIT-PRO Extension reporting guidelines for patient-reported outcomes in clinical trials. These included: variation in follow-up timepoints, high attrition rates, lack of attrition rate reporting and not defining a minimally important difference.

5 of the 9 RCTs reported no change in HRQoL despite evidence of higher erythroid responses or increase in Hb in patients as a response to the supportive care treatment. No studies demonstrated a worsening HRQoL with supportive care treatment.

Summary/Conclusions: This systematic review highlights the uncertainty of HRQoL and physical function outcomes in studies investigating anaemia interventions in patients with MDS, and the limitations of many studies to date. Our findings raise the question of whether anaemia treatment alone is effective for improving HRQoL. Given the importance of maintaining HRQoL and physical function in MDS patients, it is important that future trials of anaemia treatments include such outcomes to correlate with Hb responses, and include robust reporting of results.

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P451 | Revitalizing platelet crossmatch assessment by Solid-Phase Red Cell Adherence Assay (SPRCA) in pediatric hemato-oncology patients: A study from a tertiary care oncology centre

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Background: Platelet transfusions are vital for pediatric hemato-oncology patients. Pre-transfusion platelet cross matching is a direct means of identifying compatible donors for the alloimmunised recipient. Platelet cross matching by SPRCA is relatively low cost technique with the advantage of quick availability of compatible platelets.

Aims: To assess the platelet crossmatch results by the SPRCA method and correlating its results with post-transfusion platelet count increments in pediatric patients.

Methods: This prospective observational study was conducted for a period of 6 months in pediatric patients with hematological malignancies after approval of the Institutional Ethics Committee (IEC) and registration with the Clinical Trial Registry of India (CTRI). Total of 80 pediatric hematological patients with a history of packed red cell or platelet transfusions of more than or equal to 2 occasions were included in the study after obtaining written informed consent from patients and/or their parents. Patients having fever, sepsis,

P451 - Table 1: Correlation of Compatibility with CCI and PPR

Crossmatch Result	CCI Adequate (≥7500)	CCI Inadequate (<7500)	PPR Adequate (≥30 %)	PPR Inadequate (<30 %)
Compatible ($n = 69$)	63 (91.3 %)	6 (8.7 %)	44 (63.8 %)	25 (36.2 %)
Incompatible ($n = 11$)	8 (72.7 %)	3 (27.3 %)	1 (9.1 %)	10 (90.9 %)
Total ($n = 80$)	71	9	45	35
p value (2 sided)	0.103		0. 0.001	
Sn (%)	88.7		97.7	
Sp (%)	33.3		28.5	
PPV (%)	91.3		63.7	
NPV (%)	27.2		90	

CCI = Corrected Count Increment * p-value calculated by Fisher Exact test.
Sn = Sensitivity; Sp = Specificity; PPV = Positive Predictive Value; NPV = Negative Predictive Value

splenomegaly, disseminated intravascular coagulation (DIC), and patients receiving amphotericin B were excluded from the study. The inbuilt sample pouch of the SDP bag was used to preserve the donor platelet sample till the time the cross-match with the intended patient's serum was performed which was processed in batches at a later period of time. SPRCA was used to assess the platelet compatibility, and post-transfusion response was measured after transfusion of ABO identical SDP units. Corrected Count Increment (CCI) and Percent Platelet Recovery (PPR) of ≥7500 and ≥30% respectively was considered adequate. Dose of platelet transfusion was 10-15 ml/kg body weight of the patient The dose of platelet transfusion for children weighing less than 15 kg is 10–20 mL/kg while those above 15 kg received a single apheresis donation.

Results: Platelet crossmatch was found compatible in 86% (69/80) of patients. Patients with compatible crossmatches had a higher rate of adequate CCI and a statistically significant association was found between crossmatch compatibility and PPR as described in table 1. These findings highlight the importance of selecting compatible platelets from the available inventory to ensure better patient outcomes.

Summary/Conclusions: The SPRCA method is a rapid and effective tool for identifying crossmatch compatible platelets and improving the success of platelet transfusions in pediatric hemato-oncology patients. By implementing this method, healthcare providers can better manage the requirements of multiple platelet transfusions and ensure positive outcomes in the patients. However, there is a need for more evidence-based uniform guidelines, especially for the pediatric population for monitoring the success of platelet transfusions.

P452 | Convalescent plasma in treatment of moderate COVID-19 patients admitted in a covid hospital at a tertiary care medical institute in North India

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Background: Convalescent plasma (CP) refers to plasma that is collected from individuals following resolution of covid infection and development of neutralizing antibodies against SARS-CoV-2. These antibodies bind to virus, thereby neutralizing its infectivity directly. During peak coronavirus disease 2019 (Covid-19) in 2020-21 the patients with moderate category of Covid-19 disease who were not improving despite use of steroids were given this therapy by our hospital in line with then recommendations of the government.

Aims: To see the feasibility of collection and effectiveness of convalescent plasma to treat moderate adult patients of Covid-19 at our hospital.

Methods: Plasma (500 ml) was collected by apheresis using cell separators. A chemiluminescence (CLIA) based assay for detection of IgG antibodies against Spike protein of the virus (S/CO ratio ≥12) to qualify as high titre CP was used. Usually 200 mL single dose of CP was given slowly. Necessary testing for transfusion transmitted infections was carried out and in selected cases thromboelastography (TEG) for detection of hypercoagulable profile was performed.

Results: 72 CP donations including 54 voluntary, 18 replacement were performed by plasma apheresis in a period of 3 months from selected patients who had recovered from the disease. Time from RT-PCR negative to donation was 14-62 days. About 76.7% patients had fever, 51.2% had respiratory symptoms, 25.6% had loss of smell. Chemiluminescence based assay was used to detect IgG. These SARS-COV-2 IgG have high concordance with Virus Neutralization Test. Mean IgG S/Co ratio of donated CP units was 14.63 (High Titre). IgG S1 antibodies were detected in 12.5% of the patients within 7 days, in 77.7% of patients within 7-14 days and in 100% after 14 days in our cohort. One hundred and sixteen patients received CP till with recovery seen in 64 patients (55%). A hypercoagulable TEG profile was also observed in majority of the samples from Covid-19 patients.

Summary/Conclusions: Majority of individuals generate higher titres of antibodies ≥ 14 days after resolution of symptoms. Hypercoagulable profile is seen in many such patients even after recovery. The administration of convalescent plasma does not seem to be effective after 2-3 weeks of the onset of the disease as many patients already have formed IgG antibodies without plasma transfusion.

P453 | Abstract withdrawn

P454 | Blood loss in transcatheter aortic valve implementation (TAVI) patients in University Hospital Dubrava

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Background: Blood loss in TAVI patients became of interest to us since a significant rise of the crossmatch to transfusion (C/T) ratio was noticed for the period 01.01.2022.-30.06.2022. for cardiology patients. C/T ratio exceeded 2 and our assumption was that it was due to the fact that some clinicians kept ordering red blood cells (RBCs) for TAVI as it was an open heart surgery. It was then agreed that we limit the order to 2 units of RBCs for TAVI.

Aims: Our goal was to determine the real need for RBCs in TAVI and to reduce C/T ratio.

Methods: We analysed the need for blood transfusion in all our TAVI patients until the end of 2022 (first procedure was done on 15.07.2020.).

Results: In the next 6 months (01.07.2022.-31.12.2022.) C/T ratio was reduced to 1,44.

There was a total of 138 TAVI patients, 71 male and 67 female. 16 of them received RBC transfusion on the day of the procedure, 7 male and 9 female. Analysis showed that 11,6% patients that underwent TAVI received a RBC transfusion.

Summary/Conclusions: With total percutaneous approach, less invasive temporary pacing and optimal procedural planning, TAVI has become a minimally invasive procedure, with seldom need for urgent transfusion. The fact that 11,6% patients that underwent TAVI received a RBC transfusion makes TAVI procedure in University hospital Dubrava ideal for implementing type and screen (T&S) procedure. With T&S procedure for TAVI patients we could further reduce C/T ratio because unnecessary crossmatch testing would be avoided.

P455 | The impact of transfusion education in improving transfusion appropriateness in patients with Sickle Cell Disease (SCD)

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Background: Sickle Cell Disease (SCD) is the most common genetic haemoglobinopathy in sub-Saharan Africa. The patients are more predisposed to receiving a blood transfusion in their lifetime either to relieve an emergency or improve their quality of life. Curative therapy for SCD patients is costly and blood transfusion remains one of the affordable therapy currently available. However, they are more at risk of developing adverse reactions among which are alloimmunization, haemolytic transfusion reactions, and transfusion-transmitted infections. In addition, they require varying and special transfusion considerations different from the general population. However, there is a scarcity of data describing the clinical transfusion practice for these patients in our setting.

Aims: To assess the impact of transfusion education in improving transfusion appropriateness in patients with SCD in Baptist Hospital Mutengene, Cameroon

Methods: A hospital-based cross-sectional study was carried out from January to December 2022 at Baptist Hospital Mutengene, Cameroon. Physicians received training on blood transfusion practice for SCD patients following the updated American and British societies of hematology guidelines. Pre and post-training data were collected which included the total number of transfusions, transfusion inappropriateness, indications for transfusion, and transfusion reactions. Also, patient transfusion data were collected-age, including gender, blood group, history of previous transfusion, indication for transfusion, type of blood component, and the number of units requested. Data were entered and analysed with EPI Info version 7, statistical significance was given for $p < 5\%$.

Results: Overall 41 SCD patients were transfused, 51.2% females with a mean age of 11.3 ± 9.6 years. All the transfusions were top-up. Pre-training we recorded 27 blood units transfused with 29.6% of them being inappropriate with the highest indication being uncomplicated vaso-occlusive crisis (77.7%). Also, 5 transfusion reactions occurred with 4 febrile non-haemolytic transfusion reactions (FNHTR) and a case of transfusion-related acute lung injury (TRALI). Post-training, 19 blood units were transfused with a single inappropriate transfusion request, indicated for uncomplicated vaso-occlusive crisis (5.26%, $p = 0.04$). A FNHTR was the only transfusion reaction recorded ($p = 0.19$).

Summary/Conclusions: There was a decrease in the total number of transfusions, transfusion inappropriateness, and adverse transfusion reactions, post-transfusion education. Continuous transfusion education among clinicians would help in improving clinical care among patients in constant need of blood components such as SCD.

P456 | Evaluating transfusion appropriateness and utilisation pattern of blood in Baptist Hospital Mutengene (BHM), Cameroon

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Background: Availability of blood and blood products is scarce in low-middle income countries (LMICs). Appropriate utilisation is necessary

for effective blood transfusion services in such settings. Inadequate knowledge and training in transfusion medicine, can lead to blood component misuse. Moreover, improving patient safety requires timely administration of the right blood component to the right patient for the right reason. Furthermore, this can influence the blood demand and thus blood supply inventory.

Aims: To evaluate the transfusion appropriateness and blood utilisation pattern in Baptist Hospital Mutengene (BHM).

Methods: A prospective hospital-based cross-sectional study was done from January 1 to November 30th, 2022 at BHM. Data were collected, entered, and analysed into EPI info version 7. This data included; the requesting wards, patient's gender, age, blood group, clinical condition, history of previous transfusion, type of blood component needed, and the number of units. Transfusion appropriateness was assessed by a criterion-based method which involved the trigger haemoglobin or platelet count, clinical condition, and decompensating factors. Significant blood usage was defined with a crossmatch to transfusion ratio (C/T) of ≤ 2.5, a transfusion probability (TP) ≥ 30%, and a transfusion index (TI) ≥ 0.5.

Results: A total of 1967 units were transfused to 1123 patients. Whole blood was the most common blood type transfused (99.2%) compared with packed red blood cells (pRBCs, 0.6%) and platelets (0.2%). The overall transfusion appropriateness was 100% for platelets and pRBC with 87.7% for whole blood. The overall C/T, TP, and TI were 1.27, 68.1%, and 1.19 respectively. The ICU was the service with the most efficient blood use with C/T, TP, TI of 1.04, 90.1%, and 2.12 respectively. Conversely, the orthopedic ward had the least efficient blood utilisation with C/T, TP, and TI of 1.47, 54.2, and 0.98 respectively.

Summary/Conclusions: There was a high transfusion appropriateness and significant blood usage in the institution. The most common blood component issued was whole blood. Implementing a maximum surgical blood ordering schedule (MSBOS) could improve the efficiency of blood usage in the orthopedic unit.

P457 | Autologous plasma reservation experience in obstetrics

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Background: It is known that one of the causes of obstetric bleeding in childbirth and the postpartum period is coagulopathy: disorders in the haemostasis system in parturient and puerperant. In low income countries the prob. of provision with immunological, infection-safe and effective blood components from regular unpaid donors with low rates of trans.-associated infections has not been solved; residual risk of transfusion-associated infections and immunological reactions remains. Clinical practice haemovigilance systems and alternative transfusion methods during planned caes. section in high-risk pregnant women are needed. Blood-saving methods such as autologous plasma reservation and intraoperative cell salvage reduce or eliminate the need for perioperative allogeneic blood transfusion and minimise the risk of transfusion reactions.

Aims: To show the effect of plasma donation in the pregnant woman and fetus and to evaluate the effectiveness of autologous plasma trans. on the caes. section outcomes.

Methods: The analysis includes the results of plasma donation in 412 pregnant women with caes. section in obstetric Republic of Tajikistan institutions, with the risk of obstetric bleeding due to gestosis, placenta previa, uterine scar, multiple pregnancy, polyhydramnios, bleeding during previous childbirth; transf. reactions because of the presence of anti-erythrocyte and anti-HLA antibodies; and patients who refused allogeneic transf. In 360 women at 32-36 weeks of pregnancy as a part for caes. section preparation, autologous plasma was prepared by plasmapheresis (Haemonetics PSC-2, Haemofenix or discrete intermittent plasmapheresis) in the Republican Blood Research Center and other medical institutions at a dose of 600 ± 50 ml. In 52 patients with gestosis and hypercoagulation, a dose of 850 ± 35 ml was prepared. Plasma was resuspended with crystalloid solutions, and in patients with a tendency to hypo- and dysproteinemia – with 100-200 ml 20% albumin solution. Plasmapheresis in all cases occurred after receiving the informed consent of pregnant women. The plasma was rapidly frozen and stored at -30C in transfusion therapy rooms until caes. section.

Results: Haemodynamic parameters in pregnant women with hyper- and eukinetic types of blood circulation did not change significantly: cardiac output was in the range of 5960.6 ± 46 6.7 ml/m^2 before plasma donation, 6064.3 ± 390.8 ml/m^2 at the end of the donation, stroke volume – 68.5 ± 5.9 ml/m^2 and 69.5 ± 4.5 ml/m^2 respectively, mean arterial pressure – 89.7 ± 3.1 and 88.1 ± 2.4, total peripheral vascular resistance – 1243.2 ± 71.6 and 1192.8 ± 61.3. Hypertension due to gestosis had a positive effect on mean arterial pressure and total peripheral vascular resistance which had decreased and renal filtration rate increased. Oxygen delivery index was stable and within physiological norms. Plasma donation did not cause changes in complete blood count, biochemical blood test, or the haemostasis system. The parameters of central haemodynamics did not change in normotensive women, a positive effect was noted in pregnant women with hypertension, gestosis (especially in patients who received albumin transf. for plasma volume substitution), improvement of placental-fetal blood circulation. There was no manifestation of hypoxia in newborns. Surgical blood loss during ceas. section was 389.0 ±51.1 ml.

Summary/Conclusions: In pregnant women with risk of bleeding, autologous plasma reservation at a dose of 600-900 ml in the third pregnancy trimester with subsequent trans. during ceas. section is an effective option to reduce maternal mortality due to prevention of haemostasis system disorders, inc. pathological bleeding and transf. reactions.

P458 | Type and Screen decision going bad

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Background: Cross-matched red blood cell (RBC) is often included in many patient's assessment protocols despite numerous audits demonstrating that these prepared RBC are never transfused. Type and screen (T&S) protocol is an important policy used by blood banks to reduce unnecessary reservation of RBC and increase blood inventory available for emergent cases. In our hospital, T&S requests are never routinely performed and it depends on transfusion medical staff to adapt and changed the RBC order into a T&S.

Aims: This study aimed to analyse all RBC orders changed, by transfusion medical staff, into T&S testing that afterwards need crossmatching. The purpose was to have a better understanding of which were the causes for cross-matching after T&S decision.

Methods: Two years of retrospective data were collected, 2021 through 2022, which included: patient demographics, diagnostic, all peri-operative blood tests including T&S samples and subsequent cross-matching. Cross-matched patients were reviewed to assess transfusion requirements, haemoglobin levels and clinical course.

Results: 8172 RBC requests made between 2021 and 2022. Among all RBC requests, 1446 (17.7%) were transformed in T&S. Of these, subsequent cross-match occurred in 136 (9.4%) and actually transfused in 115 (84.6%).

T&S decision was based in haemoglobin levels prior to request, median value 12.9 g/dL (range 6.5-18.2). In elective patients, haemoglobin level was obtained on average 26 days prior RBC request (range 0-175 days).

From 136 T&S cross-matched, 132 (97%) belonged to surgical patients 63% of whom were emergencies. Main reasons for cross-matching: postoperative anaemia (56%), haemorrhagic shock (12%), preoperative anaemia (11%), septic shock (7%), high-risk bleeding patient (7%), acute posthaemorragic anaemia (3%), chronic anaemia (3%) and acute gastrointestinal haemorrhage (2%). Mean haemoglobin levels at the time of T&S was 11.9 g/dL (range 6.8-16.3), and by the time of cross-matching was 8.3 g/dL (range 4.2-15.8). Mean of 1.6 RBC units prepared (range 1-6).

Regarding 115 T&S cross-matched and transfused, mean haemoglobin levels at the time of T&S was 11.9 g/dL (range 7.6-16.3), and by the time of transfusion was 7.9 g/dL (range 4.2-10.7). Mean of 1.5 RBC units transfused (range 1-6).

No surgery or patient's care were delayed due to lack of available blood.

Summary/Conclusions: Most patients who are typed and screened will not require a transfusion, which means unnecessary patient burden and costs. It would be efficient to further classify patients according to their risk of transfusion using objective and easy obtainable information. Moreover anticipating the potential need based on the transfusion medical staff own experiences and blood sample taken too far in advance of the scheduled surgery introduces problems due to limitations on accurate patient's status.

P459 | Over-ordering is a counter intuitive practice even with type and screen protocols

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Background: Blood and its components are costly, scarce and critical health care resources. With that said accurate estimation of surgical transfusion risk, based on transfusion medical staff expertise can reduce excessive cross-matching and unnecessary use of materials and workforce. Additionally, most patients who are typed and screened (T&S) before elective procedure will not require a transfusion, which means unnecessary patient burden and costs.

Aims: To analyse the burden of over-ordering type and screen protocols.

Methods: A retrospective analysis was made between 2021 through 2022, which included red blood cells (RBC) requests, T&S decisions, T&S samples afterwards cross-matched and/or transfused. Cross-matched orders were review to assess transfusion requirements. The overall cost of routine pre-operative blood typing was analysed. The cost associated to labour work were not included.

Results: The retrospective data from 2021 and 2022 showed 1446 transfusion orders converted to a T&S protocol. Of these, 1310 (90.6%) had no further testing and patient was discharged from the hospital without needing blood cross-matched. From the 136 T&S that subsequently were requested to be cross-matched a mean of 1.7 RBC units were prepared (range 1-6) and a mean of 1.5 RBC units were transfused (range 1-4). Twenty-one T&S protocols requested to be cross-matched had RBC that were never transfused, and returned to blood bank inventory. In these cases, a mean of 1.4 RBC units were cross-matched (range 1-2).

The overall cost of routine T&S requests was 10.637,2 euros (8,12 euros x 1310 tests). The costs of RBC cross-matched and not transfused was 9,3 euros (0.31 euros x 30 RBC cross-matched).

Summary/Conclusions: T&S may be indicated in patients in whom there is a low to intermediate intra-operative transfusion probability, as literature reveal that approximately 60% of all blood transfusions are given intraoperatively. Unnecessary testing for and ordering of blood products adds to overall healthcare costs.

Our hospital should evaluate transfusion requirements and build from scratch its own Maximum Surgical Blood Ordering Schedule to minimise unnecessary requests and thus improve its blood utilisation.

P460 | Epidemiology of blood transfusion recipients and trends in blood utilisation

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Background: Blood transfusions are common to treat patients with anaemia, traumatic bleeding, cancer, and chronic renal disease. However, over the past several decades, the trend of utilisation of blood has changed. Epidemiological studies of blood transfusion recipients can provide a more demographic information for future transfusions, which may help improve the effectiveness of blood component usage.
Aims: The aim of this study is to analysis the prevalence and characteristic of blood transfusion recipients and also trends of blood usage at hospital.
Methods: From 2011 to 2020, data on blood transfusion recipients and information on blood components were retrieved from Taichung Tzu-Chi Hospital in Taiwan. All patients aged 21 to 99 years who received blood transfusions were included, and major diagnosis (ICD-10) and demographic data were collected.All blood products were supplied by the Blood Center of Taiwan Blood Services Foundation. A total of 26,859 patients from 2011 to 2020 were included in this study, with exclusion criteria of age less than 20 years and missing diagnosis information. Detailed analyses were performed by sex and age (21-30, 31-40, 41-50, 51-60, 61-70, 71-80, and 80+ years).
Results: The prevalence of blood recipients increased significantly in the elderly, from 17.54% in 2011 to 28.15% in 2020. The average age of male recipients increased from 64.2 ± 15.6 to 67.3 ± 15.4, and female recipients increased from 65.9 ± 15.3 to 69.4 ± 16.2. The three most common diseases related to transfusion were cancer (26.88%), chronic kidney disease (17.27%), and diseases of the esophagus, stomach, and duodenum (16.16%). In this study, hospitalized patients accounted for 61.65% of the blood recipients, with surgical patients, accounting for 16.76%, intensive care unit patients, accounting for 7.94%, and emergency / outpatient patients accounting for 6.84% and 6.80%, respectively.
Summary/Conclusions: The demand for blood transfusions among the elderly is gradually increasing, particularly in the ageing population with a high prevalenceof anaemia and other chronic diseases such as chronic renal disease. In clinical, blood transfusion is a critical and supportive therapy in patients with cancer and gastrointestinal bleeding. The epidemiological study and the trend of blood utilisation help us understand more about the changes in transfusion characteristics.This study underlines the clinical awareness of transfusion and is useful for the future predictability of the utilisation of blood components.

P461 | Abstract withdrawn

P462 | Platelet transfusion and RhD alloimmunization, is prophylaxis still required?

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Background: Managing platelet inventory is one of the most challenging activities in the transfusion medicine service. Matching the ABO and the Rh-D type could sometimes be unfeasible due to limited inventory and high demand. Theoretically, platelet transfusion may induce Rh-D sensitization due to residual RBCs in the whole blood-derived platelets or apheresis platelets. In our practice, if Rh-D negative platelets are unavailable for Rh-D negative patients, anti-D immunoglobulin is issued for males under 18 years old and females with childbearing potential to cover 5 units of platelets. In this study, we wanted to assess the incidence of Rh-D alloimmunization for Rh-D negative patients who received Rh-D positive platelets without receiving anti-D immunoglobulin prophylaxis.
Aims: Our objective was to assess the incidence of Rh-D alloimmunization rate in all our Rh-D negative patients who received Rh-D positive platelet transfusions (apheresis or whole-blood derived) and did not receive anti-D immunoglobulin prophylaxis.
Methods: We retrospectively reviewed the medical records of all our Rh-D negative patients who received Rh-D positive platelets and did not receive anti-D immunoglobulin prophylaxis in the period between January – December 2022 and recorded the antibody screen until the first of March 2023.
Results: A total of 111 patients were reviewed. A follow-up antibody screen was available for 83 patients only, 70 males and 13 females. The median age is 49 years, range (4 months-84 years). During this period, 509 platelet units were transfused. The median number of units transfused per patient is 2, range (1-65). The median period between the last transfusion and the follow-up antibody screen is 26 days, range (1-384). No patient has developed anti-D during the follow-up period
Summary/Conclusions: The risk of Rh-D alloimmunization due to platelet transfusion is minimal. This outcome encourages re-evaluating the need for anti-D immunoglobulin prophylaxis in our patient population, who are usually admitted for a long period, may require a large number of platelet transfusions, and may receive large doses of Rh-D immunoglobulin prophylaxis.

P463 | Prediction of short-term and mid-term platelet transfusion using viscoelastic test in trauma patients

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Background: Platelet products are the most difficult to manage due to their limited storage time (five days). Rotational thromboelastometry (ROTEM; Tem International GmbH), one of the viscoelastic test, is a global coagulation test that guides the evidence-based platelet transfusion of trauma patients. However, studies on ROTEM parameters directly related to platelet transfusion are very limited.

Aims: This study aims to evaluate ROTEM parameters for predicting short-term (24 hours) and mid-term (five days) platelet concentrate (PC) transfusion in trauma patients.

Methods: The maximum clot amplitudes after 5, 10, and 15 minutes (A5, A10, and A15) of fibrin-specific ROTEM (FIBTEM) and extrinsically activated ROTEM (EXTEM) were retrospectively collected from 82 stable trauma patients with hospitalization after successful initial resuscitation, excluding the patients who expired within five days or who were transfused due to surgery. PLTEM was calculated by subtracting FIBTEM from EXTEM. The result of conventional coagulation tests was collected and platelet transfusion for 24 hours and five days after ROTEM testing was reviewed.

Results: Respective areas under the curve using EXTEM, FIBTEM, and PLTEM were 0.841–0.854, 0.824–0.835, and 0.623–0.769, respectively, for predicting short-term PC transfusions of over four units, and 0.878–0.896, 0.915–0.923, and 0.551–0.735, respectively, for predicting mid-term PC transfusions of over 12 units. All EXTEM and FIBTEM parameters were comparable to platelet count and PFA-100 (Siemens) results (per platelet count) in the short-term prediction, and to fibrinogen, fibrinogen/fibrin degradation product, D-dimer, and antithrombin III in the mid-term prediction. High correlations (r>0.7) were noted between platelet counts and EXTEM (A5, A10, and A15) or PLTEM (A5), between platelet function (per platelet count) and EXTEM (A10 and A15), and between fibrinogen level and all FIBTEM parameters.

Summary/Conclusions: EXTEM and FIBTEM can be reliably used to predict mid-term and short-term PC transfusions in trauma patients. Also, EXTEM, FIBTEM, and PLTEM parameters correlate with the result of conventional coagulation tests related to platelet or fibrinogen.

P464 | Transfusion needs after CD19 CAR T-cells for large B-cell lymphoma: Predictive factors and impact on outcome

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Background: CART-cells targeting CD19 have been approved for the treatment of relapse or refractory (R/R) large B-cell lymphoma(LBCL). Patients undergoing CART-cell therapy may experience cytopenias due to lymphodepleting chaemotherapy and/or CART-cells, which may require red blood cells (RBC) and/or platelets (PLT) transfusions. Transfusion needs represent a surrogate marker of severe cytopenias after CART-cell therapy. Furthermore, transfusion needs may impact patients' quality of life. Finally, transfusions may impact CART-cells efficacy through transfusion-related immunomodulation.

Aims: To describe the transfusion needs of patients receiving commercial CD19 CART-cells for LBCL, identify predictive factors associated with transfusion needs and search for correlations between transfusions and CART-cells efficacy.

Methods: Clinical data were collected form the DESCAR-T registry which is the French national real-life registry for all patients treated with commercial CART-cells. Transfusion data were collected from the French national blood bank. Merging these two databases allowed us to study the relationship between CART-cell therapy and transfusions. We included patients with at least 6months follow-up. Patients were censored for transfusions at relapse, new treatment onset, or death.

Results: Between Aug.2018 and Sept.2022, 671 patients registered in the DESCAR-T registry met the eligibility criteria: 429 (63.9%) treated with Axi-cel and 242 (36.1%) with Tisa-cel. At the time of infusion, median age was 63 years (range, 18-82), 15.3% of patients had a performance status (PS) ≥ 2, and 45.6% were refractory for first line

therapy. Median number of prior lines was 2, 17.6% had received a prior autologous stem cell transplantation (ASCT). Overall, 82.7% of patients received bridging therapy, including 69.9% chaemotherapy. The CAR-HEMATOTOX score was 0, 1 or ≥2 in 32.9%, 37.8% and 29.3% of patients, respectively. Overall, 382 patients (56.9%) received at least one transfusion after CART-cell infusion, either at the early phase (i.e. within the first month) or at the late phase (i.e. beyond one month). The mean number of RBC and PLTs transfusion per patient were 3.5 (range, 0-90) and 5.1 (range, 0-127), respectively. At the early phase, 359 patients (53.5%) received at least one transfusion: 317 (47.2%) patients received RBC and 252 (37.6%) patients received PLTs transfusions. At the late phase, 208 patients (37.8%) received at least one transfusion (32.5% RBC and 33.3%PLTs). Factors associated with transfusion needs after CART-cells therapy : Interestingly, age, primary refractory status, prior ASCT, and severe CRS were associated with early but not late transfusion needs. No association was found between transfusions and best overall response rate after CART-cell therapy. However, early transfusions (RBC and/orPLTs) were associated with a shorter progression-free survival (median PFS = 3.2 vs 6.0 months, $p = 0.0168$) and overall survival (median OS = 9.3 vs 23.6 months, $p < 0.0001$). Late PLTs transfusions were associated with decreased PFS (median = 5.6 vs 12.0 months, $p = 0.0072$) and OS (median = 13.8 vs not reach, $p < 0.0001$) whereas late RBC transfusions did not impact PFS nor OS.

Summary/Conclusions: In our study, 56.9% of patients received transfusions after CART-cell therapy, including 53.5% during the 1st month and 37.8% beyond month 1. We identified specific risk factors associated with early and late transfusion needs. Early transfusions (RBC and PLTs) and late PLTs transfusion (but not late RBC transfusion) were associated with worse survival (PFS and OS). Our data shed light on the mechanisms of early and late anaemia and thrombocytopenia, and on the potential impact of transfusions on CART-cells efficacy.

P465 | Criteria for defining refractoriness to platelet transfusion: A prospective clinical study

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Background: Patients requiring extensive platelet transfusion support may develop a platelet transfusion refractoriness (PTR). The etiology of PTR may be immune or non-immune. Immune PTR is related to the presence of circulating anti-HLA-I alloantibodies, or to a lesser extent anti-HPA antibodies, which results in rapid elimination of transfused platelets. The non-immune PTR is linked to any other platelet consumption factors as bleedings or inflammation. In France, PTR is defined as two repeated transfusion failures with fresh, ABO-compatible platelets in an amount appropriate to the patient's weight. To assess transfusion efficiency, the increase in corrected platelet count (CCI) is measured 1 hour and 24 hours after transfusion. A satisfactory CCI at 1 h, but low 24 h later is considered as a non-immune PTR whereas a poor CCI at both 1h and at 24 h is considered as an immune PTR. Moreover, the diagnosis of an immune PTR cannot be drawn if non-immune factors are present. All of these strict criteria may lead to an underestimation of the incidence of PTR in France.

Aims: To evaluate the occurrence of PTR in a hematology-oncology department and re-assess the criteria required to establish a diagnosis of PTR.

Methods: A prospective clinical study on transfusion yield within the Hematology Department of the "Institut de cancérologie Strasbourg Europe (ICANS)" was conducted. Patients included in this study received chaemotherapy for hematopoietic stem cell transplantation (allogenic or autologous) or for acute leukemia treatment (induction or consolidation). Platelet counts at 1h and 24h post transfusion, non-immune factors explaining potential platelet consumption, patients weight/height, and platelet concentrate characteristics (platelet quantity, storage duration, ABO phenotype) were collected online, by completing a secured structured database. Presence of anti-HLA I antibodies was measured in sera samples.

Results: First results indicated that 74% of platelet transfusions (34 out of 46) did not lead to a satisfactory increase in platelet count at 1 hour and/or 24 hours. Out of these, anti-HLA-I antibodies were found in sera from 17 patients either in combination with (7) or without (10) non-immune factors (i.e. inflammation and/or bleedings). Interestingly, among those 10 patients with anti-HLA-I antibodies identified as the only factor explaining the PTR, 5 of them had satisfactory 1-hour CCI values. In addition, 12 transfusions had inadequate CCI values at 1 hour and 24 hours without evidence of anti-HLA I antibodies or anti-HPA, 4 of which had no factors associated with refractoriness.

Summary/Conclusions: These first results obtained in the frame of this clinical study suggest an underestimation of the incidence of immune PTR in France and highlight the unreliability of 1-hour and 24-hour CCI values to distinguish immune from non-immune PTR. A larger cohort should corroborate the need to reassess diagnostic criteria for PTR.

P466 | Abstract withdrawn

P467 | How liberal are we being with the Hemato-Oncology Department

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Background: Red blood cell (RBC) transfusion plays a vital role in the supportive care of patients receiving therapy for hematological disorder or chronic transfusion-dependent anaemia. Currently, insufficient

evidence is available to recommend a restrictive RBC transfusion approach in these patients. The lack of evidence-based guidelines leads to variable transfusion practices among clinicians. The benefits of transfusing blood products are obvious in life-threatening low blood cell counts or bleeding, but it is becoming apparent that deliberate blood transfusion in some cancer patients can trigger negative clinical outcomes.

Aims: To audit transfusion trends in Hemato-Oncology Department. The purpose was to have a better understanding of how liberal the blood bank is regarding these RBC requests.

Methods: Data on RBC requests and consumption were retrospectively collected from patient's database and blood bank records between 2018 to 2022. Clinical and laboratory records in which variables such as sex, age, clinical diagnosis, RBC requested, RBC transfused and haemoglobin level were analysed.

We performed a descriptive analysis using Microsoft Excel 2016. Two parameters were calculated from the data: Cross-match to Transfusion (C/T) ratio and Transfusion index (Ti) (number of transfused RBC units /number of cross-matched RBC units).

Results: A total of 1335 transfusions requests, corresponding to 176 patients (91 women, 85 men), were analysed. A median of 3 transfusion request per patient was observed (range 2 - 20). Patient's median age was 81 years old (range 48-99) and the following clinical diagnosis were observed: 44 multifactorial anaemia, 44 myelodysplastic syndromes (MDS), 18 Non-Hodgkin lymphoma, 18 Multiple myeloma, 13 acute leukaemia, 10 iron deficiency anaemia, 9 symptomatic anaemia of unknown cause, 4 chronic anaemia, 4 chronic lymphoproliferative disorders, 4 chronic leukemia, 2 Hodgkin lymphoma, 2 myelofibrosis, 2 autoimmune haemolytic anaemia, 1 megaloblastic anaemia and 1 pancytopenia due to liver disease.

Among all transfusions requests, 98.8% (1319) resulted in RBC transfusion and from these 81% (1069) were adequate order considering the patient's clinical status and haemoglobin level. From the remaining 250 transfusions requests that resulted in RBC transfusion and were considered overordered, 179 were for one RBC unit, 66 for two RBC units and 5 for three RBC units.

Regarding RBC units ordered a mean of 2 units/patient (range 1-5) was observed. 2109 RBC units were crossmatched and 2075 were transfused, C/T ratio of 1,02 and Ti of 0.98.

Haemoglobin evaluation showed a median value from all RBC requests was 7.2 g/dL (range 3.5-16.6) and for those patients that were actually transfused 7.1 g/dL (range 3.5-9.5).

Summary/Conclusions: In our Hemato-oncology department we observed a tendency to over-transfuse in haemodynamically stable outpatients. This practice is probably caused by an attempt to reduced symptom burden. In haemodynamically stable patients, when haemoglobin level is the sole consideration, a restrictive transfusion approach (haemoglobin threshold of 7-8 g/dL) should be used

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Clinical transfusion

Haemorrhage and massive transfusion

P470 | Transfusion management of massive bleeding patients – a single-center experience

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Background: Massive bleeding (MB), which often leads to trauma induced coagulopathy (TIC), continues to be a primary cause of potentially preventable deaths, if not diagnosed and treated on time. For a successful treatment of MB patients each hospital should have algorithms for initial resuscitation of patients and blood components provided according to massive bleeding protocols (MTPs). For the ongoing treatment, point of care (POC) viscoelastic haemostatic assays (VHA) should be used as a goal directed therapy.

Aims: The aim of this study was to analyse characteristics of patients with MB, blood components used for the treatment and outcome of these group of patients.

Methods: This is a single-centre retrospective analysis of patients with MB. The data was collected from the transfusion and hospital information system. Analysis was conducted for patients over 18 years who received massive transfusion (MT) in a period of one year (1.1.2021. – 31.12.2021.).

Results: In total, 207 patients (67% male) were analysed at the median age of 65 (18-87) years. Fresh frozen plasma (FFP) was transfused to 94.2% patients with the ratio of red blood cell (RBC) units to FFP of 1:0.6. Platelet concentrate (PC) was transfused to 54.1% patients with the RBC:PC ratio of 1:1.3. Mean number of units transfused per patient were: 11 RBC, 7 FFP and 14 PC. Fibrinogen concentrate (FC) or cryoprecipitate (CRYO) received 77.8% patients, prothrombin complex concentrate (PCC) 18%, while only 6% received factor concentrate XIII (FCXIII) and 5% factor concentrate VII (FCVII). PCC and FCXIII were mainly transfused at the Department of Surgery and FCVII at the Department of Cardiac Surgery. Out of all analysed patients, 42 (20.3%) patients died in 30 days, while 4 (1.9%) of them died in the first 24 hours due to bleeding. In total 32 (15.5%) patients received ≥ 10 RBC units in 24 hours.

Summary/Conclusions: Following ratios of RBC:FFP and RBC:PC transfusion treatment as defined by MTPs has been very beneficial in improving outcome of MB patients. Low death rate of MB patients in first 24 hours indicates a very effective aggressive treatment by POC goal directed therapy.

P471 | Development of an immunodeficient mouse model of massive haemorrhage with acute thrombocytopaenia to validate the haemostatic effect of human platelet transfusion

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Background: As massive haemorrhage often causes acute thrombocytopaenia and/or platelet dysfunction, platelet transfusion is critical for rapid haemostatic control in massively bleeding patients. Due to the short shelf-life of platelet concentrates (PCs), it is challenging to conduct inventory management and timely transfusion of PCs in such urgent situations. Thus, more efficient PCs such as cold-stored PC suspended in an adequate platelet additive solution (PAS) with a longer shelf-life and better haemostatic functions should be developed. For this purpose, the establishment of animal models, in which the functions of human PAS-PCs containing human plasma can be evaluated, is crucial as a preclinical investigation.

Aims: This study aimed to develop an immunodeficient mouse model of massive haemorrhage with acute thrombocytopaenia and validate the haemostatic effect of human PC transfusion in the model.

Methods: Using wild-type ICR mice under general anaesthesia, the frequency and volumes of arterial exsanguination required for developing massive haemorrhage with acute thrombocytopaenia were investigated. Afterwards, the established strategy was applied to immunodeficient NOD scid gamma (NSG) mice to develop a model, which mimics trauma-induced acute thrombocytopaenia and is feasible to transfuse human PCs. After intravenous infusion of 10 ml/kg PAS-PC ($1x10^9$platelets/mL) or equal amount of 5% human albumin as a control, the thrombocytopaenic NSG mice underwent punch biopsy for liver bleeding and subsequent periodic compression by a balloon occlusion catheter until achieving haemostasis. The time from liver punch to haemostasis was measured. Human PAS-PCs suspended in 65% PAS-E (T-PAS+, Terumo BCT) with 35% plasma were prepared by pooling several PCs with the same ABO blood type or single-donor apheresis PCs. The study protocol was approved by the IACUC of the Jikei University.

Results: After general anaesthesia and tracheal intubation, wild-type ICR mice underwent surgical placement of arterial catheters into left and right femoral arteries for exsanguination and arterial pressure monitoring, respectively, and a central venous catheter into right femoral vein. After 5 times of 10 ml/kg exsanguination from left femoral artery and infusion of 10 ml/kg 5% albumin to right femoral vein, the exsanguinated mice showed platelet counts of $366 \pm 46 \times 10^9$/L while those of non-exsanguinated mice were $820 \pm 89 \times 10^9$/L ($n = 3$ each). The bleeding times of these non-exsanguinated and exsanguinated mice were 2.7 ± 1.2 and 12.0 ± 2.0 minutes, respectively. In the setting of immunodeficient mice, the bleeding time of non-exsanguinated NSG mice was 3.3 ± 1.2 min ($n = 3$). Exsanguinated mice transfused with human PAS-PC showed shorter bleeding time compared with albumin-treated exsanguinated mice (5.3 ± 2.3 vs 13.3 ± 1.2 min; $n = 3$ each). Additionally, the PC-transfused exsanguinated mice had platelet counts of $1{,}300 \pm 154 \times 10^9$/L, of which human platelets were 61.4 ± 3.0%.

Summary/Conclusions: We developed a new immunodeficient mouse model of massive haemorrhage with acute thrombocytopaenia, mimicking class IV haemorrhagic shock in terms of haemodynamics and blood loss (>40%). It should be noted that the haemostatic effect of human PAS-PCs containing human plasma can be evaluated using this model, in contrast to the previous animal models of massive haemorrhage (Morgan, JAMA Surgery, 2015). Investigating the haemostatic function of human cold-stored platelets is underway in this model.

P472 | An approach to emergency transfusion in trauma and haemorrhage: analysis of urgent blood supply cases in Slim River Hospital Malaysia

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Background: A good understanding of the physiology of haemorrhagic shock and the implications of treatment strategies is essential to achieve a favourable outcome in such patients. Transfusion support is vital for a patient of trauma with haemorrhagic shock.

Aims: We aimed to characterise trauma cases that require urgent blood in this hospital.

Methods: This was a retrospective study performed from 1st January 2023 until 10th February 2023. A data collection will be based on transfusion record in Pathology and Transfusion Department for one-year period (January until December 2022).

Results: The patients who required urgent blood (packed cells) were categorized according to discipline, gender and diagnosis. Urgent blood supply showed 109 units out of 2,640 total blood in stock (4.13%) in year 2022 in this hospital. The patients who received urgent blood (83 patients) was categorized in three (3) disciplines; Emergency and Trauma, 64 out of 83 (77.11%), Surgical and Anaesthesiology, 13 out of 83 (15.66%), and Medical, six (6) out of 83 (7.23%). Gender differences showed male, 46 out of 83 (55.42%), female, 35 out of 83 (42.17%) and non-applicable, 2 (two) out of 83 (2.41%). The diagnosis differences showed trauma cases, 77 out of 83 (92.77%), and medical base (COVID19), 6 out of 83 (7.23%).

Summary/Conclusions: The data serve as a hospital database to revise our safe and critical level of blood stock. The trauma cases that required urgent blood in this hospital have been categorized.

P473 | Retrospective audit analysis: effective tool to ensure patient safety for massive transfusions

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Background: Maintaining transfusion safety is a huge and uphill task in a tertiary care set-up, based mainly on replacement donations. Our incessant challenge is not only to ensure timely and safe product delivery for heavy surgical, oncology, dialysis and complex obstetric patients, but also to serve the special demand areas like liver, bone marrow & kidney transplants in a 500 bedded JCI accredited hospital. We still are in the process of establishing real-time/prospective blood utility audits for improving our special needs, meanwhile retrospective blood audits are the main tools to help us forecast precisely the product type, quantity & special requests. We analyse regularly our retrospective audits of emergency issuance and massive transfusions besides reviewing transfusion reactions, consents and transfusion notes. A manual form is essentially filled by the physician incharge to document the most likely cause of massive transfusion.

Aims: to improve & enhance patient safety by augmenting our preparedness & planning, after analysis of retrospective haemovigilance record of massive transfusion

Methods: data collection & analysis.

We share our review of massive transfusion data since January 2012 till December 2022, with the eligibility criteria of issuance to a single patient of more than or equal to five red cell concentrates(RCC) within 24 hours. We have excluded the living donor liver transplant procedure of this data as their routine pre-operative demand is 10 RCC units.

Results: A total of 813 patients received massive transfusion, age ranged between 7-99 years with an average of 52 ± 16 years. The number of RCC ranged between 5-32(7 ± 3) units, issued to 489 male (61%) and 314 female (39%) patients. Specialty specific distribution showed maximum demand from General surgery 203, followed in sequence by hepatobiliary 121, Gynae & obstetrics 73, cardiac surgery 57, Neurosurgery:42 & Plastic surgery 41, Oncology:6 patients, who received massive transfusion. Rest of the 270 cases of massive transfusions were from different medical units including dialysis.

Summary/Conclusions: Massive transfusion record review is an authentic tool for improving & re-defining the future needs to specific, acute and critical service areas. Our decade long data though retrospective, but still reasonably indicate the demand patterns and can guide us to improve our level of preparedness for critical patients. In future we are adding the procedure & surgeon in-charge specific filters to make it more effective & reflective of the purpose.

P474 | Circulating humoral mediators in nonhuman primate model of controlled blood loss

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Background: There is currently a global shortage of human blood for medical use. Substitutes for human red blood cells are being developed. One alternative is artificial red blood cells derived from stem cells, which are currently limited by high production costs. Xenotransfusion of red blood cells from genetically engineered pigs is also emerging as an alternative. Non-human primates (NHPs) are primarily used prior to human clinical trials due to their similarity in anatomical structure and physiological characteristics to humans. However, little work has been done on xenotransfusion. In this study, we established a controlled bleeding loss model for NHPs before performing xenotransfusion in NHPs.

Aims: The main aims of this study were (1) to investigate the immune response during bleeding in the NHP model and (2) identify appropriate biomarkers for follow-up.

Methods: Ten cynomolgus monkeys (*Macaca fascicularis*; *5 females and 5 males*), 2 years 9 months to 3 years 4 months old, 2.19–2.70 kg body weight, were used. To develop the NHP bleeding model, each 10%, 14%, 18%, 22%, and 25% amount of the total blood volume was removed from the monkeys as interventions. The blood parameters of the monkeys were analysed on days -14 (as baselines, at least 2 weeks before the intervention), 0.0, 0.1 (immediately after the bleeding intervention), 1, 3, 5, 7, 14 and 21. Humoral mediators in the blood were measured by flow cytometry: interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, monocyte chaemoattractant protein-1(MCP-1) and tumour necrosis factor. IFN-γ, IL-1α, and IL-15 levels were measured by enzyme-linked immunosorbent assay. To assess the extent of complement activation and thrombosis development, C3a, C4a and factorBb were measured. All data are presented as the mean±SD of two independent experiments. Statistical analysis was performed by Pearson correlation and mixed effect model.

Results: Proinflammatory cytokines such as IL-1α, IL-8 and IL-15 peaked before bleeding on the day of intervention, and peaked again on day 5 after intervention. Complement activation of C3a and C4a showed a similar pattern to the proinflammatory cytokines. IFN-γ decreased after intervention (on day 0.1), and gradually returned to baseline levels. In the process of returning from reduction, there was a tendency to peak on day 5. The greater the degree of controlled blood loss, the lower the IFN-γ levels. Compared with the 10% blood loss condition, the IFN-γ level decreased significantly with higher blood loss (14%, 18%, 22% conditions). Meanwhile, the MCP-1 levels peaked on day 1 after the intervention and gradually decreased to the baseline levels. The higher the degree of blood loss, the higher the MCP-1 levels. Compared to the 10% blood loss condition, the MCP-1

level increased significantly when the blood loss was higher (14, 18, 22% conditions).

Summary/Conclusions: In the NHP model of controlled blood loss, the presence of bleeding alone triggered an immune response. The MCP-1 showed a reactive pattern after the blood loss. It is necessary to further investigate the role of MCP-1 in the transfusion process and this study could serve as the basis for a future model of NHP transfusion.

P475 | Variability of thrombelastometry EXTEM assay parameters in samples with systematically manipulated hematocrit and platelet count

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Background: Thromboelastometry is a well-established method for assessing the haemostatic capacity of bleeding patients, as well as guiding the treatment in patients undergoing massive transfusion. A previous study from Holland (Noorman and Hess, Transfusion, 2018) found a successively increased strength of blood cloths as estimated by thrombelastography parameters to be associated with declining hematocrit (EVF) and with increasing platelet count (PLTC) in samples consisting of blood components mixed systematically in various ratios. Inspired by this study, we aimed to investigate how different levels of EVF and PLTC in manipulated blood samples affect parameters in the ROTEM EXTEM assay.

Aims: To investigate the impact of variations in EVF and PLTC on ROTEM EXTEM assay parameters: Clot time (CT), clot formation time (CFT), amplitude 10 minutes (A10) and maximum clot formation (MCF)

Methods: One unit of fresh frozen plasma (FFP), and one unit of red cell suspension in SAGM were retrieved from the Odense University Hospital Blood Bank. Thawed plasma was platelet-depleted using a high-speed centrifuge, and the red cells in the SAGM unit were isolated by centrifugation followed by removal of most of the suspension-medium, thus creating packed red cells. Hematocrit of the packed red cells and PLTC in the platelet-reduced FFP were measured prior to use.

With the purpose of having fresh platelets, blood samples in 2,7 ml whole blood in citrate anticoagulation tubes were collected from 2 healthy volunteers. Mixtures of packed red cells, platelet-reduced FFP, and fresh citrate-stabilized whole blood were created based on a spreadsheet algorithm to produce 21 manipulated samples, each simulating whole blood from a bleeding/transfused patient, with PLTCs of 0, 15, 30, 50, 75, 100, and 175 10^9/L and EVFs of 25%, 35% and 45%. All EXTEM tests were run on a ROTEM delta using the standard EXTEM assay settings in simultaneous triplicates for a minimum of 30 minutes.

Results: All EXTEM parameters to some extent varied systematically as a function of EVF and PLTC. Clot time measurements proved difficult to reproduce as there was a high variation between triplicates at low PLTCs. For the lowest EVF (25%), all triplicate values was at the low end of the CT reference range, even at PLTC 0. For CFT, the measurements were more reproducible. At EVF 45 the CFT was within reference range at PLTC 100, at EVF 35% it was at PLTC 50 and for EVF 25% it was at PLTC 30. Both A10 and MCF varied systematically and in a reproducible manner with EVF and PLTC. In both cases, the curves (amplitude in mm as a function of PLTC) representing EVF 25% and EVF 35% were practically identical and the curve representing EVF 45% was "behind" the two others. As a consequence, the amplitude was within reference range for A10 for EVF 25/35% at PLTC 75 and at PLTC 175 for EVF 45%. Results for MCF were similar, although reference level amplitude was reached at lower PLTCs; EVF 25/35% at PLTC 50 and EVF 45% at PLTC 100.

Summary/Conclusions: By manipulation of samples with regard to EVF and PLTC it was demonstrated that regarding the EXTEM parameters, CT, CFT, A10 and MCF there was a clear dependence on sample EVF and PLTC. Overall, low EVF appear to "compensate" for low PLTC in a manner that at the lowest EVF, the samples were within EXTEM reference ranges even at very low PLTCs. This phenomenon must be taken into account when evaluating ROTEM EXTEM curves from patients with low hematocrits.

P476 | Abstract withdrawn

Clinical transfusion

Adverse events, including TRALI

P477 | Appropriateness of transfusion reaction investigations

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Background: It is important to distinguish between minor transfusion reactions and serious, even life-threatening reactions. When a transfusion reaction occurs, the transfusion should be interrupted immediately and an assessment of the patient's clinical state. In patients with mild reactions, transfusions may be carefully recommenced with symptomatic treatment only, whereas for more severe reactions thorough investigations to determine the cause may be required. These should be targeted to the differential diagnosis. For example, culturing

the unit, culturing the patient and serological investigations may all be required for a marked fever.

Aims: To determine whether transfusion reaction investigations are ordered appropriately and consistently in a single tertiary centre.

Methods: This study was a retrospective analysis of transfusion reactions reported to the laboratory in an Australian tertiary referral centre between 2015-2020. The centre has compulsory training in transfusion and transfusion reactions. There is a transfusion policy that includes management of adverse events and the recommendations for managing transfusion reactions were also readily available with clinical transfusion documents at the point of care. Due to past inconsistencies, the laboratory protocol is to culture all units returned to it where suitable. Data were collected from all transfusion reactions reported to the laboratory. Transfusions were grouped into likely reactions based on the presenting signs and symptoms of the suspected reaction. The requesting of investigations was compared to local recommendations for the possible reactions being investigated.

Results: Over the six year period there 274 suspected transfusion reactions. Of these 226 reported symptoms of fever or had a temperature rise of more than $1^{O}C$. Of the requests reporting symptoms of fever, 67 did not have a temperature rise of $1^{O}C$ or more and 3 had a fall in temperature from pre-transfusion levels and 38 did not have a temperature reach $38^{O}C$ or more. However, of the 226 patients investigated due clinical features including fever, 224 (99%) had follow up blood counts and creatinine measurement. Apart from repeat serology conducted on all pre and post transfusion samples in accordance with laboratory protocols, markers of haemolysis were less reliably requested with bilirubin measured in 212 (94%), but LDH and haptoglobin in 142 (63%) and 123 (54%), respectively. Sources of infection were also less reliably requested with 197 (87%) of patients having blood cultures and 119 (53%) having urine cultures requested. For minor allergic reactions ($n = 15$) the corresponding rates were similar with 15 (100%) having blood counts and creatinine, 13 (87%) had bilirubin, 9 (60%) had LDH and 8 (53%) had haptoglobin. Blood cultures were performed in 9 (60%) and urine cultures in 6 (40%).

Summary/Conclusions: Investigation for transfusion reactions was incomplete (not all recommended tests for each differential diagnosis were requested) and indiscriminate (the requests frequencies were similar for suspected febrile and minor allergic reactions). Improved guidance for clinicians at the point of care is required.

P478 | Collection errors reported to SHOT leading to the wrong component being transfused or the wrong patient receiving a transfusion 2017-2021

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Background: Wrong component transfusion (WCT) errors continue to be reported to Serious Hazards of Transfusion (SHOT) the UK's independent, professionally led haemovigilance scheme. When collecting the component, the staff member is required to take documentation containing the patient's core identifiers to the designated storage device. Further checks must include the correct component type, expiry date and on receipt in the clinical area a check that the correct blood component has been delivered.

Aims: To identify key themes in error reports relating to collection of blood components resulting in a WCT.

Methods: A retrospective analysis of reports submitted to SHOT between 2017-2021 was conducted to identify incidents where the primary error occurred at the collection of the component from the hospital transfusion laboratory (HTL) or a remote issue storage area.

Results: WCT errors accounted for 413 out of 13,714(3.0%) errors reported to SHOT. Collection errors accounted for 83/413(20.0%). The component was collected from the transfusion laboratory in 51(61.4%) reports, remote issue storage 18(21.7%), electronic release 1(1.2%), handed over by laboratory staff in 6(7.2%) and no data in 7(8.4%) reports.

In 56(67.5%) cases the patient received the wrong component, in 20(24.1%) the transfusion was given to the wrong patient, 2(2.4%) were a D-mismatch and there were 5(6.0%) ABO-incompatible (ABOi) red blood cell (RBC) transfusions. RBC were prescribed in 64(77.1%) cases, platelets in 12(14.5%), 5(6.0%) plasma components and 2(2.4%) platelets and plasma.

The following was the distribution of healthcare professionals involved in the errors in collecting blood components: porters 24(28.9%) and nurses 23(27.7%), with healthcare assistants (HCA) 17(20.5%). In 57(68.7%) cases the error was made by a member of staff deemed trained and competent in the collection of blood components, in 6(7.2%) reports they were not.

There were 35(42.2%) emergency transfusions, 21(25.3%) urgent, 24(28.9%) routine and there was no data in 3(3.6%) of reports. Most errors, 35(42.2%) occurred on general wards and 10(12.0%) Intensive Care Units, and 9(10.8%) in Emergency Departments. There were 15(18.1%) paediatric reports and 64(77.1%) adult, 4(4.8%) cases with no age details.

In 38(45.8%) of reports the collection was a manual process and in 18(21.7%) electronic, where the system was either available but not used or used incorrectly e.g., overriding alerts.

Short staffing, poor skill-mix and busy workloads were mentioned as contributory factors in 13(15.7%) cases, with over-reliance on agency or locum staff mentioned in 3(3.6%).

In 61(72.5%) cases the patient fully recovered but there were 3(3.6%) cases of major morbidity.

Summary/Conclusions: Preventable collection errors continue to occur and have the potential to cause patient serious harm or death. Most errors occurred when the transfusion was required in an emergency, putting the staff member under increased time pressure to collect the component. This combined with untrained staff collecting the component, increases the likelihood of an error. Training and competency assessment of relevant staff is vital to reduce this risk. A checklist may reduce error risk at this stage but whilst they are important, they may not pick up all errors.

IT systems were used in 18 instances but still resulted in an error. They are helpful, but to be effective they must be well designed and used appropriately, with staff avoiding overreliance on it.

P479 | Evaluation of clinical outcomes and healthcare resource utilisation in patients with sickle cell disease before and after suspected alloimmunization: Real-world evidence from 2011 to 2021

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Background: Red blood cell transfusions are a critical component of care for patients with sickle cell disease (SCD), with 90% of patients receiving at least one transfusion in their lifetime. While transfused SCD patients are at high risk (40–50%) for alloimmunization, real-world evidence demonstrating the impact of alloimmunization on clinical outcomes and healthcare resource utilisation remains limited.

Aims: To describe characteristics, outcomes, and healthcare resource utilisation trends among patients with SCD and who are suspected to be newly alloimmunised.

Methods: We utilised Cerner Real-World Data, a deidentified United States electronic health record database with records from >100 million patients. We selected all encounters (1/2011–12/2021) with patients aged ≥12 years who had a diagnosis of SCD. We then selected patients that had laboratory testing performed in a manner consistent with the detection of clinically significant minor RBC antibodies. The primary encounter where alloimmunization was suspected was used as the index date, at which patient characteristics were evaluated. We further restricted to patients who had available data for at least 1-year pre- and 1-year post-index date. Outcomes of interest were evaluated in the year before and after index and included rates of sickle cell crises and the number of inpatient hospital days among survivors, as well as mortality in the year after index.

Results: We identified 27,548 patients ≥12 years with SCD, of whom 288 were suspected to be newly alloimmunised (1,316 encounters). Of these, 191 patients met the data availability inclusion criteria and were examined as the final cohort. The mean patient age was 36 years (SD: ±16) and 126 (66%) were female. The majority of patients in the cohort were Black ($n = 173$ [91%]) and lived in the Midwest ($n = 107$ [56%]). Among patients who survived one year after suspected alloimmunization, the median number of inpatient hospital days was higher in the year following suspected alloimmunization versus the year preceding suspected alloimmunization (median [IQR]: 6.1 [13.9] days vs. 0.8 [7.9] days). The rate of patients with ≥1 sickle cell crises was comparable in the year before versus after suspected alloimmunization ($n = 110$, 58% versus $n = 111$, 58%). In the year after suspected alloimmunization, 13 patients (7%) died.

Summary/Conclusions: Because alloimmunization is not a structured variable in real-world datasets, it is relatively complex to identify alloimmunised patients. Using a methodology designed to identify newly alloimmunised patients with SCD, an increase in the total number of inpatient hospital days was observed in the year following alloimmunization compared to the year prior to alloimmunization. However, no increase in pain crises was detected. Patient mortality in the year after alloimmunization was 7%. While the study was not designed to assess causation, the findings do suggest that prevention of alloimmunization may carry important health benefits for patients with SCD.

P480 | Abstract withdrawn

P481 | TRALI: party of one; real-time haemovigilance demonstrates multiple event free transfusions

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Background: Transfusion-related acute lung injury (TRALI) is a potentially life-threatening transfusion related adverse event (TRAE). It is characterised by hypoxemia, hypotension, and fever. Both HLA and HNA antibodies have been implicated as causative agents. Real-time haemovigilance (RTHV) surveillance programs are uniquely positioned to capture data surrounding these events and aid in diagnosis.

Aims: We aim to highlight the diagnostic utility of a RTHV system and demonstrate the critical role it plays in patient care.

Methods: Our institution is a 678-bed hospital and ambulatory center which dispenses blood components for 100,000 patients annually. We have a RTHV system that monitors for TRAE. This system consists of a digital dashboard monitored by specialty trained nurses in a remote Haemovigilance unit (HVU). Patients receiving a transfusion have vital signs recorded at regular intervals in the electronic medical record (EMR). This data live stream populates on the dashboard and generates a transfusion risk score during and up to 12 hours posttransfusion. If a reaction is suspected, a transfusion medicine (TM) advanced practice provider is deployed to bedside to assess the patient. The encounter is then reviewed by a TM physician using the NHSN Haemovigilance Protocol to classify the event. Stored data is accessible for retrospective review. Once a TRALI case was recognised, quarantine proceedings prompted a retrospective review of the donor's history, the HVU dashboard and the EMR for five tangentially related patients.

Results: Over the course of a one-month period, the implicated donor donated three single donor platelets which were split into six products. HLA antibody testing performed prior to each donation was negative. Our RTHV system confirmed five out of the six patients did not exhibit a hypoxemic event. The isolated hypoxemic event was noted to have transient neutropenia, bilateral infiltrates on imaging and hypotension. Four out of six patients had no evidence of a TRAE. One patient, with a history of severe allergic reactions, was documented to have an allergic reaction characterised by itching, hives, and hypotension. While the patient was put on oxygen, no desaturation event was captured and posttransfusion imaging was unremarkable. Subsequent donor testing demonstrated the presence of HNA antibodies.

Summary/Conclusions: Informatics systems expand the realm of available data and eliminate underreporting of TRAE. Neither lack of antigenic target nor failure of clinical recognition account for the paucity of symptoms reported in the tangentially related cases. These observations further highlight the importance of patient factors in the pathophysiology of TRALI.

P481 – Table 1

	Initial HVU Score	Highest HVU Score	Initial Vitals	Cessation Vitals
Donation (5/31)				
1	0	8	BP: 100/45 RR: 18 O2Sat: 94% RA	BP: 83/55 RR: 29 O2Sat: 100% 2L/NC
2	0	0	BP: 119/65 RR: 20 O2Sat: Not measured	BP: 115/64 RR: 20 O2Sat: Not measured
Donation (6/7)				
3	1	1	BP: 164/69 RR: 18 O2Sat: 100% RA	BP: 160/97 RR: 14 O2Sat: 100% RA
4	0	4	BP: 108/59 RR: 18 O2Sat: 97% RA	BP: 102/72 RR: 17 O2Sat: 96% RA
Donation (6/21)				
5	0	16	BP: 113/73 RR: 18 O2Sat: 99% RA	BP: 90/60 RR: 24 O2Sat: 90% RA
6	1	1	BP: 110/51 RR: 18 O2Sat: 98% 2 L/NC	BP: 117/57 RR: 18 O2Sat: 95% RA

P482 | Case report: acute haemolytic transfusion reaction as an atypical presentation of paroxysmal cold haemoglobinuria

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Background: Case of a 94-years old female with chronic lymphocytic leukaemia diagnosed in 2016. During the summer of 2022 the patient was getting increasingly anaemic and ultimately required transfusion of red blood cells (RBCs). About half way during transfusion of the first unit the patient developed symptoms of an acute transfusion reaction including chills, dizziness, dyspnoea, hypertension, nausea and flank pain. The transfusion was immediately stopped, and the patient subsequently examined with regard to an acute haemolytic transfusion reaction. The analysis did not reveal anything conclusive regarding serological incompatibility. However, when transfusion with RBCs was repeated 4 weeks later the patient again developed symptoms of an acute transfusion reaction.

Aims: To elucidate the reason of the patients repeated acute transfusion reactions.

Methods: Routine work-up of the transfusion reaction including check of ABO compatibility, serological crossmatch, direct antiglobulin test (DAT) on a specimen obtained post-transfusion, visual inspection of serum with regard to haemolysis. Biochemical testing for haemolysis including serum haptoglobin, lactate dehydrogenase (LDH), and bilirubin. Further, a number of non-immunological reasons for haemolysis (e.g. accidental heating of the RBC-unit) were excluded. Finally, Donath Landsteiner's (DL) test was carried out. The DL test tests for the presence of biphasic antibodies (biphasic antibodies fix complement C1q to RBCs at below body temperature, dissociates and subsequently triggers haemolysis when complement is fully activated to completion at 37°C): The DL test is carried out by incubating the patient's serum with blood group O donor RBCs at 4°C. Prior to this donor blood group AB-serum may be added to ensure the presence of complement proteins. Next, the mixture is incubated at 37°C. If haemolysis occur only after incubation at 37°C, DL test is positive.

Results: The AB0 compatibility test and serological cross-match were negative, the DAT was completely negative (IgG and C3). The pre transfusion specimen was visually without signs of haemolysis but plasma of the post transfusion specimen was obviously haemolytic. Furthermore, laboratory findings regarding the post transfusion sample demonstrated a haemolytic process as well, including decreased serum haptoglobin, elevated bilirubin and elevated LDH. Following the negative immuno-haematological investigation, a number of rare non-immunological causes, e.g., haemolysis in the RBC unit before transfusion, bacterial contamination or untoward injury of the RBCs during transfusion, were investigated and ruled out. Unexpectedly, the DL test was positive indicated by a clear and distinct visual haemolysis after incubation at 37°C. A positive DL test is diagnostic for paroxysmal cold haemoglobinuria (PCH).

Summary/Conclusions: Extensive laboratory investigation revealed a positive DL test thus biphasic antibodies and the diagnosis of PCH as the cause of anaemia and repeated haemolytic transfusion reactions in an elderly, female patient. The mechanism for the transfusion reactions was probably that the RBCs units for transfusion are stored at 4°C and the transfusion of these cold RBCs induced haemolysis in the patient due to the biphasic antibodies in her circulation. In patients with PCH transfusion with RBCs should be administered by use of a blood warmer in order to prevent binding of the biphasic antibodies to and subsequent haemolysis of the transfused RBCs.

P483 | Transfusion reactions frequency in the Republic of Tajikistan

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Background: Improving the quality of erythrocyte antigens typing, screening of donor's and recipient's anti-erythrocyte alloantibodies, the implementation of standard operating procedures for patient identification and automated data recording systems, medical staff training, reduce the complications associated with transfusion care. However, given the biological nature of blood transfusion, which is allogeneic tissue transplantation, there is a risk of developing acute transfusion reactions (TR) – the main cause of serious blood transfusion complications.

Aims: To analyse TR cases in the Republic of Tajikistan medical institutions for 1989-2020.

Methods: A retrospective analysis of reporting documents of the republican and regional blood safety committees, hospital transfusion committees, blood transfusion stations and centers, blood transfusion hospital's departments, including data on the disposal of expired blood components and patient's medical histories, was carried out. TR cases were classified in accordance with the definition of the International Society of Blood Transfusion (ISBT) in 2013, according to the Serious Hazards of Transfusion (SHOT), actual from January 2022, the blood transfusion control module of the National Health Safety Network and the US Centers for Disease Control and Prevention from March 2021.

Results: During the reporting period, 4 058 687 allogeneic donor blood and components transfusions were carried out in the Republic of Tajikistan medical institutions. 1 953 cases of TR – 1 case per 2 078 transfusions were identified and registered. Among them are transfusion circulatory overload 1 : 88 232 transfusions ($n = 46$), TRALI – 1 : 60 577 ($n = 67$), transfusion–related dyspnea ($n = 7$ cases, registered since 2019), allergic reactions – 1 : 24 015 ($n = 169$), hypotension – 1 : 76 579 ($n = 53$), febrile non-haemolytic reaction – 1 : 3 566 ($n = 1\,138$), acute haemolytic reaction – 1 : 43 177 ($n = 94$),

delayed haemolytic reaction – 1 : 135 289 ($n = 30$), transfusion «graft versus host disease» – 1 : 312 206 ($n = 13$), delayed serological reaction ($n = 5$, in 2019-2020). There were also 336 cases of TP that were not included in the list of ten recommended types of TP, including posttransfusion purpura ($n = 13$), transfusion of haemolysed erythrocytes ($n = 6$), bacterial septic reaction ($n = 25$), other reactions ($n = 286$). Almost all cases of TRALI were associated with transfusion of plasma harvested from donors-women who had a history of multiple pregnancies. In many cases, the clinical manifestations of TR were not registered by the attending physicians, they were revealed during the analysis of the recipients' medical histories by transfusion medicine specialists during an external quality transfusion care audit.

Summary/Conclusions: According to the analysis, the most common cause of acute haemolytic transfusion reactions was ABO-incompatible blood transfusion as a result of mistakes during transfusion procedure. In 25-30% of cases, TR was registered in patients with inadequate indications for blood transfusion according to actual clinical blood management recommendations and protocols. The most effective solutions to reduce the rate of TR are the implementation of a restrictive blood transfusion strategy, the male donor's procurement for plasma-containing blood components preparation for TRALI development prevention and the creation of a national blood transfusion control service with a widespread TR cases electronic database.

P484 | Abstract withdrawn

P485 | Abstract withdrawn

P486 | Association of anaphylactoid or anaphylactic reaction with low haptoglobin without haemolysis in adverse blood transfusion reaction in an Asian population

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Background: Patients with anaphylactic non-haemolytic transfusion reaction are well known to be associated with IgA deficiency. However, relatively less known is their association with haptoglobin deficiency. Low haptoglobin or anhaptoglobinemia, a condition that has high incidence in Asia, may cause anaphylactoid reaction or anaphylaxis in severe cases.

Aims: We study the association of non-haemolytic low haptoglobin with anaphylactoid or anaphylactic transfusion reaction among patients reported for blood transfusion reaction at Singapore General Hospital (SGH) in Singapore.

Methods: A total of 263 cases of voluntarily reported blood transfusion reactions to the Blood Bank at SGH over a 30-month period from January 2017 – June 2019 were studied retrospectively to identify their presenting signs and symptoms. We analysed the results of serum bilirubin and serum haptoglobin, with high bilirubin accompanied by low haptoglobin as indication of haemolysis. The reference range of total bilirubin was 7-32 umol/L and haptoglobin was 0.37 – 2.70 g/L.

Results: Out of 263 patients reported with transfusion reaction, 48 patients (18.2%) had low serum haptoglobin. Only 19 patients with low haptoglobin presented with haemolysis (7.2%) and 29 patients with low haptoglobin without haemolysis (11.0%). In the latter group of 29 patients, 10 patients presented with anaphylactoid reaction or anaphylaxis with hypotension, dyspnea, wheezing or chest pain. Of these 10 patients, 6 had serum haptoglobin < 0.10 g/L and 4 patients had > 0.10 but < 0.37 g/L. For the remaining 19 patients, 15 of them presented with allergic transfusion reaction and 4 patients with febrile non-haemolytic transfusion reaction. Low haptoglobin without haemolysis was associated with 3.8% (10/263) occurrence of anaphylactoid or anaphylactic non-haemolytic transfusion reaction and 5.7% (15/263) of allergic transfusion reaction among those reported for blood transfusion reaction.

Summary/Conclusions: Our study showed the importance of recognising the association of non-haemolytic low haptoglobin with anaphylactoid or anaphylactic transfusion reaction among patients with blood transfusion reaction in an Asian population. Further studies are required in haptoglobin-deficient patients to elucidate the mechanism underlying the transfusion reactions, in particular anaphylaxis in order to institute appropriate transfusion strategy.

P487 | Abstract withdrawn

P488 | Abstract withdrawn

P489 | What is the role of testing for donor leukocyte antibodies in cases of suspected antibody-mediated TRALI?

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Background: Anti HNA and HLA antibodies in donated blood products are established causes of transfusion related lung injury (TRALI), but demonstration of antibodies is neither necessary nor sufficient for to diagnose TRALI. However, asking if the reaction was caused by leukocyte antibodies remains important for haemovigilance and blood product safety. The question of whether to test donors for antibodies is therefore not identical to the question of whether the case meets definition criteria for TRALI, but instead could consider whether the benefits of testing are greater than the disadvantages. It may be unclear how the benefits of increased confidence in TRALI diagnosis and prevention of future TRALI cases could be balanced against the consequences in terms of donor deferral in making a testing decision. Therefore, a formal decision making model has been developed in an attempt to improve transparency and consistency of decision making.
Aims: To develop a formal decision model to indicate whether to test cases of suspected antibody mediated TRALI.

P489 - Table 1 Post test probability of antibody mediated TRALI (assuming a donor antibody prevalence of 0.05 and sensitivity =0.9)

Pretest probability	Number of implicated donors			
	1	5	10	20
0.975	1.0	.99	.99	.98
0.85	.99	.96	.93	.89
0.5	.95	.8	.7	.6
0.15	0.76	0.42	0.29	0.21
0.025	0.32	0.095	0.057	0.037

P489 - Table 2 Proportion increase in detection of donors with antibodies compared to testing a similar number of random donors (assuming a donor antibody prevalence of 0.05 and sensitivity =0.9)

Pretest probability	Number of implicated donors			
	1	5	10	20
0.975	19	3.7	1.9	0.93
0.85	16	3.2	1.6	0.81
0.5	9.5	1.9	0.95	0.48
0.15	2.9	0.57	0.28	0.14
0.025	0.48	0.095	0.047	0.024

Methods: A Bayesian probability model is used to estimate the benefits of donor testing and recipient testing quantitatively. The model is used to construct a formal decision making algorithm to calculate whether the benefits of testing are greater than the drawbacks when given the number of indicated donors and level of suspicion (pre-test probability) of antibody-mediated TRALI.

Results: The model suggests that the gain in diagnostic information on whether the case is likely to be antibody mediated TRALI is optimal in cases where there is diagnostic uncertainty and fewer implicated donors [table 1]. In contrast, the increased detection of donors with antibodies compared to testing donors at random is optimal where there are fewer implicated donors but high suspicion that the reaction was caused by donor antibodies [table 2]. Calculation of whether the overall benefit of testing is greater than the drawbacks requires a judgement on the relative value (utility) of diagnosis in the recipient, prevention of future reactions, and donor deferral. The output of the decision algorithm is sensitive to choice of utility values.

Summary/Conclusions: The modelling illustrates the complex relationship between the pre-test probability, the number of implicated donors, and the benefit of testing, which would be difficult to calculate informally in a consistent way. There can be no objectively correct algorithm to optimise the risk-benefit balance of testing because assumptions of the relative utility value of different outcomes are subjective. Nevertheless, operational use a formal model may help consistency of decision making once underlying assumptions have been agreed, and promote transparency because of the need to develop a consensus on those assumptions.

P490 | Factors associated with mortality in transfusion associated circulatory overload

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Background: Transfusion associated circulatory overload (TACO) surpassed transfusion-related acute lung injury (TRALI) making it the leading cause of death amongst respiratory transfusion reactions.

Aims: Here we retrospectively determine risk factors associated with TACO from a haemovigilance database.

Methods: Non-pregnant adults (age > 18 years) with possible to definite TACO reactions from 3 quaternary urban hospitals from 2010-2020 were analysed (REB#1000080295). Of 407 suspected TACOs, 19 were ruled out, while 17 were excluded as massive haemorrhages. To assess TACO risk factors the following parameters were analysed: age (years), sex, received-red blood cells (RBC)s (Y/N, platelets- (Y/N), plasma- (Y/N), TACO severity (1: minor [required no treatment or only agents that nursing can provide from automatic orders], 2: moderate [required MD review ± unplanned treatment], 3: severe [escalation in care/ disposition change now needing major supplemental oxygen], and 4: life-threatening/fatal [requirement for life-saving care such as intubation]), grade (high severity: 3+4; or low: 1+2), 28 day mortality, in-hospital mortality and new ICU admission. TACO reactions were judged on the confidence spectrum as either ruled out/doubtful, possible, probable, most likely, or definite per previously described paradigms. The potential additional diagnosis of TRALI was also noted when applicable. Descriptive statistical tests were conducted with SPSS software. A. $p \leq 0.05$ was considered statistically significant.

Results: Of the 371 TACO cases (63 ± 16.8 years; mean ± S.D.) overall in-hospital mortality (IHM) was 17.5% and the 28-day mortality (28M) was 12.9%. Both 28M ($p = 0.04$) and IHM ($p = 0.04$) were significantly different between the 179 males and 192 females. The odds of surviving were 0.55 times lower in males (95% CI: 0.32 – 0.95). Severity grading revealed 31 (8.4%), 83 (22.4%), 204 (55%), and 53 (14%) life-threatening, severe, moderate, and mild reactions respectively. Platelet-involving encounters were associated with severe TACO. Among cases, 274 (73.9%), 121 (32.6%), and 28 (7.5%) involved RBC, platelets, or plasma respectively. Mortality data were available for 369 patients. Platelet transfusion was found to be associated with both 28M ($p = 0.035$) and IHM ($p = 0.013$). Survival odds were 1.97 times greater (95% CI: 1.14-3.40) in patients with no platelet transfusion compared to those platelet-transfused. We assessed concomitant TRALI as a possible confounder as we found 29 TACO cases that had an additional diagnosis of TRALI which was also associated with mortality ($p < 0.05$). Of interest 10 of those were transfused platelets. New ICU admission (data available for 356 cases) amongst the survivors

and non-survivors was also found to be significantly associated with 28M ($p = 0.001$) and IHM ($p = 0.00$).

Summary/Conclusions: TACO remains underrecognised. While TACO may generally associate often with RBC transfusion, here we show TACO mortality associations with platelet transfusions, male recipients, and disposition escalation to the ICU. Our findings are preliminary and limited by the retrospective and uncontrolled nature of the study. Gaps included details on circulatory fluid status as well as renal or cardiac disease. Future prospective mechanistic and larger epidemiological studies are needed to confirm our findings. Potential sex differences and product-related factors may influence not only the incidence but also the severity (i.e. mortality) of TACO.

P491 | Analysis of the causality of adverse reactions related to transfusions of blood components received from the Regional Blood Center in Poznan in 2011-2021

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Background: Patients treated with blood components are a heterogeneous group. They present different symptoms due to their clinical condition, which are overlapped by adverse post-transfusion reactions. Determining the correlation between the symptoms and transfusions is often a substantial problem

Aims: The aim of this paper was to analyse the causality of post-transfusion reactions

Methods: The study was retrospective and included patients treated with blood components in hospitals under the supervision of the Regional Blood Center in Poznan (BC). Patients included in the study received transfusions between 2011-2021 and were reported with adverse reactions (AR). The analysis contained: the data from the adverse reaction report forms sent to BC, the results of diagnostic tests performed in hospitals and BC. In the case of a serious adverse reaction, additional information regarding the patient's clinical condition was obtained and transferred to the Institute of Hematology and Blood Transfusion in Warsaw (IHBT) for consultation. After analysing the available documentation, each report was classified into one type of AR on the basis of the criteria available in the literature, and then was rated in terms of the correlation with transfusion, according to the descriptions below. The following criteria were analysed: recipient's age, diagnosis, type of component, time of reaction, symptoms, treatment, severity of reaction, patient's condition before transfusion, transfusions in the past, presence of antibodies.

Correlation between symptoms and AR imputability levels according to Polish criteria:

NA Not Assessable - when there is insufficient data for imputability assessment

0 Excluded/Unlikely - where there is conclusive evidence beyond reasonable doubt for attributing the adverse reaction to alternative causes/ where the evidence is clearly in favor of attributing the adverse reaction to causes other than blood or blood components

1 Possible - when the evidence is indeterminate for attributing the adverse reaction either to the blood or blood components or to alternative causes

2 Probable - when the evidence is clearly in favor of attributing the adverse reaction to the blood or blood component

3 Certain - where there is conclusive evidence beyond reasonable doubt for attributing the adverse reaction to the blood or blood component

Results: The studied population consisted of 867 patients. Data on the causality of the response are presented below:

Total causality: number of adverse reactions, n(%): **NA** $n = 4(0.5)$ **0** $n = 91(10.5)$ **1** $n = 395(45)$ **2** $n = 225(26)$ **3** $n = 152(18)$

FNHTR febrile non-haemolytic transfusion reactions $n = 378(44)$: **NA** $n = 1(0.3)$ **0** $n = 11(3)$ **1** $n = 275(72.7)$ **2** $n = 4(1)$ **3** $n = 87(23)$

Allergy $n = 323(37)$: **0** $n = 6(2)$ **1** $n = 78(25)$ **2** $n = 203(63)$ **3** $n = 36(10)$

TACO transfusion associated circulatory overload $n = 38(4)$: **0** $n = 1(3)$ **1** $n = 14(37)$ **2** $n = 16(42)$ **3** $n = 7(18)$

Haemolysis $n = 29(3)$: **0** $n = 1(49)$ **1** $n = 3(10)$ **3** $n = 12(41)$

TAD transfusion-associated dyspnea $n = 23(3)$: **0** $n = 1(43)$ **1** $n = 3(57)$

TRALI transfusion related acute lung injury $n = 11(1)$: **2** $n = 2(18)$ **3** $n = 9(82)$

PTP post-transfusion purpura $n = 6(1)$: **0** $n = 4(67)$ **1** $n = 1(17)$ **3** $n = 1(16)$

Infections $n = 10(1)$: **NA** $n = 3(30)$ **0** $n = 6(60)$ **1** $n = 1(10)$

Other $n = 49(6)$: **0** $n = 39(80)$ **1** $n = 10(20)$

Summary/Conclusions: Almost half of the reported adverse reactions could not be determined whether the reaction was caused by the transfusion or by other reasons. A probable and definite association of adverse reactions with transfusion was stated for more than forty percent of the reports.

Clinical Transfusion

Haemovigilance and patient safety

P492 | Causes of blood components wastage in a teaching hospital in Taiwan: A retrospective analysis

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Background: Blood transfusion is a critical component of modern medicine. In most countries, the majority of blood and blood components come from unremunerated blood donation. Due to Taiwan's declining birthrate and ageing population, many hospitals may experience blood supply shortages. Therefore, clinical physicians have to use the blood properly. In our hospital, we set up several guidelines and protocols to decrease the blood wastage includes education programs for physicians and other medical staff ever year, as well as inspecting the transfusion procedure in hospital wards, and monitoring the shipping time form blood bank to bedside wards.

Aims: The purpose of this study was to determine the rate and causes of wastage of blood products in our hospital.

Methods: This study was conducted in 600-beds teaching hospital in-Taiwan. A retrospective review of the causes of blood product wastage from Jan 2011 to Jan 2021. During the study period, we reviewed every blood waste report to find out the main causes. Data were analysed using descriptive statistics.

Results: The average amount of 34,797 units of blood components were transfused every year. The wastage rate has significantly decreased in recent years, going from 0.29% in 2011 to 0.05% in 2021. In this study, FFP (33.5%), platelet concentration (19.4%), pack red cell components (17.1%), and cryoprecipitate (9.9%) made up the majority of the blood components that were discarded. The most common causes of blood wastage were including excessive order/ Inappropriate evaluated of patient's condition (26.2%), order error of blood components (15.6%), inability reissue to another patient before usable period (12.5%), incorrect storage temperature of platelet components (11.0%), and blood products expired (10.6%).

Summary/Conclusions: In many countries, the blood supply is extremely limited, as it is in Taiwan's blood supply system. Unfortunately, human error and unforeseen circumstances might result in certain blood components being wasted during blood transfusion procedures. To prevent blood wastage, we provide training courses and information for healthcare professionals. Although the evidence on whether continuing education changes the behavior of medical staff in clinical practice is limited, this outcome demonstrates a decline in the hospital's blood waste rate. Through this study,we hope to draw attention on using blood products properly, as medical professionals, no blood products should ever be discarded in clinical settings.

P493 | Abstract withdrawn

P494 | Analysis of the reported adverse transfusion reactions

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Background: Haemovigilance as a set of surveillance procedures covering the whole transfusion chain from the collection of blood and its components to the follow-up of its recipients, is required to identify and prevent occurrence or recurrence of transfusion related unwanted events to increase the safety, efficacy and efficiency of blood transfusion.

Aims: The aim of this study was to analyse the adverse transfusion reactions that were reported to the Quality Assurance and Quality Control (QAQC) Department of the Institute for Transfusion Medicine of Republic of North Macedonia – Skopje (ITM).

Methods: Retrospective analysis of the reported adverse reactions to the QAQC department in the period 2007-2022 from its monthly and yearly registries.

Results: The most frequent adverse reactions that were reported were mild allergic and febrile non-haemolytical transfusion reactions with urticaria, rash, fever and vomiting, just a few of them were moderate and severe with dyspnoea, headache, hypotension and tachycardia. In 2007 were 2 reported adverse transfusion reactions (Cryoprecipitate and Fresh Frozen Plasma (FFP)), in 2008- 1(FFP), in 2009 – 1, in 2010 -1(FFP), 2011 – 3 (2 (RBCSAGM and 1 FVIII conc.), in 2012 - 1 (Cryoprecipitate), in 2013- 2 (RBC-SAGM and Cryoprecipitate), in 2014 – 1 (Cryoprecipitate), in 2015 – 1 (FFP), in 2018 - 4 (1-FFP and 3-RBC-SAGM), in 2019 – 3 (FFP), in 2020 - 4 (1-platelet concentrate, 3 RBC-SAGM), in 2021 – 1 (RBC-SAGM) and in 2022 – 4 (RBC-SAGM). There was no mortality associated with blood transfusion in the last 16 years.

Summary/Conclusions: The hospital transfusion committees and an active surveillance program have a key role in enhancing patient safety by making changes to prevent reoccurrence and management of adverse reactions to blood transfusion. Improving the reporting of transfusion related adverse reactions, analysis of the reports for the blood components use and adverse transfusion reactions will help us to focus on safe transfusion and upgrade of the legislative with a by-law for haemovigilance. The information from a good functioning

haemovigilance system can be used as a quality indicator for blood transfusion safety and can contribute significantly to evidence-based transfusion medicine.

P495 | Abstract withdrawn

P496 | Abstract withdrawn

P497 | Abstract withdrawn

P498 | Evaluation of transfusion reactions in patients following transfusion of blood products containing antibodies to HLA class I

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Background: Transfusion-related acute lung injury (TRALI) is characterised by lung edema and hypoxia developing within 6 hours following transfusion of plasma rich blood products from single donors. Although a leading cause of transfusion-related deaths, the condition is most likely underdiagnosed. In most cases, the pathophysiology includes an immunological component, with transduction of antibodies to human leucocyte antigens (HLA) as a precipitating factor in a predisposed patient. Following a fatal TRALI induced by a thrombocyte concentrate collected by apheresis, Oslo Blood Center implemented screening for HLA-specific antibodies in all thrombapheresis donors. The screening is routinely performed when whole-blood donors are recruited to thrombapheresis, and repeated after pregnancy or transfusion. Identification of donors with antibodies to HLA class I/II leads to deferral from donation of products rich in plasma. From December 2014 to December 2016, 1369 apheresis donors were screened for HLA-specific antibodies. Antibodies above cut-off (Normalized background (NBG)-ratio >10) were found in 200 donors (35 men), who had donated apheresis or whole blood 5-100 times each. In theory, the risk of TRALI in the recipients of blood products from these donors with HLA-specific antibodies present in plasma, should be increased.

Aims: The current study was conducted to evaluate our practice and attempt to estimate the risk of TRALI when blood components containing HLA-specific antibodies were transfused. In a retrospective manner, we wanted to investigate whether transfusion reactions had been reported for patients receiving the blood components donated from these donors.

Methods: By use of the blood bank information system (ProSang) we first identified the blood products (erythrocyte concentrates, buffy coat and apheresis-derived thrombocyte concentrates) from the donors in question. Secondly, we tracked the recipients of these transfusions and investigated whether transfusion reactions of any type were reported.

Results: We have mapped 150 donors who were deferred from donation of plasma rich blood product due to detection of HLA-specific antibodies in the screening. Altogether they had donated thromapheresis or whole blood 2829 times before screening results were available. Of 2684 donations investigated this far, 457 were not used (outdated, returned or discarded) and 165 products were sold to other blood banks. We investigated the reports from 2357 transfusions of products obtained from these donors in the laboratory system. Of these, 101 transfusion confirmations were reported as incomplete. The number of transfusion reactions reported was 11, mainly mild reactions. No case of TRALI was reported in these patients.

Summary/Conclusions: Following transfusion of 2357 blood products from blood donors in whom HLA-specific antibodies later were found, only 11 cases of transfusion reaction were reported back to the blood bank, of which none were TRALIs. This supports the notion of TRALI being rare, or seriously under-reported. However, the usefulness of screening and deferring donors with HLA-specific antibodies with the purpose to reduce TRALI remains uncertain.

P499 | The Slovenian Haemovigilance Network: 20 years of experience (2002-2022)

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Background: The Slovenian Haemovigilance Network has been organised since 2002. For 20 years, this organised service has been collecting and processing data on adverse reactions, events, and donor reactions.

Aims: Overview of statistics for adverse reactions, events, and donor reactions from 2002 till 2022.

Methods: The National Haemovigilance Office has been collecting data from 26 Slovenian hospitals (2 university, 12 general, and 12 specialised hospitals). The unique information system enables traceability from donor to recipient for every issued unit. All adverse recipient reactions are stated per thousand issued blood units (/1000 issued blood units) because there is only 60% feedback if the issued unit has actually been transfused to the patient or discarded instead.

Results: Recipient haemovigilance: there were 2285 reactions (1.15 reaction/1000 issued units) reported to the Haemovigilance Office. The majority of cases (85%) were mild allergic and nonhaemolytic febrile reactions, followed by transfusion-associated circulatory overload (TACO) cases. There were 9 transmissions of hepatitis B (HBV) infection. There were no fatalities or cases of acute haemolytic transfusion reaction, post-transfusion purpura and graft *versus* host disease reported.

The majority of all reported cases were mild reactions. Less than 10% of reported reactions were life-threatening: predominantly TACO, followed by anaphylactic reactions, and cases of transfusion-related

acute lung injury (TRALI). There were only a handful of long-term illnesses, all of which were due to HBV transmission. More than 70% of the reactions in absolute number were attributed to erythrocyte units, which were the most frequently issued blood component. However, platelets, per 1000 units issued, were the most common unit causing a reaction.

Donor haemovigilance: there were 14,119 donor reactions (8.91 reactions/1000 donations) reported. At the beginning, there was very poor reporting with only 2.77 reactions/1000 donations reported. But with education and encouragement of staff involved in donations, reporting rate has risen to around 14 reactions/1000 donations in the past years. The vast majority represented vasovagal reactions, followed by hematomas and citrate reactions. Most of the reactions were mild and improved after prolonged post-donation rest and hydration (*per os*). Very rarely, medical intervention was needed.

Adverse events: true adverse events were very rare, and represented less than 1% or our reports. The vast majority of cases were near misses, dominated by incorrect patient data or misinterpretation of the ABO bed-side test. The rate of reported events related to blood units issued (after they left the blood establishment) was very low (0.47%). We were very rarely informed about such events, or found out about them by chance. The vast majority of these were due to improper handling of the issued blood units (e.g. failure to provide a cold chain, transfusion of blood units later than 4 hours after issue, improper thawing of fresh frozen plasma, etc…).

Summary/Conclusions: The Haemovigilance Network, which was launched in 2002, is an ongoing process that needs to be constantly adapted and improved. Over 20 years, reactions' reporting has become a routine process in hospitals. On the other hand, reporting on adverse events will need additional encouragement and education of staff involved in the transfusion chain.

P500 | Analysis of transfusion-related errors reported to the Korean Haemovigilance System

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Background: We have established and operated a national haemovigilance system, which is supported by the Korean Ministry of Health and Welfare but operated independently by the Korean Society of Blood Transfusion since 2007.

Aims: We intended to analyse the transfusion-related errors reported to the Korean Haemovigilance System from 2011 to 2022.

Methods: Transfusion-related errors were classified according to the order of process from before product check-in to after product release. We analysed the month, day, and time zone in which the errors occurred, also.

Results: Total 1,495 transfusion-related errors were reported during this period. Of these, 1,121 cases were near-miss incidents, 346 cases were incidents related to transfusion but no transfusion reaction, and 28 cases were incidents related to transfusion with transfusion reaction. Forty-two percent (628 cases) occurred in related to sample collection and 37.6% (562 cases) occurred during transfusion in the ward. Errors related to pre-transfusion testing (108, 7.2%), blood processing (75, 5.0%) and ordering the blood (58, 3.9%) followed. Twelve cases (0.8%) of errors before product check-in and 52 others were also reported.

The average number of error reports per month was highest in May (12.2, 9.9%), followed by March (11.8, 9.6%). October's (9.2, 6.9%) and February's (8.3, 6.7%) were lower than other months, and there was no significant difference in other months. Analysis by day and time period was available for data from 2011 to 2021. By day of the week, errors occurred the most on Monday (20.0%), and were lower on Saturday (7.9%) and Sunday (8.3%) than on other days. When analysing the time of occurrence, the incidence was higher during the day (67.1%) than at night (32.9%).

Summary/Conclusions: Transfusion-related errors occurred at every point in the transfusion process. Most errors were near-miss incidents, but there were also cases that led to transfusion reactions. Most errors arise from human behavior, which may be preventable and require efforts to prevent.

P501 | Abstract withdrawn

P502 | Abstract withdrawn

Clinical transfusion

Alternatives to blood transfusion

P503 | Abstract withdrawn

P504 | Abstract withdrawn

Clinical transfusion

Patient blood management

P505 | Erythropoietin to reduce blood transfusions, results of the first 115 cases

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Background: Preoperative anaemia is associated with adverse clinical outcomes after cardiac surgery such as increased perioperative packed red blood cell transfusions, acute kidney injury and death. Intraoperative packed red blood cell transfusions in patients having cardiac surgery is related to longer hospital stay, increased incidence of mortality and morbidity, including ischemic and infectious complications and increased hospital costs.

A recent double-blind randomised controlled trial examined the effect of an ultra-short-term treatment protocol based on erythropoietin before cardiac surgery in patients with anaemia or iron deficiency on reducing perioperative packed red blood cell transfusions. The study reported a significant reduction of perioperative packed red blood cell transfusions in the treatment group during the first week postoperative compared with the control group.

Our treatment protocol is based on this study results and on the guidelines of the European Association for Cardio-Thoracic Surgery and the European Association of Cardiothoracic Anaesthesiology.

Aims: To assess whether implementation of an immediate preoperative treatment in anaemic patients could result in fewer perioperative packed red blood cell transfusions and improved outcomes in a real-world setting.

Methods: Starting on January 1st, 2020, we implemented a perioperative protocol for anaemic patients (women Hb < 11.5 g/dL, men Hb < 12.5 g/dL), which included subcutaneous erythropoietin alpha, intravenous Iron, intramuscular vitamin B12 and per os Iron and folic acid given once a day postoperatively. We retrospectively compared all patients receiving the protocol to all eligible patients who were operated on in the four years prior to implementation of the protocol. Primary outcome was amount of packed red blood cell transfusions during surgery and index admission. Secondary outcomes were other blood product administration.

Results: In the 22 months after protocol implementation, 115 patients who received the treatment protocol were compared to 188 anaemic patients in the four years prior who did not receive the protocol. The treatment reduced total packed red blood cell use (treatment group median 5 [3-8] units vs. control 2 [1-3] units, $p < 0.0001$) and the incidence of post-operative blood products transfusions (treatment group 58 patients, 50.4% vs control group 141 patients, 75%, $p < 0.0001$). Haemoglobin prior to discharge was higher in the protocol group (treatment median 9 [8.35-9.5] g/dL vs. control 8.6 [8.1-9.1] g/dL, $p = 0.0086$). In addition, the incidence of postoperative acute renal failure was lower among the protocol group (treatment group 5.26% vs control group 12.23% $p = 0.0462$).

Summary/Conclusions: Implementation of a perioperative treatment protocol for anaemic patients significantly decreased intraoperative and postoperative packed red blood cell transfusions. Despite the use of a cheaper type of IV iron (ferric hydroxide sucrose) compared to previously described protocols, the protocol was still effective in reducing the intake of packed red blood cell. The study results support that the protocol is also effective in real world setting.

P506 | Abstract withdrawn

P507 | Evaluation of a closed loop blood sampling system in intensive care: a pilot randomised controlled trial.

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Background: Management of critically ill patients involves extensive diagnostic testing and procedures to inform clinical decision making. This is commonly facilitated by an arterial catheter (AC) connected to a pressurised administration set that maintains patency and facilitates continuous monitoring. Blood sampling is enabled via this system but can result in blood wastage and contamination.

Aims: To test the feasibility of conducting a randomised controlled trial (RCT) to evaluate the impact of a closed-loop blood sampling system and conservation bundle

Methods: Single site, parallel group, pilot RCT comparing open system sampling (OS) to closed system sampling (CS) and conservation bundle aligned with national guidelines. Participants were ≥18 years who had AC inserted in intensive care. Randomisation was generated by statistician and then via opaque envelopes. Key outcomes included trial feasibility, blood sample loss, haematocrit (HCT) change, and transfusion (PRBC) use.

Results: Eighty patients were randomised ($n = 39$ OS group, $n = 41$ CS group). Characteristics in each group were equal at baseline with overall mean age 60 years [IQR 48.6-70.4], 58% male, and median APACHE score 16 [IQR 11 - 22]. The proportion of patients eligible was 29% and missed eligible 65%. Otherwise, feasibility criteria were met with proportion of eligible patients agreeing to enrolment 99%, 100% of patients receiving allocated treatment, and only 1% data

missing. Analysis demonstrated a significant reduction in daily blood sample losses (OS 32.7 (SD 1.58) mL vs CS 15.5 (SD 5.79) mL, $t = 8.454$, df = 78, $p < 0.001$). There was no significant difference in HCT levels. The proportion of patients with PRBC transfusion use was less in the Intervention group (Intervention 20% vs Control 29%; $t = 1.57$, $p = 0.120$).

Summary/Conclusions: Critically ill patients are at risk of iatrogenic anaemia and other harmful sequelae from repeated blood sampling. Results from this study demonstrated that a large, multi-site trial evaluating blood conservation intervention is feasible with enhanced eligibility criteria, increased recruitment support, and using a cluster design. The intervention studied reduced daily blood sample volumes and PRBC transfusion use. Further trials are required to provide both effectiveness and implementation outcomes.

P508 | Application of Patient Blood Management (PBM) principles for more accurate evaluation of blood demand: Case of Baptist Hospital Mutengene, Cameroon

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Background: Patient Blood Management (PBM) is defined as a patient-centred, systematic, evidence-based approach to improve patient outcomes by managing and preserving a patient's own blood, while promoting patient safety and empowerment. Among its proven benefits of patient safety and cost-saving, it also has impact in improving blood supply through minimizing inappropriate blood demands. However, its limited knowledge and lack of implementation leads to significant stress in the blood transfusion services, already battling with limited availability of safe blood components.

Aims: To evaluate the effect of PBM principles on blood demand in a hospital setting at Baptist Hospital Mutengene

Methods: A hospital-based interventional study was done from January 1 to November 30th, 2022 at Baptist Hospital Mutengene, Cameroon. The data was collected and analysed using EPI info version 7 and statistical significance at $p < 0.05$. This included the number of inpatient admissions, blood units transfused, and the number of patients transfused for each of the wards. A comparative analysis was done before (January to March 2022) and after (April to November 2022) implementing a PBM program. This involved training sessions with the physicians, nurses, and medical laboratory scientists; the creation of a hospital transfusion committee, adapting a new type and cross-match form, providing a specific transfusion guideline, a multidisciplinary team approach (involving a transfusion medicine physician), and informed patient consent.

Results: In total, we recorded 1967 blood units transfused to 1123 patients over the study period. Pre-PBM program implementation we had an average number of in-patient admissions, patients transfused and the number of units transfused being 504.7 ± 96.3 patients, 128.3 ± 11.9 patients, and 228.3 ± 32.6 blood units respectively. Post its implementation we found out that the average number of in-patient admissions, patients transfused and the number of units transfused were 533.8 ± 58.1 patients, 92.3 ± 21.7 patients ($p < 0.0001$), and 160.3 ± 50.8 blood units ($p < 0.0001$) respectively.

Summary/Conclusions: Even with an increasing number of in-patients hospitalization, we recorded a significant decrease in the number of patients transfused and blood units distributed after implementing a contextualized PBM program. This thus helps us to better anticipate the supply needed with a more accurate blood demand.

P509 | Transfusion in the emergency unit of a tertiary hospital. Development of a Patient Blood Management program

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Background: Patient Blood Management (PBM) is a systematic evidence-based approach that aims to optimise patient management and transfusion to ensure effective and quality patient care. There is little literature on the usefulness of PBM in hospital emergency departments (HED).

Aims: The objective of the study was to analyse the characteristics of patients who received red blood cell (RBC) concentrates in HED and to assess possible PBM strategies.

Methods: Analytical, observational, cross-sectional study of patients transfused with RBC in the HED of a tertiary hospital. We included all patients who received RBC in the HED in January 2022. Data were analysed using the statistical program SPSS.

Results: 131 transfusion episodes were analysed, 216 RBC transfused to 110 patients. Mean age 72.29 years (SD 18.37) and 44.3% women. Mean Charlson comorbidity index 6.08 (SD 2.93). The main diagnoses were anaemia (92.4%) and haemorrhage (2.3%), however, according to clinical history were anaemia (74.8%), bleeding (21.4%) and others (3.8%). 48.1% of patients had the diagnosis of anaemia in their clinical records. 25.6% underwent oral anticoagulant treatment: 11.2% with

P509 - Table 1

Adverse Effect	n	%
Heart Failure	6	4,58
Hepatic decompensation	2	1,52
Fever	1	0,76
Dypsnea	1	0,76
Pneumonia	1	0,76

P509 - Table 2

patient	Number of RBC	Time (hours) 1° RBC	Time (hours) 2° RBC	Time (hours) 3° RBC
1	2	2	1	-
2	2	2	1,5	-
3	1	2	-	-
4	2	2,5	1	-
5	3	1,5	3	3

antivitamins K (VKA) 14.4% with direct-acting oral anticoagulants (DOAC). The reason for transfusion was haemorrhage in 32.2% of anticoagulated patients vs. 18.9% of noncoagulated patients (p= 0.122). No differences were found in haemoglobin values (Hb) between the two groups. Patients in VKA treatment bled more than patients in DOAC group (38.5% vs 27.8%; p 0.53). 33.6% of patients had received iron (Fe) during the last year and 21.4% had been transfused in the previous three months. The mean Hb was 6.9 g/dL (SD 1.4); medium 7 g/dL, p25 5.9 g/dL and p75 7.9 g/dL. The mean Hb of patients with bleeding was 7.7 g/dL (SD 1.6) versus anaemia 6.7 g/dL (SD 1.3) $p = 0.001$. The incidence of transfusion-associated adverse effects (AEs) is described in Table 1. There was no correlation between Charlson index and Hb level, reason for transfusion or incidence of Aes. Heart failure (HF) was the most frequent AE, 6 patients (4.58%). The infusion time was mostly 60-90 minutes (52.04%), the infusion times of HF patients are shown in Table 2. 71% of patients received diuretics before (23.7%) or after transfusion (47.3%). AEs in patients without diuretics were observed in 1.8%, with diuretic 13.2% p= 0.01. In 56% of cases the patient received 2 RBC. Patients were discharged at home (40.5%) and hospitalized (59.5%), with bleeding 75% and anaemia, 55% (p 0.06).

Summary/Conclusions: The transfusion-associated AEs observed in our study were higher than those reported in the literature. The implementation of PBM strategies in HED could improve patient care. Transfusion of a single RBC and administration of intravenous Fe are options to consider.

P510 | Abstract withdrawn

P511 | Screening for iron deficiency among pregnant women

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Background: Patient blood management during pregnancy is essential given the high prevalence of Iron deficiency (ID) and iron deficiency anaemia (IDA) in pregnant women. Both conditions are associated with increased risk of maternal and neonatal morbidity. Although there is general agreement that haemoglobin level must be measured at multiple points during pregnancy, routine ferritin screening, for anaemic and non-anaemic women, seems not be widely adopted.

Aims: The primary aim is to evaluate the patterns of screening and management of ID and IDA among pregnant women in an academic tertiary care center. The secondary aim is to describe prevalence of anaemia and iron deficiency in every trimester.

Methods: Retrospective record review of electronic health records of women who delivered at the study site from the 1st of January to 31st of March 2022. Women who did not follow up before delivery, and women with haemoglobinopathies or chronic inflammatory disorders were excluded. Anaemia was defined as haemoglobin less than 11 g/dL, and anaemia severity was classified into mild (Hb 10-10.9 g/dl), moderate (Hb 7-9.9 g/dl), and severe (Hb <7 g/dl). Iron deficiency was defined as ferritin <30 ng/mL or transferrin saturation less than 20%.

Results: Out of 400 women who delivered at the study site during the study period, 336 met the inclusion criteria. A complete blood count to screen for anaemia was performed in 38.4% of women in the first trimester, in 67.6% in the second trimester, and in 69.9% in the third trimester. Haemoglobin was less than 11 g/dL in 31.7% of screened women in the first trimester, in 55% of those screened in the second trimester, and in 51.9% in the third trimester. Mild anaemia was more common in all trimesters than moderate anaemia, with only 2 patients having severe anaemia.

Patients were rarely screened for iron deficiency. Less than 5% of women had ferritin testing if they had normal haemoglobin. Ferritin testing was performed in 19% of patients with anaemia in the 1st trimester, 6% in the 2nd trimester, and 18.8% in the 3rd trimester. When ferritin testing was performed, most women (69%) had ferritin level <15 ng/ml.

When charts were reviewed for management of pregnant women with anaemia, it was noted that almost all patients were prescribed oral iron: 41/41 (100%) in the first trimester, 124/125 (99.2%) in the second trimester, and 121/122 (99.2%) in the third trimester. IV iron was also prescribed in 6/41 (14.6%) in the first trimester, 12/125 (9.6%) in the second trimester, and 16/122 (13.1%) in the third trimester. Blood transfusion was given to 2 patients with severe anaemia in the third trimester.

Summary/Conclusions: Iron deficiency and iron deficiency anaemia are common among pregnant women, but screening for them remains suboptimal. We recommend surveying primary care physicians, obstetricians, and midwives to evaluate attitudes and barriers to improved practices. Policy makers are encouraged to develop additional guidance for screening and management of ID, even without anaemia, during pregnancy.

P512 | Preoperative patient evaluation and strategy to reduce the number of perioperative transfusions

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Background: New evidences for transfusion medicine emphases of the preoperative assessment of the patient, assessment of the risk for transfusion, and the use of adjunct medications to prevent and/or treat bleeding.

They include use of pharmacologic therapies to minimise blood transfusions, such as erythropoietin for the anaemic patient, prothrombin complex concentrates for urgent reversal of warfarin, and intraoperative antifibrinolytic therapy during selected cardiac and noncardiac procedures having a high risk for bleeding.

Preoperative evaluation of a patient to identify risk factors for requiring a blood transfusion includes reviewing past medical history and existing laboratory test results (e.g., haemoglobin, hematocrit, coagulation tests). All of information may be predictive of perioperative blood loss, the risk of transfusion.

Aims: In our study we aimed to assess the preoperative evaluation of patients who undergo surgeries in a year period for bleeding risks and actual blood transfusion needs of these patients, then evaluate the transfused blood unit number retrospectively in order to assess the rational use of blood supply.

Methods: In a year period, all blood orders for patients who will undergo for a surgery to the transfusion center were evaluated retrospectively and for patient hospital records were assessed for the bleeding risks. Actual transfusions were compared with the orders to see the rational use.

Results: In 2022, 2015 units of blood was ordered for 1231 patients after preoperative evaluations. 1464 units in 2015 were transfused (72.7 %). Among all, 30.3% of units were transfused to patient haemoglobin level was between 6-8 mg/dL, and 29.7 % for patients of haemoglobin levels were 8-10 mg/dL and 6.4 % to whom haemoglobin were 10-12 mg/dL.

Neurosurgery department was one of the departments that the blood orders / transfusion ratio was the highest (421/125), which was followed by orthopedics (110/28).

When the blood orders were evaluated retrospectively, It was observed that blood order was made mainly according to haemoglobin values in patients with low bleeding risk.

Summary/Conclusions: Preoperative evaluation of a patient to identify risk factors for requiring a blood transfusion and to review alternative treatment methods will reduce the number of blood uses and unnecessary orders.

P513 | Knowledge, Attitude and Practices Study (KAP) on Patient Blood Management among medical faculty and residents at PGIMER Chandigarh, India

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Background: Patient blood management is a multidisciplinary and multimodal approach to reduce the need of allogenic blood exposure to patients and improve their clinical outcome. The decision to transfuse is at primarily based on the clinical judgement of the clinicians. Thus, it is important to understand the knowledge and attitude of clinicians towards patient blood management

Aims: 1. To analyse the current transfusion practices being followed in our institute.

2. To assess the knowledge of clinical residents and faculty members regarding Patient Blood Management (PBM) with the help of the validated questionnaire with the aim to implement Patient Blood Management in future.

Methods: Study design: Cross-sectional (KAP) study

To analyse and to understand the attitude of current transfusion practices, a questionnaire was prepared which constituted 30 Questions. It was validated by thirteen faculty members from the Medical and Surgical specialties. Once, it was validated, pre-tested in the pilot mode. The study was conducted through online mode.

Results: Knowledge:

It was observed that overall knowledge score for medical and surgical specialists was similar, However medical faculty and senior residents scored better than their counterparts in surgical specialties. Whereas junior trainees in surgical specialties (MKS = 12) scored higher than Medicine junior trainees (MKS = 11). There was a significant difference in knowledge score between senior residents of Medical and Surgical specialties ($p = 002$), in which SRs from medical specialties (MKS =12) scored higher than their counterpart (MKS = 9). Among the clinicians with 3-10 years of experience, medical specialists scored higher (11) as compared to the same category participants from surgical specialties ($p = 0.002$).

Attitude

About 64% of clinicians were in favour of single unit transfusion policy, as well 35% of clinicians have mentioned that it would be applicable only to elective patients (p>0.95) and 52% expressed that it is difficult to reassess the patient after every transfusion in a tertiary hospital as it requires huge resource and manpower.

Practice

About 26% of JRs and 17% of SRs ($p < 0.001$) have a practice of transfusing one unit of PRBC pre-operatively, irrespective of the haemoglobin of the patient. Overall, about 78% of clinicians follow ratio-based approach, whereas 22% of clinicians follow non-ratio based approach for blood component transfusion.

Summary/Conclusions: Although the majority of the clinical colleagues were aware about the patient blood management. However, the practice of pre-operative anaemia assessment, single and

restrictive transfusion policy, bleeding risk assessment and treatment of anaemia by medical methods needs to be emphasized for the implementation of PBM.

P514 | Outdated (expired) blood units in the period from 2014 to 2022 year

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Background: The racional usage of blood is very immportant, but sometimes there are outdated (expired) blood units.

Aims: Monitoring outdated (expire) blood units before utilisation in period from 2014 to 2022 year at the Institute of Transfusion Medicine of Republic of Macedonia). – Skopje (ITM Skopje)

Methods: A retrospective study performed by using data from official records of Department for immunohematology and distribution of blood components and information system E-Delfin.Using formula for development trend y=a+bx, where a and b are koeficients of trend; x is time; N- is number of years which we researched ;a=∑y/N and b=∑xy/∑x2.

Results: In 2014 year were expired data 1556 units, in 2015 expired data 1389 units, in 2016 expired data 1320 units, in 2017 were expired data 1612 units, in 2018 were expired data 2796 units, in 2019 were expired data 1418 units, in 2020 were expired data 718 units, in 2021 were expired data 569 units and in 2022 were expired data 563 units.Using formula for development trend y1=1326,7+(-1328)x(4)=1858; y2=1328,7+(-1328)x(3)=1725,2; y3=1591.6; y4=1459.64;y5=1326,7; y6=1194; y7=1061; y8=927,6; y9=795,8.

Summary/Conclusions: The percentage of the outdated blood units before utilisation was in range of European Guideline (4, 9%). Last 3 years this number fall down to 1-2,5 %.Analysis of the reason and undertaking corrective measures toward better inventory management is essential to minimise outdating of blood units. Pandemic with COVID -19 made big change in collection of blood units, rational usage of blood and minimise outdate blood units. Calculation of development trend with using formula for next three years is in trend to fall down.

P515 | A tertiary center experience with intraoperative cell salvage autologous transfusion as a patient blood management strategy

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Background: Perioperative cell salvage (PACS) is a key strategy of the Patient Blood Management (PBM) concept, being an effective blood conservation technique. It is indicated in surgeries with high estimated blood loss, to maintain postoperative haemoglobin concentration.

Aims: The aim of this study is to share our experience with intraoperative cell salvage as a key component of the PBM strategy in the perioperative setting in a reference hospital in the city of São Paulo, Brazil. Although our ICS experience dates more than 30 years, our focus was on the last 10 years´ data, considering the recent evolution of surgical techniques.

Methods: This is a descriptive study reporting our cell salvage experience, analysing the percentage of surgeries performed, processed volume, surgery time, infused volume and the number of avoided allogeneic red blood cells (RBC) transfusions.

Results: The average surgeries with cell salvage per year was 108, representing 1,188 procedures in the last ten years. From these 1,188 procedures with cell salvage, 985 were processed because the volume of blood aspirated from the surgical field was considered sufficient for reinfusion (82%) and 203 were not processed or infused (18%). Most of the surgeries were cardiovascular (928; 94.2%), followed by orthopedics (44; 4.5%) and others (13; 1.3%). Of the 1,188 procedures that cell salvage was used, 20 were performed in children (< 10 years) and 1,168 were adolescents/adults. Regarding the procedures done in children, all of the 20 surgeries were cardiovascular and processed. The children´s mean age was 4 years ± 2.8, (range 0-8), while the adults´ mean age was 62 years ± 15.5 (range 10- 94). The mean processed volume of cardiovascular adult surgeries was 3,303 mL ± 1,835 (range 481-19,349) and the mean infused volume was 557 mL ± 549 (range 119-5,911). Orthopedics adolescent/adults procedures were: column ($n = 38$; 3.9%) and hip surgeries ($n = 6$; 0.6%); with a mean processed volume of 3,480 mL ± 2,282 (range 801-10,021) and mean infused volume of 400 mL ± 2,264 (range 124-1,235). Other surgeries in adults included: liver transplantation (0.8%), exploratory laparotomy (0.2%) and lung surgery (0.2%). In these cases, the mean processed volume was 5,062 mL ± 3,244 (range 1,342- 13,863) and the mean infused volume was 1,129 mL ± 817 (range 238- 2,819). The total infused volume over the last 10 years was 546,509 mL, with 514,216 mL infused in cardiovascular surgeries, 17,604 mL in orthopedics and 14,689 mL in others. Considering that the average volume of one RBC unit is 300 mL, approximately 1,822 RBC transfusions were avoided in the last 10 years due to the use of cell salvage. Out of the 1,822 transfusions avoided, 1,714 were in cardiovascular surgeries (94%), 59 in orthopedic surgeries (3.3%) and 49 in others (2.7%). Although cell salvage is consecrated for cardiovascular surgeries, it could also be used for

other procedures, especially spinal arthrodesis, and liver transplantation.

Summary/Conclusions: This study reaffirms the evidence for cell salvage as part of the PBM program to decrease RBC demand, ensuring a lower risk of erythrocyte alloimmunization and transfusion reactions. In our service, cell salvage recovery equipment and trained personnel are available 24 hours a day for patients undergoing surgeries in which blood loss is a major potential complication, and has been an important PBM strategy for the past thirty years.

P516 | Abstract withdrawn

P517 | Abstract withdrawn

P518 | Preliminary evaluation of the implementation of Patient Blood Management in Obstetrics & Gynecology

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Background: Patient blood management (PBM) is a standard of care that seeks to maximise the use of a patient's own blood and avoid unnecessary blood transfusions during surgery by optimizing haemoglobin and iron levels, securing ideal haemostasis and minimizing blood loss, thus improving patient outcomes. It uses transfusional alternatives like iron, vitamin B12, folic acid and erythropoietin stimulating agents.

Aims: We aim to show how PBM in Obstetrics & Gynecology (OBGYN) is of a particular interest due to the prevalence of anaemia and hematinic factors deficit in this population.

Methods: retrospective analysis of 100 patients referred by OBGYN to our clinic for pre-operative PBM.

Results: In the 100 women followed, the median age was 39 years old (y.o.) (19-79 y.o.). The most frequent diagnosis was anaemia in pregnancy (34%); followed by abnormal uterine bleeding (AUB) due to myomas (32%); AUB with no identifiable cause (19%); oncological diseases (5%) in which 3 had primary cervical carcinoma, 1 ovarian carcinoma and 1 endometrial adenocarcinoma; post-partum haemorrhage (3%); AUB due to uterine polyps (2%); AUB with congenital factor FVII deficiency (1%) and AUB in a Turner syndrome patient (1%).

In this study, 85% of women had anaemia in the first consultation, with haemoglobin (hb)levels ranging from 5,6g/dL to 13,9g/dL. Of these patients, 49% had microcytic hypochromic anaemia, all with absolute iron deficiency. The median ferritin value on the first evaluation was 29ng/mL, with a minimum of 1,6ng/mL and maximum level of 700ng/mL. Only 5 patients had ferritin levels above 50ng/mL. Of all 100 women, 23 had folic acid deficiency and 12 had vitamin B12 deficiency. 30% of women had previously taken oral iron medication with either no change in absolute iron levels or intolerance to the treatment.

To improve the haemoglobin and hematinic factor levels 99% required intravenous iron administration, 47% were prescribed folic acid tablets and 11% vitamin B12 correction. Only 5% needed transfusional support with red blood cell transfusion, 3 because of anaemia in oncology patients under chaemotherapy and 2 pregnant women with alarm signs (difficulty breathing and chest discomfort). In 6 cases we prescribed oral tranexamic acid therapy to control AUB. None of the women took erythropoietin stimulating agents.

In the follow-up observation, there was a rise in haemoglobin levels in nearly all women, with a median hb level of 12,2g/dL (9,7g/dL-14,6g/dL) and median ferritin level of 200ng/ml (20-843ng/mL).

Of the 66 non pregnant women, 14 undergone surgery as of today and 23 are under medical treatment with oral contraceptives to reduce bleeding.

Summary/Conclusions: Anaemia and hematinic factor deficits in OBGYN are frequently seen and should be treated as soon as possible. Transfusional alternatives in this group of patients greatly reduce transfusional needs by rapidly increasing haemoglobin and iron levels.

P519 | Autologous Transfusion REquirements in bone MArrow harvest: preliminary results of the ATREMA study.

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Background: High-volume harvest of bone marrow (BM) for donation purposes in cases of adult recipients, poses donors at risk of anaemia. To prevent allogeneic transfusions and improve post-donation recovery. BM donors enrolled in the adult transplant program at our hospital usually undergo one or two autologous blood unit donations, in the 2 or 3 weeks before the harvest. Despite the practice of perioperative autologous donation (PAD) being widely used in this setting, it is not supported by strong clinical evidence and has been abandoned by some transplant teams. The auto-transfusion program in the setting of high-volume BM donation undeniably constitutes a controversial issue. The principal argument argued against PAD practice is that lower Hb levels at harvesting have been reported in donors with autologous blood pre-deposits in comparison with those without.

Aims: To expand the evidence that BM harvest can be safely performed even without PAD, we started a prospective study enrolling familial BM donors recruited at Fondazione Policlinico A. Gemelli IRCCS (Autologous Transfusion REquirements in Bone MArrow Harvest: The ATREMA Study NCT04355130).

Methods: The recruitment started on April 2020, with an originally planned sample size of 25 subjects. Inclusion criteria were the qualification to donate BM and the acceptance to participate in this study. Exclusion criteria consisted of abnormalities in the health status and/or laboratory tests of the donor, even though it did not prevent him from donating. One week before donation, all enrolled donors received vitamin and iron supplementation (i.v. infusion of 500 mg of ferric carboxymaltose in saline 100 ml, s.c. injection of 1 mg of vitamin B12, and oral supplementation of acid folic 5 mg/die until day +15 after BM donation). BM collection was carried out under general anaesthesia with a maximal target dose of 20-22 ml/Kg of the donor's body weight. Laboratory data (iron status and hematological parameters) were collected at baseline, at hospital admission before BM harvesting, the morning after BM harvest, at discharge, and at day + 7 outpatient control.

Results: From April 2020 to December 2022, seven total BM donors were included in the study. The under-recruitment was imputable to changes that occurred after the COVID pandemic, with preferential use of mobilized stem cell collections instead of BM graft even in haploidentical transplants. All donors were male, with a mean age of 32 ±2 years and a mean body weight of 78 +3.8 kg. The harvested BM volume was 20.8±0.8. Table 1 summarises laboratory parameters recorded at the time of vitamin supplementation, at BM harvest, at discharge, and at day+7. No adverse event occurred, no allogeneic transfusions were administered, and the median hospitalization duration was 2 days.

Summary/Conclusions: Our data suggest that even in high-volume BM donations, iron and vitamin supplementation can allow to avoid PAD, likewise preventing allogeneic blood transfusion.

P519 - Table 1. BM donor biochemical and hematological parameters.

	Day -7	**BM harvest**	Discharge	Day +7
Haemoglobin (g/dl)	14.5 (0.3)	14.2 (0.4)	10.5 (0.6)	9.6 (0.4)
Reticulocytes (10^9/L)	78.36 (4.7)	73.78 (8.8)	109.5 (38.7)	37.6 (3.7)
Mean corpuscular volume (fL)	85.3 (0.8)	85.7 (0.8)	85.1 (1.1)	85.0 (1.0)
Platelet count (109/L)	207.4 (20.5)	203.6 (19.5)	185.4 (38.7)	149.9 (19.8)
Iron concentration (mcg/dL)	84.0 (9.1)	102.4 (6.0)	nd	nd
Serum ferritin (mcg/dL)	170.9 (33.1)	488.0 (74.7)	nd	nd
Total iron binding capacity (mcg/dL)	269.4 (27.4)	261.3 (26.7)	nd	nd
Transferrin saturation (%)	32.3 (3.9)	41.8 (5.1)	nd	nd

P520 | Transfusion of packed red cells in patients heart failure: analysis of N-terminal Pro-brain natriuretic peptide and high sensitivity troponin

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Background: Background: Patients with chronic heart failure, are often accompanied by anaemia. The management of anaemia includes blood transfusions. Blood transfusions have a high risk of fluid overload and even Transfusion-Associated Circulatory Overload (TACO). There is no effective clinical evidence yet to prevent fluid overload, even TACO. Therefore, biomarkers development is needed, including N-Terminal pro-Brain Natriuretic Peptide (NT-ProBNP) and high sensitivity troponin I (hs-troponin I).

Aims: To know the kinetics of NT-proBNP and hs Troponin-I in heart failure patients receiving blood transfusions Packed Red Blood Cell (PRC) especially at Saiful Anwar Malang Hospital.

Methods: Methods: This study was a cross-sectional study with twenty-four heart failure patients who were transfused by PRC and treated in the Intensive Coronary Care Unit of dr. Saiful Anwar Hospital, Malang. NT-ProBNP levels were checked using ECLIA method, while hs-troponin I using CMIA method. Comparative test was used to see differences in levels of NT-ProBNP and hs-troponin I, correlation test between PRC blood bag and the levels of PRC's NT-ProBNP and hs-troponin I, also the correlation between Hb levels with NT-ProBNP and hs-troponin I.

Results: Results: The mean age of twenty-four patients is 61 years. From this study, the levels of hs-troponin I biomarker were increased significantly after blood transfusion ($p < 0.01$), whereas 12 patients (50%) increased 1.5 times. There was a significant difference in NT pro-BNP levels before and after blood transfusion ($p < 0.01$), which was an increase of 1.5 x in 14 patients (58.3%). There was no correlation between the PRC blood bag and the increase in hs-troponin I ($p = 0.827$) and NT pro-BNP ($p = 0.52$) levels. There was no correlation between Hb levels and increased levels of hs-troponin I ($p = 0.736$) and NT pro-BNP ($p = 0.089$).

Summary/Conclusions: Conclusion: (1) There was a significant increase in the levels of NT-ProBNP and hs-troponin I after PRC transfusion in patients with heart failure. (2) There was no correlation between the levels of NT-ProBNP and hs-troponin I with PRC blood bag in patients with heart failure. (3) There was no correlation between Hb levels and the levels of NT-ProBNP and hs-troponin I.

Clinical Transfusion

Clinical / laboratory interface–Transfusion practitioner (TP) initiatives

P521 | Screening of antenatal patients for anaemia and haemoglobinopathies

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Background: The causes of anaemia could be nutritional deficiency, due to blood loss, chronic disease, and so on. Nutritional anaemia is a worldwide problem with the highest prevalence in developing countries like India.The most common cause of nutritional deficiency is Iron Deficiency Anaemia. Haemoglobinopathies are a group of inherited disorders because of abnormalities in haemoglobin synthesis or structure. Thalassemia and Sickle cell anaemias are the most prevalent haemoglobinopathies and a national health burden. To reduce this burden, detecting them in the carrier stage is crucial. The health of pregnant women determines the future of the pregnancy. Hence identifying these disorders during the antenatal period is necessary to take the appropriate measures.

Aims: To assess the prevalence of anaemia and other haemoglobinopathies in antenatal patients by screening the for low haemoglobin (<10.0 gm /dl) value and investigation for causes related to low haemoglobin and also evaluate other factors related to haemoglobinopathies. We have also assessed the severity and morphologic type of Anaemia and encouraged patients to come back for follow up, so that proper treatment can be given.

Methods: A prospective study of screening for haemoglobin variants in Antenatal Patients due to low haemoglobin and evaluation of other causes was performed for 1 year with 570 samples. In patients with low haemoglobin CBC, Reticulocyte staining and sickling test was performed. Haemoglobin analysis was done by HPLC BIO RAD VARIANT II. In Iron Deficiency Anaemia (IDA) Serum ferritin & Serum Iron level were done and in Megaloblastic Anaemia(MA) Vitamin B12 level were done.

Results: Prevalence of anaemia in antenatal patients was found to be 90.25%; Out of this, Iron Deficiency Anaemia was 84.21%, Megaloblastic Anaemia was 4.73% and 1.27% was Dimorphic Anaemia. Prevalence of haemoglobinopathies in our study was 9.75%. Beta Thalassemia Minor was 5.08%, Sickle Cell Trait 4.03%, Hb D Punjab 0.52% and Hb Q India 0.17%.

Summary/Conclusions: Antenatal screening for haemoglobinopathies aims to reduce the burden of these diseases by offering information to individual with a high likelihood of giving birth to affected babies and giving parents more choice regarding their appropriate decisions. For this High Performance Liquid Chromatography forms a rapid,

accurate, and reproducible tool for the early detection and management of haemoglobinopathies and variants.

P522 | Alloantibody profile in partial better matched versus usual matched transfusions in thalassemia patients

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Background: Prevention and management of alloimmunization in thalassemia patients is a controversial topic for which no standard guidelines are available. Better match (BM) approach proposes to do red cell matching for Rhesus and Kell antigens along with ABO and D antigens, with the hope that prophylactic antigen matching will reduce the risk of formation of alloantibodies. On the other hand, traditional usual match (UM) approach does matching only for ABO and D antigens, which doesn't prevent formation of alloantibodies.

Aims: To study the alloimmunisation profile in thalassemia patients managed with partial better match (PBM) approach versus usual match (UM) approach.

Methods: This cross sectional observational study was done to evaluate alloimmunisation status in thalassemia patients enrolled at our institute. Antibody screen and identification was done at AHG phase in LISS. We have been providing partial better matched leucoreduced RCCs (phenotype matched for c, E and K antigens along with ABO and Rh D matching), for all patients since April 2009.After excluding NTDTs, Sickle/beta thal, irregularly transfused and expired patients, 339 TDT patients were included in this study.

Patients were divided into 3 categories:

Group 1: Patients who had been receiving transfusions as UM before 2009 and were shifted to leucoreduced PBM approach after 2009. This also included patients referred from other centres or were intermittently receiving transfusions from outside. These patients were variably getting leucoreduced/ nonleucoreduced RCCs/whole blood.

P522 - Table 1: Clinical Characteristics of Thalassemics in 3 groups

	Group 1 ($n = 198$)	Group 2 ($n = 38$)	Group 3 ($n = 103$)
Age: <15 yrs	27	35	97
>15 yrs	171	03	06
Diagnosis: B Thal Major	184	37	100
HbE-B Thal	14	1	3
Splenectomy: IAT Positive	4/23	0/3	0/3
IAT Negative	22/175	1/35	0/100

P522 - Table 2: Antibody Specificity in various groups

Antibodies	Group 1 ($n = 23$)	Group 1 ($n = 3$)	Group 1 ($n = 3$)
E	8	3	0
K	4	0	0
c	3	0	0
Kp^a	3	0	0
C^w	2	0	1
D	2	0	1
Mia	2	0	0
Jka	1	1	0
S,C,Ina	1,1,1	0	non specific ab

Group 2: Limited UM transfusions (less than 5 transfusions at less than 18 months of age) (leucoreduced/nonleucoreduced;RCC/WB) before registration at our center.

Group 3: Patients who had received only PBM leucoreduced RCC transfusions from beginning.

Results: Clinical details and alloantibody specificities in 3 groups are described in Table 1 and 2. Of 339 TDT patients, 23/198(11.62%) patients in group 1 had alloantibodies. This is not true reflection of prevalence as many patients were referred from other centres. After shifting to PBM, four patients formed new antibodies: Two patients developed Mia,one patient developed In^a and one developed Jk^a. Group 2 patients had limited UM transfusions, and 2/38 had alloantibodies. Anti E was found to be most prevalent alloantibody with Rh and Kell system contributing to 81.25% of all alloantibodies in group 1 and 2. There was significant difference in alloimmunization rates between splenectomised and non-splenectomised patients. In group 3, 3/103(2.91%) were alloimmunised, however, specificities of antibodies were different, with none of the patients having anti c, E or K, though one patient developed anti D.

Summary/Conclusions: The PBM approach helps in reducing the incidence of alloimmunization, particularly to Rh and Kell antigens. However, it might also be due to the simultaneous shift to universal leucoreduced RCC transfusions. Better matched approach, though expensive and time consuming, is preferable for management of thalassemics.

P523 | Challenges of hyperhaemolysis syndrome management in a sickle cell disease pregnant patient: a case report

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Background: Hyperhaemolysis syndrome (HHS) is a severe life-threatening haemolytic transfusion reaction commonly associated with sickle cell disease(SCD) and β-thalassemia major.

Aims: We report a case of a 36-years old female with homozygous SCD complicated by HHS after red blood cell exchange (RBCx).

Methods: case report

Results: We report the case of H.E, gravida 2 para 1, diagnosed with SCD since the age of 12, with a family history of a deceased brother with SCD at the age of 32 of acute chest syndrome (ACS).The 1st pregnancy in 2015 was complicated by severe vaso-occlusive crises, a pulmonary embolism and HELLP Syndrome leading to urgent fetal extraction at the 8th month.

In 2022, H.E was hospitalized for an episode of vaso-occlusive crisis resistant to analgesic treatment with a chief complaint: bilateral gonalgia. The patient was pregnant at 12 weeks of amenorrhea. Complete blood count showed: HB = 10,9 g/dL, WBC = 17840 X 10^6 /µL and Cytobacteriological examination of the urine showed leucocytes = 16000 /mL and a contaminated urine culture. Therefore, she was put on antibiotics and an RBCx session was performed. The patient's blood group is A RH:-1,-2,-3,4,5 KELL:-1. She received 5 phenotyped leukodepleted and crossmatched PRBC. HB Electrophoresis showed a drop of HB S from 65.9 to 33.8% before and after RBCx session, respectively.

On day 3 post- RBCx, H.E reported dyspnea, polypnea, tachycardia and a blood oxygen saturation at 91%. Due to the unavailability of a pulmonary scintigraphy, we conducted a CT angiography that showed no signs of pulmonary embolism. Because of the history of PE and the high risk, the patient was put on anticoagulation.

Simultaneously, CBC showed a deglobulisation, her HB decreased from 10.9 to 6.6 g/dL with high LDH and bilirubin. Direct antiglobulin test came positive (negative before RBCx), Irregular antibody Screening showed an association of anti-KEL1 and anti-MNS4. The diagnosis of an acute HHS was retained.

The management for HHS requires holding transfusions to avoid further haemolysis and using steroids and IVIG, erythropoietin and iron sucrose injection to stabilize HB levels.

In this case, a specific protocol of management of the pregnancy was established requiring a regular monitoring of the patient, every 15 days or every month. At each visit, in addition to clinical monitoring, a complete blood count and irregular antibody screening were performed. Furthermore, we underwent cross matching of compatible KELL:-1, MNS:-4 RBCs which have been reserved for the patient to be transfused in case of need.

Our patient gave birth at full term. She did not require any transfusion during pregnancy and after childbirth. Her HB levels were stabilized around 9 g/dL and dropped to 7g/dL after giving birth.

Summary/Conclusions: This case report emphasizes the importance of preventing and diagnosing HHS in patients with SCD and to properly manage transfusions in these cases. This case highlights the importance of cooperation between clinicians and biologists in treating patients with HHS.

Cellular therapies

Stem cell and tissue banking, including cord blood

P524 | Establishing a homograft bank with vascular allografts within the blood- and tissue transplant service in the region of Southern Denmark

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Background: Infection of artificial blood vessels and heart valves is life-threatning and requires urgent surgery with replacement of the infected implants. Despite antibiotic treatment the risk of new infection is significant due to the ongoing infection. Many studies report considerable durability and robustness when using heart valves and blood vessels (vascular allografts) from deceased donors. Vascular allografts have been primarily used for aortic valve replacement, peripheral bypass, haemodialysis graft, and aortic prosthesis infections, and the prognosis when using allografts seem to be much better regarding survival, durability of reconstruction and thereby absence of multiple interventions.

Aims: The aim was to establish a homograft bank with vascular allografts at Odense University Hospital, as a collaboration involving the blood- and tissue transplant service at the Department of Clinical Immunology and Department of Cardiac, Thoracic and Vascular Surgery. As far as we know, it would be the first in Scandinavia handling both blood vessels and heart valves.

Methods: A steering group consisting of doctors and other academic personnel from Department of Clinical Immunology as well as surgeons from Department of Cardiac, Thoracic and Vascular Surgery planned and implemented the structure and set-up a homograft bank with vascular allografts. The work was based on excursions to other European tissue banks with vascular allografts besides communication and workshops with experts of the field. A quality management system and a laboratory information system for traceability of the vascular allografts was developed by using already existing systems at the blood- and tissue transplant service both systems fulfilling the requirement in the EU directives of Tissues and Cells.

Results: Within less than 3 years a well-organized homograft bank has been established. Nurses and surgeons were trained in selecting donors and in the procurement procedures of the vascular grafts immediately after the removal of organs for transplantation. Personnel from the blood- and tissue transplant service were trained in the processing, sterilization, freezing and quality control procedures of vascular allografts. A validation of the quality of a graft, before and after cryopreservation, as well as a validation of the whole workflow including traceability regarding both donor, graft and recipient in the laboratory information system

was performed. Primo 2021 the homograft bank was authorised by the Danish Health authorities. By January 2023, 110 grafts have been harvested from 19 donors. 49 grafts were transplanted and only 1 graft discarded due to positive microbiology after sterilization. Among the 26 recipients of the grafts, 21 received a homograft due to graft infection of an aortic valve, ascending- or abdominal aortic artificial graft. Two died within 30 days postoperatively, both due to bleeding.

Summary/Conclusions: Establishing a homograft bank with vascular allografts in an existing blood- and tissue transplant service has several advantages. Most critical processes are handled by personnel with experience of working with the regulation of blood and tissues. Collaboration of departments within the same hospital, and a homograft bank partly driven by surgeons that are the end users of the grafts, in our opinion heightens the quality insurance of the grafts. Finally, the cost effectiveness of using systems already in place at the hospital is considerable.

P525 | Abstract withdrawn

P526 | Unrelated hematopoietic stem cell donor recruitment of the Korean Red Cross Blood Services (2019∼2022): The effect of repeat blood donor targeted recruitment

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Background: The National unrelated hematopoietic stem cell (HSC) donor program of Korea was established by the Korean Ministry of Health and Welfare (MoHW) in 1994. The Korean Red Cross Blood Services (KRCBS) has been participating as a recruitment organization from the beginning, targeting repeat blood donors as potential HSC donors. From 1994 to 2022, a total of 420,217 potential HSC donors were registered, of which 159,042 (37.8%) were recruited by the KRCBS. The proportion of repeat blood donors among KRCBS recruited HSC donors was 99.9%. Before the COVID-19 pandemic, the annual recruitment target of KRCBS has been about 6,000 donors, accounting for about 32% of the total recruitment target.

Aims: To analyse the effectiveness of the recruitment method using individual counselling targeting repeat blood donors and to compare KRCBS's performance with other recruitment organizations.

Methods: Statistics on registration, donor demographics, and retention rates of potential HSC donors recruited by the KRCBS from 2019 to 2022 were analysed using the registry of the Korean Network for Organ Sharing (KONOS) of the MoHW.

P526 - Table 1. HSC donation retention rates of HSC donors recruited by the KRCBS (2019∼2021)

	2019	2020	2021
KRCBS	67.7%	65.0%	72.0%
Total*	57.2%	56.5%	58.0%

Results: During the COVID-19 pandemic, other recruitment organizations, relying on group recruitment rather than individual recruitment, experienced difficulties in recruiting potential HSC donors due to lockdown policies. To meet the recruitment goal, the MoHW reassigned targets for recruitment for each recruitment organization in 2020. As a result, recruitment of potential HSC donors of the KRCBS increased steadily; 5,071/17,000 (29.8%) in 2019, 6,200/17,000 (36.4%) in 2020, 7,200/17,000 (42.4%) in 2021, and 7,350/17,000 (43.2%) in 2022. As of 2022, The ratio of men and women in potential HSC donors recruited by KRCBS was 65.6% and 34.3%, respectively. The age distribution at the time of recruitment was as follows: 18-19 years (12.5%), 20-29 years (69.2%), 30∼39years (18.3%), age criteria for HSC donor registration is 18 ∼ 40 years. As of 2022, 9,756 cases of unrelated HSC transplantation were conducted in Korea, of which 4,961 cases (50.9%) were donated by HSC donors recruited by the KRCBS. Retention rate of potential HSC donors recruited by the KRCBS was consistently higher than the total retention rate.

*Data of five recruitment organizations including KRCBS. At the time of writing, data for 2022 was not available.

Summary/Conclusions: Higher retention rate of HSC donors recruited by the KRCBS is the result of recruiting potential HSC donors through individual counselling by trained nurses and strategically targeting repeat blood donors. In addition, the KRCBS was able to play a pivotal role in recruiting HSC donors when group recruitment was limited due to the COVID-19 pandemic.

Cellular therapies

Collection, processing, storage and release

P527 | Abstract withdrawn

P528 | Overview of apheresis collection of periferal blood stem cells in hematologic patients and healthy donors

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Background: Peripheral blood stem cell (PBSC) products have replaced bone marrow as the hematopoietic stem cell component for autologous transplantation and are frequently used for allogeneic transplantation.

Aims: The aim of this study is to present our experience with apheresis collecting of mobilized PBSC in hematologic patients and healthy donors.

Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of North Macedonia and University Clinic for Hematology; the data was obtained from our database for period from 2000 till 2022. All patients were fully informed on the donation procedure and signed an informed consent for donation. Minimum dose required to ensure successful and sustained engraftment was $2x10^6$/kg CD34+ cells and/or $2x10^8$/kg mono-nucleated cells (MNC). PBSC collection was performed with continuous flow cell separator Baxter C53000, COBE Spectra and Terumo BCT Spectra Optia using conventional-volume apheresis processing the 2 - 2.5 total blood volumes per apheresis. A femoral catheter was used for collection of PBSC and Acid Citrate Dextrose formula A (ACD-A) is used for anticoagulation. Mobilization regimens included granulocyte colony-stimulating factor (G-CSF) alone or combination of G-CSF and disease-specific chaemotherapy.

Results: There were 977 apheresis collections of PBSC in total, of which 792 (81%) performed in 448 hematologic patients (aged 16-65) and 185 procedures (19%) performed in 120 healthy donors, mostly siblings of the treated patient, including 5 unrelated voluntary donors (aged 16-63). The single procedure usually took 180-270 minutes and the volume of collected stem cells was 50-400 ml. The needed number of MNC and CD34+ cells was successfully collected by 1.8 apheresis in autologous donors and 1.5 apheresis in allogeneic donors. Apheresis procedures were generally well tolerated. The only adverse effects were bone pain, as reaction of G-CSF and numbness of the extremities as reaction of ACD-A, which occur rarely and were very mild. The main indications for autologous stem cell transplantation in our patients were: multiple myeloma - 229 (51.1%), acute myeloid leukemia 83 (18.5%), non-Hodgkin lymphoma – 61 (13.6%), Hodgkin disease – 60 (13.4%), acute lymphoblastic leukemia – 11 patients (2.5%), chronic lymphoblastic leukemia – 3 patients (0.7%) and 1 patient with Ewing Sarcoma (0.2%), while indications for allogeneic SCT were: acute myeloid leukemia - 67 patient (55.8%), acute lymphoblastic leukemia - 17 patients (14.2%), chronic myeloid leukemia – 9 patients (7.5%), severe aplastic anaemia – 7 patient (5.8%), myeloproliferative disorders – 6 patients (5%), myelofibrosis – 5 patients (4.2%), non-Hodgkin lymphoma – 4 patient (3.3%), multiple myeloma – 3 patients (2.5%) and one patient with Hodgkin disease and chronic lymphoblastic leukemia.

Summary/Conclusions: The apheresis collection of peripheral blood stem cells in patients and donors is a safe and effective procedure.

P529 | Validation of cryopreservation method for autologous hematopoietic stem cells without hydroxyethyl starch

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Background: To secure viability of cryopreserved hematopoietic stem cells, it is vital to protect cells from extracellular and intracellular water crystal formation during the cryopreservation process. For many years, our department has used a combination of 5% dimethyl sulfoxide (DMSO) and hydroxyethyl starch (HES) as cryoprotective agents, followed by direct cryopreservation in -80°C freezer. Unfortunately, EU has decided to suspend HES solution from the market from 21.09.2022. Cryopreservation solely using DMSO is a well-established method, but cryopreservation is most often performed by controlled rate freezing, to avoid crystal formation.

Aims: The aim of the validation was to establish freezing curves for a cryopreservation method with 5% DMSO in -80°C freezer without HES, and to compare cell viability in the product frozen with and without HES.

Methods: We established freezing curves in -80°C freezer by logging temperature in two different sizes of CryoMacs bags (250 and 500ml) each containing the intended minimum and maximum volume of pooled buffycoat product (30 and 70 mL for the 250 mL bag, 50 and 100 ml for the 500 mL bag). We repeated every measurement six times. The recommended freezing rate in literature is 1-3°C/minute, especially in critical temperature points at 0°C and -40°C.

Viability was measured by pair wise comparison of six buffycoatpools, which were split and cryopreserved with either 5%DMSO+HES, or 5%DMSO alone. The same volume of each pair was transferred to CryoMACS bags and Nunc-tubes. We also performed the same viability comparison of two buffycoatpools that were frozen at minimum and maximum volumes of the two CryoMACS-bags. All were direct frozen at -80°C and stored in the gas fase of liquid N2 for one week before thawing and analysing.

The products and corresponding NUNC-tubes were thawed at 42°C. Viability testing of monocytes, lymphocytes and CD34+ stem cells by flow cytometer FACS Canto II (BD) with a panel of monoclonal antibodies against following CD markers: CD45(FITC), CD34(PE), 7AAD (PerCp-Cy5.5), CD33(APC), CD14(APC-cy7) was performed immediately.

Results: Freezing curves for three of the four product volumes showed freezing rates higher than 1-3°C/minute for more than half of the measurements. We observed that the highest temperature change rate (up to +8,4°C/min) occurred around -8/-9°C. This positive change is due to the thermic effect of DMSO.

The viability of the cells were on average 99,9% and 98,5% in products and Nunc tubes frozen with 5%DMSO+HES, and 99,7% and 98,8% when frozen with 5% DMSO alone. These results were comparable to the validation we performed in 2019, comparing two different types of HES solution. The viability in different product volumes was also comparable with the average of 99,2%, 98,7%, 99,2% and 97,8% for the 30ml, 50ml, 70 ml and 100 ml product respectively.

Summary/Conclusions: Comparing cryopreservation methods with direct freezing in -80°C freezer and 5%DMSO, with and without HES results in similar, comparable, high viability of the cryopreserved cells, despite higher freezing rate than recommended. We plan to follow up on the viability measured in the autologous stem cell products and compare this to our historical data, as well as to follow up on the subsequent engraftment in patients. Preliminary data on this matter show satisfactory results.

P530 | An alternative washing solution for the thawing of cryopreserved hematopoietic stem cells

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Background: Cryopreservation of hematopoietic stem cells (HSC) involves slow-rate cooling in the presence of a cryoprotectant (DMSO) to avoid the damaging effects of intracellular ice formation. At room temperature, DMSO is toxic for the cells making it advisable to infuse them in about 10 minutes after their thawing, furthermore, the infusion of DMSO with the thawed product has been related to adverse events. Reduction of DMSO content by washing the HSCs after thawing has been suggested as a method to avoid infusion-related side effects. Human albumin (HSA)-dextran or albumin- hydroxyethyl starch (HES) washing methods have proved useful in thawing HSC products. Dextran and HES shortages prompted us to search for suitable alternatives.

Aims: We report the results of a comparative study of the use of an albumin-only-based solution as an alternative to HES for washing thawed HSCs products.

Methods: A total of 26 peripheral blood HSC bags intended for disposal were used. Peripheral blood HSCs had been collected by apheresis from patients mobilized by recombinant human G-CSF. The leukapheresis products had been cryopreserved with 10 % DMSO within 24 h after collection at a final cell concentration $<3 \times 10^8$ cells/mL using a controlled rate freezer.

Cryobags were placed in a 37°C water bath, then the bag content was slowly reconstituted with an equal volume of the washing solution and transferred to a Cobe- 2991 processing pack and placed in the Cobe-2991 device. The standard washing solution (HES) contained HES and HSA diluted in Plasma-Lyte A, to reach a final HES and HSA concentration of 2.4 % and 4.2 % respectively and the test solution was made out of HSA (20%) diluted in Plasma-Lyte A (75%) and ACD-A (5%).

A total of 26 HPC bags cryopreserved with 10 % DMSO and intended for disposal were used. We conducted a paired study with equivalent bags at the starting point, sixteen washing procedures (of 1 or 2 bags each) were made comparing 8 procedures with our standard washing solution (HES solution) and 8 procedures with the albumin solution.

For each paired experiment, products were thawed at different times by the same technologist using the same equipment. Each final product was tested immediately after washing and after 60 min for total nuclear cell (TNC), acridine orange viability, viable CD34+ enumeration, and clonogenicity (CFUassay).

Data were expressed as the mean ± standard deviation (SD). A Student's t-test was used. All statistics were performed using Excel 2010 (Microsoft Corporation, Redmond, USA). p values were considered significant when they were less than 0.05.

Results: Results are summarised in the following table, no significant difference was found for any of the studied variables.

Summary/Conclusions: We can state that the washing solution based only on HSA is equivalent to that used in our routine practice and we can consider to use it in our routine procedures.

P530 – Table 1. Comparison of HES and albumin washing solutions.

Measurement	Washing solution	N	Mean±SD	p
Final TNC	HSA/HES	8/8	1.99 ± 1.13/1.90 ± 0.95	0.86
TNC recovery (%)	HSA/HES	8/8	88.91 ± 7.89/86.71 ± 6.22	0.548
Imm % viable CD34 cells	HSA/HES	8/8	91.74 ± 8.10/93.62 ± 4.45	0.57
1-hour % viable CD34 cells	HSA/HES	7/7	92.56 ± 5.81/93.52 ± 4.83	0.74
Immediate final CD34x10^6/kg	HSA/HES	8/8	0.48 ± 0.58/0.37 ± 0.54	0.69
Immediate CD34 recovery %	HSA/HES	8/8	101.21 ± 24.03/87.01 ± 29.75	0.312
1-hour final CD34×10^6/kg	HSA/HES	7/7	0.30 ± 0.32/0.30 ± 0.34	0.98
1-hour CD34 recovery (%)	HSA/HES	7/7	93.55 ± 21.93/91.11 ± 17.20	0.820
CFU assay (x10^4/kg)	HSA/HES	5/6	18.83 ± 28.15/18.23 ± 24.56	0.97

P531 | Abstract withdrawn

P532 | Real World Evidence (RWE) to assess effectiveness and safety of donors during and after cell and bone marrow collection

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Background: RWE regarding effectiveness and safety outcomes after bone marrow(BM) and peripheral blood stem cell(PBSC) collection are extremely relevant to determine the real impact of this act on quality of life of donors. This study describes the experience of a Portuguese collection center that serves and cares for matched unrelated donors(URD) from the National Registry and related donors(RD) of patients transplanted in one of the largest transplant units in Europe.

Aims: To evaluate real-world outcomes of donors during and after cell and bone marrow collection.

Methods: We conducted a prospective observational study with adult donors between January/22 and December/22. Clinical data was collected from medical and administrative records, at baseline, collection and 1-month after donation. Clinical characteristics were evaluated using descriptive statistics.

Results: Fifty-six donors were included in this study. URD group ($n = 40$) included 23 females and 17 males with a median age of 36(22-52)years; RD group ($n = 16$), 9 females and 7 males with 46(24-62)years. 86% of donors collected PBSC after a 5-day G-CSF mobilization period (median dose: 12ug/kg/day). Length of stay of inpatient care was 2,6 days for all BM donors. At baseline, we found a healthy population, with no associated comorbidities. The most reported symptoms, immediately after PBSC collection, were related to the mobilization (58%) and venous access (50%) with systemic and local pain (48% in the legs, 33% at the lumbar spine), respectively. 63% of BM donors reported nausea and 100% reported pain at the puncture site; poor physical performance and asthenia were also observed (BM 76%/75%; PBSC 21%/38%). At 1-month after donation, 14% of BM donors and 13% of PBSC still complained of moderate pain; however, the PBSC donors had full and faster recovery (respectively, 1 week and 2 weeks). 95% of PBSC donors had complete recovery, most of them with 1-5 days recovery time. All BM donors had complete recovery, 57% with 6-15days recovery time.

Summary/Conclusions: Understand donors' medical fitness at the first evaluation and safely prepare them for a high-risk invasive procedure is the better way to prevent risks associated with cell and bone marrow collection. RWE available at 1-month after donation will allow us to make comparisons between BM and PBSC donors and determine their long-term outcomes.

P532 – Table 1

time points	characteristics	BM donors ($n = 8$)	PBSC donors ($n = 48$)
Baseline	Haemoglobin, g/dL	14.0	14.1
	Leucocytes x10^6/ml	5.44	6.96
Collection	Time of collection, hours	01:10	03:54
	Haemoglobin, g/dL	12.1	13.7
	Leucocytes x10^6/ml	8.6	46.6
	Pain local/systemic %	100/62	50/58
1-month Follow-up	Haemoglobin, g/dL	13.7	13.9
	Leucocytes x10^6/ml	5.1	5.8
	Pain local/systemic %	43/14	8/12.5

P533 | Contingency plan for the storage of cryopreserved products: how to proceed if the N_2 tank collapses

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Background: The proper storage of hematopoietic progenitor cells (HPCs) is essential to ensure the viability of the product and, therefore, the success of the bone marrow transplantation. Due to the absence of a backup tank, a contingency plan was implemented in case of collapse of the current tank (rupture or leakage of the cylinder).

Aims: To validate the contingency plan which consists of immediately transferring the products from the liquid N_2 tank to ultra-freezers at -80°C on a temporary basis, until another liquid N_2 container is available.

Methods: On D0, all racks containing the products were removed from the liquid N_2 and immediately stored in a -80°C freezer. These samples were gradually removed from the freezer on days 0, 6, 14, 21, 30, 61, 90, 120, 150 and 180, thawed in a water bath (37°C), tested by the Trypan Blue (TB) viability test by optical microscopy, and also tested for viable CD34+ cells with 7AAD by flow cytometry. We used products from patients (or allogeneic donors), which would be discarded after the patient's authorization or after the patient's death. A total of 56 samples (31 bags and 25 segments) were studied from 10 collections of 6 patients.

Results: The viability analysis by TB showed no significant difference between the days analysed (p>0.05), with a mean of 78.8 ± 5.5%. The only difference ($p = 0.002$) was observed between D0 and D180, however we only had 2 samples available on D180. Greater variability, however, was noted by 7AAD between the samples (10 samples/day) of days 6, 30 and 90 (mean ± sd: 87.5 ± 0.9%; 84.4 ± 13.6%; 86.1 ± 13%, respectively). When comparing both methodologies, there was a significant difference only on D0 (mean 87.2 ± 5.3 by TB; 76.5 ± 12.5 by 7AAD), but both presented similar averages after D6 (mean 84.2 ± 6 by TB, 87.5 ± 10.9 by 7AAD). In the 10 samples analysed on D0, an average recovery of 72% of viable CD34+ was observed by TB and 76% by 7AAD, when compared to the pre-freezing dosage.

Summary/Conclusions: Although some authors mention that it is common cell viability in thawed samples to reach values below 70% using the TB test, in our study all samples showed acceptable viability (above 70%). During the analysis with the 7AAD test, the results were also above those found by other authors. Thus, in an emergency situation, it is feasible to use ultra-freezers at -80°C to store cryopreserved products until another liquid N2 container is obtained. Due to the criticality of the product, this storage should be done for the shortest period possible, only as a palliative solution, until another N_2 container is provided, preferably not exceeding a period of 15 days.

P537 | Reducing the DMSO concentration in the cryopreservation mixture from 10% to 5% improves cell viability, results of a validation study.

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Background: The procedure of autologous peripheral blood stem cell transplantation and some allogenic stem cell transplantation requires cryopreservation of the stem cells in a mixture containing dimethyl sulfoxide (DMSO). DMSO is necessary to secure cell viability by preventing the formation of ice crystal that damages the cells. However, its infusion may be toxic to stem cell recipients, especially in the pediatric age group. Hence, we investigated the option of reducing the DMSO concentration in our stem cell processing laboratory.

Aims: This validation study aimed to prospectively evaluate the impact of DMSO concentration reduction from 10% to 5% on cell viability.

Methods: We ran a validation study on 10 random peripheral blood and bone marrow stem cell collections in the period between February 2021 to December 2022. Each collection was cryopreserved in duplicate for 5% and 10% DMSO concentrations. The WBC, absolute CD34 count and cell viability were assessed before cryopreservation, 2 weeks, 4 weeks, and 8 weeks post-thawing. P-values were calculated using the Wilcoxon test. P values ≤0.05 are considered statistically significant.

Results: The results showed significantly lower viability when using 10% DMSO compared to 5% at 2 weeks, 4 weeks, and 8 weeks of cryopreservation ($P = 0.002$, $P = 0.002$, and 0.004), respectively.

Summary/Conclusions: In view of these results, a 5% DMSO mixture may be considered a new standard in the cryopreservation of hematopoietic stem cells for pediatric patients at the KFSH&RC stem cell processing laboratory.

Cellular therapies

Clinical applications

P534 | Abstract withdrawn

P535 | Hematopoietic stem cell transplantation in non malignant diseases- single-center experience from India

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Background: Haematopoietic stem cell transplantation (HSCT) can be used to cure or remedy variety of non-malignant diseases(NMDs). These range from inherent defects of haematopoiesis, through metabolic diseases, to severe autoimmune diseases. This protocol is based on the immunoablation with high-dose chaemotherapy followed by subsequent regeneration of naïve T-lymphocytes derived by reinfusing haematopoietic progenitor cells into the patient. Ours is a Super-Speciality Quaternary center in Northern India with a very large volume of patients from across the world, treated for various malignant and Nonmalignant diseases, some of which need Bone Marrow Transplants for treatment.

Aims: To review and analyse our 6 year HSCT data of transplants done for patients with NMDs from 2017-2022 and to get perpective into its various affecting factors for going forward.

Methods:

P535 – Table 1

Total HSCT Done	1104
Malignant Non Malignant(NMD)	816/1004(81.58% 185/1004(18.42%)
Type of Stem Cell Product used for NMDs 1. Allogenic - HLA MSD (Matched Sibling Donor) - HAPLO Matched 2. MUD (Matched Unrelated Donor)	164 108 56 21
Mean Age (yrs) Mean Weight(Kg)	20.5(Range 1-56) 54.95(Range 9-111)
Male Donors Female Donors	118 67
Mean TBV processed	2.2
Mean PreCD34+ count Mean PostCD34+ yield	104.56x10^6 cells/l (Range 21.5-577.2) 8.79 million/Kg body wt
No. of Repeat PBSC collection	09
Avg Dose Infused	5.3x10^6/ul/Kg body wt

P535 – Table 2. Various NMDs with Transplant outcomes in our Center

Disease & No. of pts	HLA MSD/ GVHD	HAPLO/ GVHD	MUD/ GVHD
Sickle cell Anaemia (38)	24/13	14/09	0/0
Beta Thalassemia (75)	42/16	21/18	12/12
Aplastic Anaemia(32)	22/09	05/03	05/05
Fanconi Anaemia(17)	09/05	06/04	02/02
Adenoleukodystrophy(03)	02/01	01/00	0/0
ChediakHigashi (02) Ch. Sideroblastic Anaemia(01) Diamond Blackfan A (02) Hunter Disease(03) Krabbe Disease(01) Cytopenia(02) Familial Lympho histiophagocytosis(01) Nieman Pick Dis(01) CGD(01)	02/01 01/0 0/0 01/0 0/0 0/0 0/0 0/0 01/01	0/0 0/0 01/01 01/01 01/01 02/02 01/01 01/01 0/0	0/0 0/0 01/01 01/01 0/0 0/0 0/0 0/0 0/0

Retrospective data of 1004 HSCT, done from Year 2017-2022, was collated and analysed. 185 transplants done for NMD patients. PBSC were collected in the centre and MUD taken from Registries. Venous access was either Femoral or Jugular vein. Collection done on Optia cell separator (Terumo BCT) and Comtec (Fresenius Kabi). Flowcytometric analysis of the samples was done for CD34+ cells. Details in **Table 1 Transplant details of Non Malignant Diseases from year 2017-2022**

Results: Of 188 HSCT done, HLA MSD had GVHD 46/104 (44.2%) patients and death within 120 days in 11/104(10.5%) cases. Haplomatched transplants had 42/56(75%) GVHD and 18/56(32.1%) death within 120 days. MUD Transplants had 21/21(100%) GVHD and 5/21(23.8%) death in 120 days. Few patients were lost to followup. No Correlation of the outcomes was found with procedural parameters.

Summary/Conclusions: Our Analysis showed outcomes in parity with the various publications for HLAMSD, Haplomatched and MUD HSCT transplants done in our center, upto a period of 120 days post-transplant, but we could not followup most of the paients for long since majority of our patients were from various countries across the world and limited our followup.

P536 | Efficacy of novel low cost Autologous Bone Marrow Aspirate Concentrate (BMAC) processing technique using quadruple blood bags as adjuvant therapy in management of AVN of hip joint

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Background: Osteonecrosis has been described as death of trabecular bone and cells within the confines of femoral head leading to subchondral collapse and deformation of articulating head surface Bone marrow derived stem cells are being used for quite a few years for early stages (Stage I and Stage II). We have used modified technique for collecting Bone marrow aspirate using quadruple blood bags and processing in the standard blood bank component preparation centrifuge to prepare a Bone Marrow Concentrate (BMC) with good results .

Aims: The treatment of ONFH has been studied extensively, and there are many options available for ONFH, including both nonsurgical treatment and surgical treatment. The most common surgical treatment is core decompression (with or without adjuvants) for early stage disease. The ideal goal of early stage treatment is to postpone or stop the progression of the disease before femoral head collapses.

Objective :

Although the mechanism of autologous BMCs combined with core decompression for ONFH treatment is unknown; simultaneous use of Concentrated Autologous Bone Marrow containing mononuclear cells as adjuvant in the pre-collapse stages of the disease is beneficial in improving the functions scores and for reducing the radiological progression of the disease .

This study is Retrospective which Aims:

1. To evaluate the efficacy of a low cost adjuvent therapy ----An in house preparation of Autologous Bone Marrow Concentrate using top and bottom quadruple blood bags processed in standard blood bank component preparation centrifuge.

2. To use a modified surgical technique of Core Decompression -Single pilot hole leading to all four quadrants and centre of femoral head with reverse drilling

Methods: We devised a simple method of Core Decompression and implantation of Bone marrow concentrate that will not destabilise the hip and help in regeneration / healing of the lesion in Stage I and stage II of AVN of femoral head. All patients having Grade I or Grade II (Steinberg Classification) were taken up for the study, irrespective of the etiology.

50 ml of bone marrow is aspirated for a single hip. If it is a B/L procedure, 100ml of bone marrow was aspirated. The aspirated bone marrow on heparinised syringes was put in the Top and Bottom quadruple blood collection bags and sent to blood bank for separation of Buffy Layer of Mononuclear cells under full aseptic precautions. This was done by removing some of the red blood cells (the non nucleated cells) and the plasma with a standard blood bank centrifuge used for the preparation of blood components from the whole blood --Hereus 6000i centrifuge at 3900 RPM for 10 min at 220C with a g force of 493.

Final volume concentrated and extracted from a 50 ml of BM aspirated for unilateral procedure was about 10 ml which amounted to 40x103 - 50 × 103 cells per hip.

A simple method of Core Decompression involving a Pilot hole and 5 tracts made to all the four quadrants and center of femoral head with very slow reverse drilling under C-arm. Bone marrow concentrate back in operation theatre is carefully filled in a 10 ml syringe. Jamshedji needle is used for injecting the concentrate making sure the tract with the sclerotic cavity is chosen under C-arm. The pilot hole is sealed with bone wax.

Results: Overwhelming results were obtained from comparison of Harris Hip Score, Visual Analogue Scale, progression of the osteonecrosis stage & radiological comparison before and after surgical procedure. Since May, 2013, 18 patients, irrespective of the aetiology of AVN, have undergone this procedure. Only one patient who was having bilateral AVN secondary to chronic alcoholism progressed to Stage III in right hip but the left hip was saved. In rest of the patients, the pain resolved and none of them progressed to later stages till now. No perioperative and postoperative complications were noted.

Summary/Conclusions: This method of multiple quadrant decompression and Bone Marrow Concentrate infiltration via single pilot hole is simple, safe and easy to replicate. The procedure can be done at any of the hospitals having a C- Arm machine and a blood bank with minimal financial implications. This is a minimally invasive technique that is simple to perform, not associated with complications and donor site morbidity. Patient is allowed to bear weight post operatively (as tolerated) so patients rehabilitation is also better.It provides excellent outcome in preserving hip anatomy, halting progression of pathology and ultimately the untimely Total Hip Arthroplasty in in early stages of osteonecrosis of femoral head (Stage 1 & 2).

Clinical immunogenetics

HLA in transfusion medicine

P538 | Association of HLA-DRB1 alleles with anti-D alloimmunization in RhD negative pregnant women

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Background: The frequency of anti-D alloimmunization in RhD negative pregnant women is still quite high in our population and was observed to be around 15% in a recent study from our centre. Human leukocyte antigen (HLA) restriction plays an important role in the susceptibility to alloimmunization against red blood cell (RBC) antigens. Certain HLA-DRB1 alleles including HLA-DRB1*15, HLA-DRB1*11

and HLA-DRB1*13 have been found to be associated with RBC alloimmunization. However, there is limited data regarding HLA type of individuals with anti-D alloantibody in the setting of RhD negative pregnancy in our population.

Aims: The study was performed to determine the association of HLA-DRB1 alleles with anti-D alloimmunization in RhD negative pregnant women.

Methods: The study was approved by the Institute Ethics Committee (No. INT/IEC/2019/001927 dated 26.09.2019). RhD negative pregnant women visiting the antenatal clinic of our institute were enrolled for the study and an RBC antibody screen (ABS) was performed using a 3-cell panel by column agglutination technique (CAT) (Bio-Rad, Switzerland). Those with a negative result were included in the 'non-alloimmunised' (NAL) group ('Control' group). If the ABS was positive, then an antibody identification was done using a 11-cell panel by CAT. If the alloantibody specificity was anti-D, with or without other alloantibody(ies), then such patients were included in the 'alloimmunised' (AL) group of the study. There were 50 patients enrolled in each of the two groups (AL and NAL). For HLA-DRB1 allele determination, first the deoxyribonucleic acid (DNA) was extracted within 5-7 days of sample collection using the DNA extraction kit by spin column-based method (QIAmp DNA, Qiagen, Hilden, Germany). Subsequently, the HLA-DRB1 typing was done by Luminex based reverse sequence specific oligonucleotide probing (SSOP) using commercial kits (HLA-DRB1 SSO Typing Kit, Immucor, USA). The HLA-DRB1 allele frequency was compared in both the groups. The anti-D antibody titre was performed using tube technique by preparing serial doubling dilutions of the serum with normal saline.

Results: There was a significant difference between the AL and NAL groups in terms of gravida status (98% versus 66% being multigravida, respectively; $p < 0.001$) and history of receiving anti-D immunoprophylaxis (86% versus 50%, respectively; $p < 0.001$). The frequency of HLA-DRB1*03 and HLA-DRB1*04 alleles was significantly higher in the AL group than the NAL group: 40% versus 18% [Odds Ratio (OR): 3.04, 95% CI: 1.21-7.6; $p = 0.015$] for HLA-DRB1*03 alleles and 18% versus 4% (OR: 5.27, 95% CI: 1.08-25.78, $p = 0.025$) for HLA-DRB1*04 alleles. In addition, the AL group had a significantly higher frequency (38%) of the specific allele HLA-DRB1*03.01 than the NAL group (16%) [OR: 3.22, 95% CI: 1.25-8.3; $p = 0.013$]. The frequency of other HLA-DRB1 alleles was not significantly different in the two groups. There was no significant association of gravida (range: 1 – 7) with anti-D titre ($p = 0.060$), although a higher anti-D titre was observed in G3 and G7 women.

Summary/Conclusions: The frequency of HLA-DRB1*03 and HLA-DRB1*04 alleles is significantly higher in RhD negative pregnant women with anti-D alloantibody, thus implicating an association of these alleles with anti-D alloimmunization. Further studies in a larger population may help exploring the role of HLA restriction in developing RBC alloimmunization.

P539 | Abstract withdrawn

P540 | Anti-HLA antibody screening of allo-exposed COVID-19 convalescent donors

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Background: At initial stage of COVID-19 pandemics, plasma obtained from COVID-19 convalescent donors, due to the presence of neutralizing antibodies directed against viral antigens, has been considered a promising therapy for the infection. Considering the scale of the pandemics, introduction of such treatment to a routine practice would require significant increase in the number of the available donors, possibly through the qualification of allo-exposed individuals to the donor pool that may pose safety issues. Although recent WHO guidelines recommend against the use of convalescent plasma to treat COVID-19, the question on the safety of allo-exposed donor plasma remains. Here, we utilised data from prospective screening for anti-HLA antibodies performed in plasma donations collected from allo-exposed COVID-19 convalescent donors with a history of pregnancy and transfusion performed during COVID-19 pandemics in Poland in 2020 to evaluate the rate of allo-immunization in this population.

Aims: Evaluation of HLA allo-immunization risk in a population of allo-exposed COVID-19 convalescent plasma donors

Methods: Since April to December 2020, the Institute of Hematology and Transfusion Medicine collected plasma samples from 1,864 allo-exposed COVID-19 convalescent donors (1844 women, 20 men) with a history of pregnancy and/or transfusion obtained who donated plasma in 19 Regional Blood Transfusion Centers in Poland. Anti-HLA-antibodies were tested using LABScreen Mixed Class I & II (One Lambda). If the results were inconclusive LABScreen Single Antigen Class I and Class II was performed on LABScan 100^{TM}(Luminex x MAP^{TM} Technology).

Results: The percentage of immunised allo-exposed COVID-19 convalescent donors with HLA anibodies was 61.9% (1154/1864, including 1152 women and 2 men with antibody against HLA class I and II). Anti-HLA class I antibody was detected in 305 (16.36%), anti-HLA class II in 236 (12.66%), and anti-HLA class I and II in 613 (32.88%) donors.

Summary/Conclusions: The prevalence of anti-HLA antibodies in allo-exposed COVID-19 convalescent donors with a history of pregnancy and/or transfusion is high and consequently plasma obtained from such donor pool pose high risk of non-haemolytic post-transfusion reactions (including TRALI) and is considered unsafe. These results confirm that the screening for HLA-antibody in all allo-exposed donors is required to exclude immunised donors from the donor pool.

P541 | Features of the three-locus HLA-haplotypes structure in Tajik nationality persons living in the Republic of Tajikistan

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Background: The polymorphism of the HLA genes makes it possible to identify population genetic differences, as well as to select the most compatible allogeneic donors for organ and hematopoietic stem cell transplantation. Against the background of a studies shortage dedicated to HLA genes in the Republic of Tajikistan, the necessity for the development of organ transplantation, and also taking into account the fact of HLA-specificity distribution variety in different populations, it is of great value to create a national register of HLA-typed donors.

Aims: To identify the HLA-A, HLA-B and HLA-DR genes prevalence in blood donors (self-identified as Tajik nationality) who expressed a willing to become hematopoietic stem cell donors if necessary in the future.

Methods: The gene structure of 300 donors was determined in a standard two-stage microlymphocyte toxic test using a set of histotyping anti-HLA serums of «Interregional Immunogenetics Center» and «Gisans» histotyping reagents on the basis of the Republican Scientific Blood Center. Using these kits, 19 antigens of locus A and 38 antigens of locus B of class I were detected. DNA typing of 600 blood donors who consider themselves Tajiks aged 18 to 30 years was also carried out. Of these, 358 are men and 336 are women. DNA samples for HLA typing were obtained from fresh whole blood (anticoagulant – EDTA) by column filtration using Protrans DNA Box 500 Fast DNA reagent kits. Molecular genetics typing (basic resolution) was performed by polymerase chain reaction with a set of sequence-specific primers (PCR-SSP). Detection was carried out in 2% agarose gel using electrophoresis. All studies were performed with standard Protrans (Germany) reagents according to the manufacturer's protocol on the basis of the Republican Blood Research Center and foreign laboratories.

Results: As a result of the conducted studies, it was noted in the distribution of HLA-alleles class I genes A2, A3, A1, A24, A1 dominate most often in the Tajik population donors. Genes mainly B35, B44, B51, B07, B49, B17, B18, B14, B12 were identified in the locus of the HLA-B gene. Among the locus of the DRB1*DR the dominated genes were DRB1*03, DRB1*15, DRB1*04, DRB1*11, DRB*05, DRB*09. Most European peoples are dominated by genes A2, A3, A24, A01, B7, B35, B18, B44, DRB1*15, DRB1*07, DRB1*13, DRB1*11, DRB1*01, DRB1*16, DRB1*18. Comparative HLA genes analysis shows the similarity of the results obtained with the data obtained in Caucasoid. At the same time, according to the loci of HLA genes, there is a significant difference in comparison with the Turkic peoples living in Central Asia, including in the Republic of Tajikistan, in particular the Kyrgyz, who often have antigens A24, A11, A26, B37, B48, B57, B63, DRB1*14, DRB1*09, DRB1*10.

Summary/Conclusions: According to HLA-genes typing, the Tajiks living in the Republic of Tajikistan revealed the proximity of the genes of the HLA-A, B, and to a lesser extent HLA-DRB1 loci with Caucasoid, and significant differences in comparison with the Turkic peoples (Kyrgyz, Kazakhs, Uzbeks) living in Central Asia and the Republic of Tajikistan. The preliminary results dictate the necessity to create in prospect a HLA-typed donors register in the Republic of Tajikistan.

P542 | Determination of genetic markers of alloimmunization: Association between the presence of alloantibodies against erythrocyte antigens and specific HLA class II alleles.

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Background: Alloimmunization against erythrocyte antigens increases morbidity and mortality in transfused patients due to the risk of transfusion haemolytic reaction. The cost of an alloimmunised patient is estimated at US$50,000 (Kacker S. Transfusion. 2014). To avoid alloimmunization, it is recommended to transfuse compatible for clinically important antigens, which could be optimised by knowing prior to transfusion which patients will be alloimmunised.

It is not known why some patients become alloimmunised (responders) and others do not (non-responders), however, many studies describe as risk factors: antigen disparity between donor and recipient, age at first transfusion, sex, severity of the underlying disease, altered immunoregulatory status, inflammation and genetic factors. Among the latter are the HLA class II genes that encode molecules involved in antigen presentation. The polymorphisms present in HLA alleles could influence whether antigen presentation is more or less efficient. An association has been found between specific HLA class II alleles and alloimmunization. No studies have been carried out in Latin American countries, except Brazil (Sippert, Transfusion, 2017).

Aims: To investigate whether the presence in a patient of a specific allele of HLA class II genes is associated with increased alloimmunization.

Methods: We studied 58 alloimmunised transfused patients corresponding to the study group and 32 non-alloimmunised transfused patients as control group.

DNA extraction from EDTA whole blood was performed using the QIAamp DNA Blood Mini Kit. Genotyping of HLA-DRB1, DQA1, and DQB1 genes was performed using allele-specific PCR combined with Luminex technology that allows fluorometric detection of specific alleles.

The frequency distribution of HLA alleles between groups was compared using Fisher's exact test to evaluate the association between

the presence of alloantibodies and HLA alleles, and the Odds ratio between groups was calculated to obtain the probability of alloimmunization of one group in relation to another.

Results: When comparing the frequency of each allele between patients with and without alloantibodies, we obtained a *p*-value = 0.0312 and an Odds Ratio (OR) of 3.5 for the DRB1*07 allele, indicating that there is a significant statistical difference between the two groups and that when this allele is present, the odds of alloimmunization (ratio between alloimmunised and non-alloimmunised) increase 3.5 times. In the DQA1 gene, differences were only found in the DQA1*04 allele, but with an OR of less than 1; DQA1*01 and DQA1*03 presented an OR greater than or equal to 2, but without significant statistical differences between the study group and the control group. In the DQB1*03 allele, an OR of 3.75 was obtained, with no significant statistical difference between the two groups.

Summary/Conclusions: The HLA DRB1*07 allele could be used as a genetic marker of Chilean patient responders, allowing to focus efforts to prevent alloimmunization in susceptible patients who present this allele, reducing the costs of having an alloimmunised patient and increasing transfusion safety.

P543 | Results of individual selection of platelets according to the HLA system in hematological patients

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Background: The most common cause of immunological refractory to donor platelet transfusion is the presence of HLA-specific antibodies in the patient, because the HLA 1 class antigens present on platelets that most often cause sensitization that develops with multiple transfusions of platelet concentrate. Sensitization to HLA antigens is defined as the presence of antibodies against HLA molecules of the selected donor in a potential recipient. Exposure to foreign HLA antigens can cause the production of HLA-directed antibodies.

Aims: To determine laboratory parameters affecting the probability of finding a compatible platelet donor.

Methods: 22 adult patients with a history of multiple transfusions and having various transfusion reactions were examined. Platelet selection was carried out in three stages: determination of the percentage and specificity of HLA antibodies by flow cytometry (One Lambda, Lab Screen), determination of the patient's HLA phenotype (FluoGene, Inno-Train) and the donor (HLA Ready Plate, Inno-Train), setting up a cross-match compatibility test by serological method.

When interpreting the results of HLA antibodies, the following parameters were taken into account: the percentage of sensitization – reflects the number of types of detected antibodies, the level of fluorescence (MFI) - determines the concentration of detected antibodies in the blood serum. According to the results of the percentage of sensitization of HLA antibodies, patients were divided into 3 groups: with low sensitization - patients who showed results from 0 to 10%, with average sensitization from 11 to 30%, and with high sensitization from 31 and above.

Results: 163 donors were examined to find a suitable donor for patients of the first group, of which 34 donors showed a positive result of the "cross-match" (20%) and 129 donors showed a negative result (79.1%). For the second group, 172 donors were examined, of which 85 (49.4%) donors had a positive result and 87 (50.6%) donors had a negative result. 152 donors were examined for the third group, of which 70 (46.1%) were positive and 82 (53.9%) had negative "cross-match" results. The prognosis of finding a compatible donor in the first group is favourable, whereas the probability of finding a compatible donor for patients with 30% and higher antibodies was 50%. In addition, in the medium sensitization group, there was a patient with a low percentage of antibodies of 12%, but with a high MFI (111744), who in 92% of cases had a positive "cross-match" with donor cells. This fact proves the need to pay attention to the MFI level when assessing the possibility of finding a compatible donor. The higher the MFI, the worse the prognosis of the possibility of having a compatible donor. In addition, there was a patient with a large number of positive "cross-match" (85%), but with a low MFI index (5356) for which the exclusion of the presence of IgM is required.

Summary/Conclusions: As a result, when selecting platelets according to the HLA system, not only the presence and specificity of the detected antibodies, but also their MFI level with IgM identification is of great importance.

P544 | Resolving next-generation sequencing-generated ambiguities in HLA typing using nanopore sequencing technology

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Background: Accurate genotyping for human leucocyte antigen (*HLA*) is crucial for hematopoietic stem cell transplantation (HSCT). The next-generation sequencing (NGS) is currently the most widely used method for HLA typing, however, the HLA typing ambiguities are increasing in clinical practice due to increasing number of HLA alleles and the short read lengths of NGS platforms. Now, the third-generation sequencing which is a technology of single-molecule, real-time and long-read length sequencing, such as nanopore sequencing technology, was introduced to the characterization of HLA, and the accuracy of nanopore sequencing is increasing these years.

Aims: To establish a method for HLA typing based on nanopore sequencing technology, and to resolve HLA typing ambiguities generated by NGS, which could be more sufficient to discover the complexity of the HLA highly polymorphic systems.

Methods: In this study, a total of 32 samples with ambiguous generated by NGS in HLA typing were collected in our laboratory during 2021-2022, and the *HLA-A, -B -C, -DRB1,-DRB3/4/5, -DQB1, -DQA1, -DPB1* and-*DPA1* loci were amplified through AllType™ NGS 11-loci amplification kit (One Lambda). The purified PCR production were

end repaired and dA tailed, ligated to Oxford Nanopore Technology (ONT) native barcodes, pooled equimolar, and ligated to ONT Barcode Adaptor Mix. The adaptor-ligated and barcoded pool was loaded onto an ONT SpotON SQK-LSK110 flow cell on the MinION MK1C, and amplicon strands were processed by MinKNOW software version 22.12.7 (Oxford Nanopore Technologies). The resulting FASTQ files were analysed and assigned using NGS engine software (GENDX).

Results: This nanopore sequencing experiment in HLA typing was successful and the average reading depth of each locus was above 800 by the NGS engine software analysis. The HLA typing results in two-field high resolution generated by nanopore sequencing in these 32 samples were conference to those by NGS. Among the collected 32 samples, there were 5 unphased novel alleles and 25 different common ambiguities with the NGS platform. Though the nanopore sequencing with long-read length, we identified 4 *HLA-DPB1*and 1 *HLA-DRB1* new alleles with exon 3 and 6 mutations, and phased 24 different ambiguities including 1, 6, 1, 1, 1 and 14 kinds of which in *HLA-A*, *-B -C*, *-DRB1*, *-DPA1* and *-DPB1* loci respectively, and got the accurate allele patterns. Exceptionally, only one allele ambiguities*DQB1**06:02, 06:41 versus *DQB1**06:03, 06:84 were not resolved.

Summary/Conclusions: Nanopore sequencing technology offers a promising solution to resolve the ambiguities generated by NGS in HLA typing.

P545 | Highly efficient knocking out HLA class I molecules in HEK-293 cell via CRISPR/Cas9 ribonucleoprotein

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Background: Human leukocyte antigen (HLA) class I molecules are highly polymorphic expressed on the surface of all nucleated cells, which play a central role in allogeneic hematopoietic stem cell transplantation. The generation of HLA universal cells by silencing HLA class I molecules via gene editing is a pathway to improve the survival rate of allogeneic grafts. CRISPR/Cas9 is now widely used for gene editing, which can induce Cas9 to cut specific target genes by changing only a short gRNA sequence. Transient expression of CRISPR/Cas9 is alternatively supplied to cells through ribonucleoprotein (RNP), which is generated using purified Cas9 protein pre-complexed with in vitro transcribed gRNA. Compared to traditional plasmid DNA, the RNP-based method is more effective with lower off-target. In this study, the high-efficiency method was developed to knock out HLA class I molecules in HEK-293 cell.

Aims: To develop a highly efficient method for knocking out HLA class I molecules in HEK-293 cell, which can provide a basis for the research of HLA universal cells.

Methods: The EasyEdit sgRNA targeting the exon 2 of β2m gene (B2M-sgRNA) was synthesised by GenScript (Nanjing GenScript Biotech CO., Ltd.). Commercial NLS-Cas9-NLS nuclease were incubated with B2M-sgRNA at room temperature for 15 min in different molar ratio (1:1∼1:4), which could form very stable ribonucleoprotein (RNP) complexes. The HEK-293 cells were either electroporated with different concentration of RNP complexes or without RNP complexes (neg control) by Lonza 4D system under optimised electroporation conditions. After transfection and cell culture, the electroporated cells were also analysed for the surface expression of HLA-I molecules at 72 h by flow cytometry, which could validate the efficiency of gene editing in different transfection groups. In parallel, the genomic DNA was extracted from the electroporated cells using commercial kit, which was used for amplifying β2m gene with the specific primers(B2M-F:5'-ACACTTGGTGCCTGATATAG-3', B2M-R:5'-ACCTGAAGCTGCCACAAAAG-3'). The PCR products were denatured and annealed in order to allow formation of heteroduplex between PCR products with or without mutations. The heteroduplex was digested with EnGen T7 Endonuclease I (NEB) and then was analysed the fragment by 2% agarose gel electrophoresis. The PCR products were also measured by sanger sequencing to further verify the efficiency of gene editing for HLA class I molecules.

Results: Compared with the control group, the negative cell populations of HLA-I molecules were detected in all the four transfection groups. More than 90% negative cell populations of HLA-I molecules were detected in the group with 1:4 molar ratio, suggesting a highest cutting efficiency. According to the result of 2% agarose gel electrophoresis, the specific PCR products were partially cleaved into two bands in all the four transfection groups by T7 Endonuclease I enzyme digestion. However, there was a single target band in the control group. Moreover, there were some overlapping peaks in the vicinity of PAM sequences of β2m gene in the four transfection groups by sanger sequencing, indicating the occurrence of the gene mutations.

Summary/Conclusions: In this study, the RNP-based method was successfully developed for knocking out HLA-I molecules from the surface of HEK-293 cell with the highly efficient of gene editing, which could provide a basis for the study of HLA universal cells.

P546 | Analysis of KIR and HLA polymorphisms with COVID-19 infection

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Background: Killer-cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins expressed on the surface of natural killer (NK) cells and a subset of T cells. They interact with polymorphic human leukocyte antigen (HLA) class I molecules playing an important role in regulating NK cells against many virus. Both KIR and HLA are encoded by polymorphic genes. They have numerous alleles encoding

unique KIR and HLA class I proteins, which affect the capacity of NK cells to recognise and kill target cells, even have different susceptibility or protection to virus infection.

Aims: In this study, we analysed the polymorphism of KIR genes, HLA genes, and the interaction between KIR and HLA for Covid-19 infected patients to investigate the association between KIR/HLA and Covid-19 infection.

Methods: 119 Delta Covid-19 infected patients were recruited and the blood were collected after they cured. Genomic DNA was extracted and an exome capture based high-throughput sequencing method was used to capture and sequence KIR and HLA, then Pushing Immunogenetics to the Next Generation pipeline was performed to analyse KIR genes, genotype, alleles, haplotypes, HLA ligands, and KIR-HLA interaction, which were then compared with healthy control.

Results: In the Covid-19 infected patients, a significant increase of KIR3DL3*00802 and a lower Bw4 alltotype encoded by HLA-B was observed. Besides that, several other factors should be noticed which were found associated with Covid-19 infection although they lost significant after P correction. They were KIR Bx3 genotype, KIR3DL3*00301, 3DL3*048, and C1+ (HLA-C), which showed significant higher frequency in Covid-19 infected patients before P correction. They may be associated with susceptibility to Covid-19 infection, which should be interpreted with caution.

Summary/Conclusions: Our findings suggest that KIR3DL3*00802 is a high risk for Covid-19 infection, and Bw4 encoded by HLA-B maybe protective to Covid-19 infection.

P547 | Relationship between HLA-*DRB*15* allele and erythrocyte alloimmunization in hematooncology patients receiving repeated transfusion

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Background: Hematooncology patients almost experience anaemia which requires repeated transfusions for disease management. Repeated transfusions carry the risk of erythrocyte alloimmunization which results in the formation of alloantibodies against erythrocytes. The appearance of erythrocyte alloantibodies can be a problem if long-term transfusion therapy is required. Erythrocyte alloantibodies can cause delayed haemolytic transfusion reactions in further transfusion. Several factors can influence the occurrence of erythrocyte alloimmunization both genetically and non-genetically. HLA-DRB1 gene polymorphism is thought to be a risk factor for erythrocyte alloimmunization in repeated transfusion patients, one of which is the HLA-DRB1*15 allele.

Aims: Analysing the relationship of the HLA-DRB1*15 allele with the occurrence of erythrocyte alloimmunization in hematooncology patients receiving repeated transfusions.

Methods: A cross-sectional study of 47 hematooncology patients receiving repeated transfusions who were treated at the Department of Internal Medicine of Dr. M. Djamil Hospital, Padang, Indonesia. The patients had received a compatible transfusion of at least three packed red cells (PRC) units in the 3 months prior to the study. Erythrocyte alloimmunization was determined based on a positive alloantibody result on the indirect antiglobulin test (IAT). Patients with a history of autoimmune disease, being pregnant, positive direct antiglobulin test results and infections were excluded from this study. HLA-DRB1*15 allele was examined using PCR-Sequence Specific Primer and electrophoresis system. Statistical analysis by using Chi square with a significance $p < 0.05$. This research was approved by the Research Ethics Committee of Dr. M. Djamil Hospital, Padang, Indonesia.

Results: A total of 47 hematooncology patients who received repeated transfusions in this study aged 44.1 ± 17.3 years old, consists of 48.9% male and 51.1% female. The most common diagnosis in hematooncology patients receiving repeated transfusions was acute myeloblastic leukaemia (31.9%), followed by chronic myelocytic leukaemia and Non-Hodgkin's malignant lymphoma were 19.1% and 12, 8% respectively.

Erythrocyte alloimmunization occurred in 12.8% (six patients) of the 47 hematooncology patients receiving repeated transfusions in this study. Five of the six patients had the HLA-DRB1*15 allele. The percentage of positive alloantibodies was higher in positive HLA-DRB1*15 alleles compared to negative HLA-DRB1*15, namely 100% versus 2.4%, which was statistically significant ($p < 0.05$).

Summary/Conclusions: There is a relationship between the HLA-DRB1*15 allele and the occurrence of erythrocyte alloimmunization in hematooncology patients receiving repeated transfusions.

P548 | How often do we get Intronic assignments right? - A study from a tertiary care hospital in South India

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Background: Enhancing graft longevity engraft graft outcome in both stem cell and solid organ transplant setting. HLA compatibility and a greater HLA match between patient and donor result in better transplant outcomes. Getting an accurate HLA typing at the allelic level is crucial in a transplant program. This facilitates donor choice and also enables the accurate definition of donor-specific antibodies if present. Currently HLA matching by allele-level genotyping is attributed based on genetic similarity between a few exons that encode the antigen recognition domain (ARD) of the HLA protein. It has been a major area of study while the role of allelic polymorphism within the Antigen recognition domain (ARD) have been cleared the question as to whether intronic and allelic definition beyond the ARD have been a point of

P548 - Table 1: Total number of Intron mismatches observed in each locus of class I allele

Locus	A*	B*	C*
Total number of Alleles	31	46	28
Total number of alleles with Intron mismatches	9 (29%)	20 (43.47%)	13 (46.42%)

P548 - Table 2: Total number of Intron mismatches observed in each locus of class II allele

Locus	DPB1*	DQB1*	DRB1*
Total number of Alleles	26	21	33
Total number of alleles with Intron mismatches	19 (73.07%)	13 (61.90%)	30 (91%)

question. However, the clinical applicability has to be defined that the definition of alleles has to be clear.

Aims: Against this background we aimed to study the prevalence of intronic mismatches and the definition of allele in a group of patient and donor HLA typed for various organ transplant setting.

Methods: High resolution HLA typing is performed using MIA FORA kits on the Illumina MiniSeQ platform. 200 high resolution HLA typing was performed for HLA Class I- HLA-A*, HLA-B* & HLA-C* and HLA Class II - HLA-DRB1* & HLA-DQB* loci and were compared to assess the similarities and differences in the intronic level mismatches.

Results: In our study, among the 200 high resolution HLA typing, All patients had one or more intron mismatches in each of the locus. HLA-A* locus had shown 16/200 (8%) intronic mismatches ranging from (1 to 37), HLA-B* locus had shown 23/200 (11.5%) intronic mismatches ranging from (1 to 182), HLA-C* locus had shown 34/200 (17%) intronic mismatches ranging from (1 to 23), HLA-DPB1* locus had shown 66/200 (33%) ranging from (1 to 493), HLA-DQB1* locus had shown 62/200 (31%) ranging from (1 to 86) and HLA-DRB1* locus had shown 172/200 (86%) ranging from (1 to 2787).

Summary/Conclusions: High prevalence of intronic mismatches using current typing methods raises the importance of addressing this issue given the emerging relevance of non ARD and intronic assignments in the transplant settings.

P549 | Nuances that make transplant safer: The role of HLA-DPB1 testing in a transplant setting

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Background: Fine-tuning of HLA typing helps achieve graft longevity. With the advent of Next Generation Sequencing (NGS), typing multiple loci of HLA alleles has become possible. The need to type HLA-DPB1 locus has been questioned due to limited evidence to support its clinical utility, and it raises the question if DPB1 typing becomes relevant for transplantation. However, in the context of Solid Oran Transplantation (SOT) or hematopoietic stem cell transplantation (HSCT), the evolving evidence of the HLA-DPB1 allele and antibodies is associated with an increased risk of graft rejection and worse transplantation outcomes.

Aims: This study aimed to study the prevalence of anti-HLA-DPB1 antibodies and to assess specificity in donor-recipient pairs.

Methods: Clinical records and electronic files of 288 donor-recipient (DR) pairs awaiting SOT/HSCT were taken over the past 12–18 months. All patients had anti-HLA antibody testing done using Single Antigen Bead (SAB) assay (Lifecodes class I and II ID panels, Immucor Gen-Probe, San Diego, CA). A bead with mean fluorescence intensity (MFI) between 500 and 1500 was considered borderline positive, and >1500 was considered positive.

Results: Of the 288 DR pairs, 110 (38.19%) were positive for HLA class I antibodies, of which 45 (40.9%) were borderline positive, and 103 (35.76%) were positive for HLA class II antibodies, of which 50 (48.5%) were borderline positive. 31 (10.76%) of them had anti-DPB1 antibodies, of which 8 (25.8%) had Donor Specific antibodies (DSA) with MFI ranging from 737 to 6669 and 30 (96.77%) had non-DSAs (MFI range 501–18,234). Except for one, the rest of the DPB1 DSAs were in combination with other antibodies (HLA-DR:4, HLA-A:4, HLA-DQ:3, HLA-B: 3, and HLA-C: 1). Of the 8 DR pairs which had DSAs, the T-cell flow cytometry crossmatch (FXM) was positive in five cases (range 0.85–20.2) and B-cell FXM positive in two (range 0.87–21.9) which correlated with the DPB1 combination of antibodies.

Summary/Conclusions: Considering the emergence of HLA-DPB1 antibodies, this data highlights the importance of accurate HLA typing and compatibility testing. Evolving literature suggests that concomitant high-resolution HLA typing and understanding the prevalence of HLA-DPB1 antibodies is vital, and it seems prudent to perform HLA-DPB1 typing in a transplant setting.

P550 | Matched platelet donors: How many to type?

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Background: Platelet refractoriness is a significant clinical problem in transfusion medicine, and can be caused by antibodies to class I HLA or HPA.

For highly immunised patients, it is often necessary to find platelets from donors with identical or almost identical class HLA- A and B, as well as identical HPA for the loci the patient has antibodies towards. For this reason, transfusion medicine centres will want to their donor pool to contain matching donors for as large parts of the population as possible.

Aims: The aim of this study was to *in- silico* examine the number of donors necessary to type in order to find a given number of HLA 4/4 matched donors, hereafter referred to as matched donors. In addition, we used a similar model to estimate the number of donors necessary to type in order to match HPA loci.

Methods: Anonymized HLA phenotype data from the Tobias Bone marrow registry ($n = 30{,}222$) was analysed to determine HLA- haplotype frequency. HLA-A∼B haplotype maximum likelihood estimates of haplotype probabilities were estimated using an expectation- maximization (EM) algorithm at a one- field resolution.

For patient phenotype {A1, A2, B1, B2} we then define the following haplotypes that can be a part of a 4/4 matched phenotype: {H1 = A1∼B1; H2 = A2∼B2; H3 = A1∼B2; H4 = A2∼B1}. It follows that the estimated proportion E of donors that are matched is

E-HLA=EH1H1+EH1H2+EH1H3+EH1H4+EH2H2+EH2H3+-EH2H4+EH3H3+EH3H4+EH4H4. Which we, assuming that the haplotypes do not deviate overtly from the Hardy- Weinberg equilibrium, can calculate from the haplotype frequencies as $E = EH1{*}EH2$ for heterozygotes and $E = H^2$ for homozygotes. In a population of $N = 10{,}000{,}000$, the number S matching donors for each phenotype can be estimated by $S = E{*}N$, which we simulate for each phenotype assuming a binomial distribution. The probability of finding K or more matched donors when typing n number of donors in a population of N with the number of matched donors is S, can be estimated by a hypergeometric model

$$P(K \geq x) = 1 - \left(\sum_{x=1}^{K-1} \left(\left(S!/(x!*(S-x)!)\right)*\left((N-S)!/((n-x)!*((N-S)-(n-x))!)\right)\right)/(N!/(n!*(N-n)!))\right)$$

P550 – Table 1.1

Number of typed donors *n*	Proportion of population with matched donors
500	22%
1000	48%
1500	58%
2000	62%
2500	64%
5000	69%

P550 – Table 1.2

	MAF	n
HPA- 1	0.833	285
HPA- 2	0.896	735
HPA- 3	0.604	48
HPA- 4	1,000	>100,000
HPA- 5	0.901	816
HPA- 15	0.516	32

Under this model, we estimate the number n necessary to attain a $P(K \geq 2) = 0.9$ for each phenotype.

Using directly measured phenotype frequencies in the data, we can then estimate the proportion, with a probability of $P = 90\%$, matched by at least $K = 2$ donors. The calculation for HPA follows all the same assumptions as above, except that with f_minor being the minor allele frequency (MAF) of each HPA- locus, then the estimated proportion of donors homozygous for the minor allele at each locus is estimated by $E\text{-}HPA = f\_minor^2$.

f_minor was estimated as the mean of different frequencies from different publicly available European datasets.

Results:

Table 1.1: The table shows the proportion of patients with a probability $P = 90\%$ of having at least $K = 2$ matched donors after having typed n number of donors

Table 1.2: The table shows the mean MAF, with the range in brackets and number of donors n necessary to type to have a probability $P = 90\%$ to have found at least $K = 5$ donors homozygous for the minor allele at each locus

Summary/Conclusions: Our model indicates that HLA typing of platelet donors hit a point of diminishing returns at 1500 typed donors. Obtaining 4/4 HLA- matched coverage of more than 60 % of the populations is unlikely to be possible without very large- scale typing projects which may not be possible for single transfusion medicine centres. This may provide some guidance in planning donor typing strategies so as to optimise patient population coverage per typed donor.

Clinical immunogenetics

Histocompatibility in organ transplantation

P551 | Pirche II scores or HLA class I or Class II derived Pirche II scores - what matters in a renal transplant setting? A study of 104 consecutive renal transplant donor recipient pairs

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Background: Prevention of rejection episodes post renal transplant is critical to graft longevity and better graft outcome. Looking past the traditional HLA matching and anti HLA antibodies, higher Pirche II scores appear to offer greater information on those who might be at a higher risk for rejection episodes. Whether greater scores contributed by class I or class II derived epitopes impacts negatively on the graft is also of interest.

Aims: To assess the Pirche II scores in a group of consecutive renal transplant donor recipient pairs and assess if there was any correlation between rejection episodes.

To study the variation of class I and class II derived pirche scores in patients with and without graft rejection

Methods: 104 consecutive renal transplant donor recipient pairs were included in the study. Follow up ranged between 3 and 15 months. All biopsy proven graft rejection events were collated. Pirche II scores were calculated using the Pirche utility software. Classs I and II derived pirche scores were also derived including locus wise differences that contributed to the pirche score. Scores contributed by each HLA locus was also analysed to see if locus based scores varied between the groups with and without rejection. IBM SPSS was used to analyse the results. Independent sample t-tests were conducted to analyse differences in pirche scores in the two groups.

Results: A total of 104 consecutive renal transplant donor recipient pairs were studied. 20 (19.23%) patients had biopsy proven rejection episodes, with either T cell mediated acute rejection, antibody mediated rejection or both in a follow up period of 3–15 months . Overall pirche scores showed no significant difference between the group of patients with rejection (72.8 ± 33.6) and without rejection (62.2 ± 39.3), $t(102) = 1.11$, $p = 0.27$. Class 1 derived pirche scores showed no significant difference between the group of patients with rejection (35.1 ± 32.1)and without rejection (35.5 ± 24.7), $t(102) = -0.67$, $p = 0.947$. Class 2 derived pirche scores were significantly higher in the group of patients with rejection (51.4 ± 23.9) as compared to patients without rejection (30 ± 25.1), $t(102) = 3.453$, $p = 0.001$. While scores contributed by difference in the HLA A, B and C loci did not show any significant difference between the groups with and without rejection, scores contributed by the DRB*1 and DQB*1 loci showed a statistically significant difference. Scores contributed by the DRB*1 locus were significantly higher in the group with rejection (20.5 ± 12.6) than without rejection (11.7 ± 10.2), $t(102) = 3.311$, $p = 0.001$. Further, scores contributed by the DQB*1 locus were significantly higher in the group with rejection (30.9 ± 14.2) than without rejection (18.3 ± 18.2), $t(102) = 2.88$, $p = 0.005$.

Summary/Conclusions: Higher overall Pirche II scores did not appear to have an effect on graft rejection. However higher Class II derived pirche scores showed a statistically significant association with graft rejection. Our findings highlight the importance of analysing class II derived pirche scores individually as the clinical impact is significant. We recommend studying larger numbers to provide more evidence. However our study demonstrates a clear trend of the impact of higher Class II derived pirche scores on graft rejection.