NHS BLOOD AND TRANSPLANT

CARDIOTHORACIC ADVISORY GROUP

H&I REPORT – cRF IN CARDIOTHORACIC TRANSPLANT

SITUATION

Concerns have previously been raised via the NHSBT CTAG Lung Advisory Group, and directly to the NHSBT OTDT H&I Lead, regarding a perceived disparity in the application of the Human Leucocyte Antigen (HLA) calculated reaction frequency (cRF) tool for cardiothoracic transplant waiting list patients between different Histocompatibility and Immunogenetics (H&I) laboratories and thereby transplant centres. It has been suggested that this impacts on equity of access and that cRF should be standardised across H&I laboratories. HLA matching is not currently considered in allocation for CT transplant, but it remains important in consideration for immunological compatibility of a patient with a potential donor.

RECOMMENDATIONS / FOR DISCUSSION

- Transplant centres to be aware of the BSHI and BTS UK guideline on the detection of alloantibodies in solid organ (and islet) transplantation (2023) to guide local practice.
- Apparent confusion in the transplant community about what calculated Reaction Frequency is and how it may be used/calculated. An explanation of cRF will be included in the upcoming BSHI BTS highly sensitised patient management guidelines. If this is considered by CTAG to be a knowledge gap, then H&I could provide additional local/national training (e.g. webinar).
- cRF should not be used in isolation to form the basis of clinical decisions. As the case study at the end of this document illustrates, interpretation of a patient's potential chances of transplant is complex and nuanced. It is recommended that the H&I team are included in patient discussions, MDTs and assessments to ensure understanding of requirements, risks and treatment plans for individual patients.
- It is suggested that a national joint meeting involving clinical teams and H&I scientists to discuss clinical risk appetite for transplantation, and how cRF is used to inform this, could be useful to improve consistency. A questionnaire being circulated to H&I laboratories is expected to provide laboratory data to inform discussion.

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BACKGROUND

Calculated reaction frequency (cRF) tools provide an assessment of a patient's likelihood of Human Leucocyte Antigen (HLA) incompatibility with the UK deceased donor population expressed as a percentage; such that a patient with a 70% cRF would be considered HLA incompatible with 70% of ABO identical deceased donors. A similar calculation, termed Panel Reactive Antibody (PRA) is based on reactivity against a panel in a laboratory assay, rather than the HLA frequency in a population.

The NHSBT-OTDT cRF calculator tool was developed to predict kidney transplant recipient compatibility against a 10,000 historic deceased donor pool (Calculators - ODT Clinical - NHS Blood and Transplant) and requires the patient's HLA type, ABO blood group and an understanding of which HLA antigens would be considered to be immunologically incompatible for the patient (based on their HLA antibody profile and prior sensitisation history). This tool can be used in different ways depending on the intent; it may be used to calculate cRF based on a single sample (antibody profile), to estimate the likelihood of receiving a compatible offer, or to consider the potential impact on cRF of removal of "low-level" antibody specificities. The output of the tool is therefore dependent upon what information is entered into it and at what time point (i.e. HLA antibody profiles change).

The application of cRF calculators for cardiothoracic (CT) transplantation is further limited as it does not take into consideration other factors that take precedence over HLA in allocation (as described in NHSBT policies; POL228 for heart and POL230 for lung), including clinical urgency, blood group prioritisation and relative size. Furthermore, it is not currently possible to include HLA-DQA or HLA-DP antigens as unacceptable in the NHSBT-OTDT cRF tool, although clinically relevant antibodies can be identified against these HLA antigens. Thus, patients' who have antibodies to these antigens are poorly assessed by the current NHSBT-OTDT cRF tool and may have falsely low cRF estimates.

The current BSHI and BTS UK guidelines on the detection of alloantibodies in solid organ (and islet) transplantation (https://onlinelibrary.wiley.com/doi/10.1111/iji.12641) discusses the current reliance on highly sensitive Luminex-based HLA antibody testing techniques for the identification of clinically relevant HLA antibodies. The mean fluorescence intensity (MFI) value generated from Luminex single antigen bead data is often clinically used as a proxy for antibody titre/strength. However, MFI is a measure of the amount of antibody bound to a particular

microbead during individual testing with multiple associated variables and therefore is not an accurate estimate antibody titre. Inter- and intra-laboratory MFI variation between laboratories when testing the same serum can be considerable. It is generally recommended that MFI should be used as a benchmark for antibody analysis rather than a definitive value (https://onlinelibrary.wiley.com/doi/10.1111/iji.12552).

As part of UKAS accreditation, H&I laboratories are required to reach consensus for HLA antibody testing assessed in external quality assurance schemes. The UK NEQAS H&I Scheme 3 assesses the ability of laboratories to determine the specificity of HLA antibodies, with consensus on the presence and absence of HLA antibodies in samples distributed throughout a 12-month cycle. As reported in the UK NEQAS H&I annual general meeting report in April 2025, between 2019-2024, all UK laboratories reached consensus for both HLA Class I and Class II antibody identification indicating that the laboratory testing and interpretation of positive reactions is consistent between laboratories, regardless of MFI. For 2024-2025, there was 96.9% antibody absence and 95.5% antibody presence concordance in reporting. Where consensus is not reached then laboratories must address the root cause of this failure and implement remedial corrective actions.

ASSESSMENT OF CURRENT SITUATION

Pre-transplant testing recommendations for patients requiring a CT transplant are discussed in the BSHI and BTS UK guideline on the detection of alloantibodies in solid organ (and islet) transplantation (https://onlinelibrary.wiley.com/doi/10.1111/iji.12641; page 43-49). These include:

- Two independent samples to be tested for HLA antibodies before listing for transplant
- Use of single antigen beads for antibody identification
- Understanding of priming source to interpret HLA antibody results
- Use of immunological risk grading based on MFI for cRF calculations. These MFI grades being 500-1999, 2000-4999 and ≥5000 MFI
- Regular antibody screening, ideally at least three monthly and following a potential sensitising event.

These guidelines were ratified by the NHSBT-CTAG prior to publication, but it is unclear how clinical teams and H&I laboratories are currently implementing the published recommendations. A questionnaire to H&I laboratories supporting CT transplant is shortly to be circulated, which could be used to assess how transplant centres are aligning with these guidelines.

The crucial step in determining a cRF estimate for a patient is establishing which HLA antibodies would be considered immunologically relevant (i.e. those which represent a risk to a transplanted organ). This requires consideration of several factors including:

- HLA antibody reactivity patterns fluctuations (e.g. mean MFI over time)
- the patient's overall level of sensitisation
- clinical urgency
- the relevance of historic and currently detected anti-HLA antibodies attributed to known sensitisation events (transplant, transfusion, pregnancy, ventricular assist devices etc)
- the local risk appetite for transplant (clinical team and patient) which can lead to different local considerations of the impact of cRF
- immunosuppressive protocols

SUMMARY

The cRF value of a patient is for local information only and does not impact on the patient's likelihood of a deceased donor organ offer. It was commented in the July 2025 CTAG Lungs meeting that there are more transplants being performed using imported lungs than from donors at the local centre and that cRF may be contributing to this. The proportion of patients who are sensitised to HLA nationally is unknown as antibody data for cardiothoracic patients is not currently held centrally within OTDT, unlike kidney transplant patients who have unacceptable antigens listed at the time of transplant registration. Therefore, although we do not currently have a complete picture of why local donor organs are not used, a proportion could be due to immunological incompatibility.

REFERENCES

NHSBT OTDT Calculator available at:
https://www.odt.nhs.uk/transplantation/tools-policies-and-guidance/calculators/

NHSBT OTDT Policies available at:
https://www.odt.nhs.uk/transplantation/tools-policies-and-guidance/policies-and-guidance/

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CASE STUDY – the challenge with cRF

Background

A female patient on the transplant waiting list for a lung transplant was tested for HLA antibodies on four samples prior to transplantation. All four samples showed consistent results, with a cRF determined as 10%. In February 2022, the patient received a double lung transplant.

Post-Transplant

Shortly after transplantation, the patient developed donor specific antibody (DSA) to both HLA Class I and Class II antigens. The peak cRF% of the samples tested during this period was 90%. The DSAs and a general increased HLA antibody reactivity lasted for approximately five months, during which time the patient was also treated for acute cellular rejection (ACR). Subsequent biopsies showed evidence of antibody mediated damage.

At six months post-transplant, the patient's antibody levels returned to baseline, with two negative HLA antibody tests in 2023 and 2024. These samples would have a reported 0% cRF. More recent testing shows a few HLA class I specificities with a cRF, returning to approximately 10%.

Re-Transplant Assessment

The lung function of the patient deteriorated rapidly to the point where they were being considered for re-transplantation. The clinical team, looking at the most recent antibody testing results, assumed that the 10% cRF reported on the most recent sample was an appropriate reflection of their immunological status for re-transplantation. However, upon discussion with the H&I Consultant Clinical Scientist, the actual cRF value for re-transplant would 99%.

Whilst this clinical team were looking at the cRF for an individual sample, the patients' sample cRF had fluctuated from 0% - 90% between 2022 and 2025 as their antibody profile varied over time. As the first graft failure was determined to have an immunological cause with both the development of DSA and the treatment for ACR, indicating allorecognition and sensitisation to the mismatched antigens presented by the donor lungs, all previous mismatches are deemed unacceptable for the purposes of re-transplantation, raising the total cRF for consideration of re-transplantation to 99%. The full HLA testing history is shown in **Table 1** below.

Table 1 – A summary of HLA antibody testing information for lung transplant recipient between 2020 and 2025. Patient was transplanted in Feb 2022 and considered for re-transplant in 2025.

Date	Class I	Class II	HLA Antibody Specificities	cRF (%)
17/02/2020	POS	POS	B45, DR8, DR12	10
30/04/2021	POS	POS	B45, DR8, DR12	10
14/07/2021	POS	NEG	B45, DR8, DR12	10
22/10/2021	POS	NEG	B45, DR8, DR12	10
09/02/2022	POS	NEG	A29, A43, B37, B45, B76	11
24/02/2022	POS	POS	A11, A29, A43, B8, B37, B45, DQ2, DQ5, DQ6	90
10/03/2022	POS	POS	A11, A29, A43, B8, B37, B45, DQ2, DQ5	72
21/03/2022	POS	POS	A11, A29, A43, B37, B45, DQ2, DQ5, DQ6	89
30/05/2022	POS	POS	A11, A29, A43, B45, DQ5, DQ6	72
25/08/2022	POS	NEG	A29, A43, B45	9
20/03/2023	NEG	NEG	-	0
08/02/2024	NEG	NEG	-	0
07/10/2024	POS	NEG	A29, A43, B45	10
08/04/2025	POS	NEG	A43, B45	2
08/06/2025	POS	NEG	A29, A43, B45	9
04/08/2025	POS	NEG	B45, Cw17	3
Antibody defined specificities deemed unacceptable for re-transplantation			A11, A29, A43, B8, B37, B45, DR8, DR12, DQ2, DQ5, DQ6	92
Other mismatches from 1st graft, also deemed unacceptable for re-transplant			A3, A33, B14, Cw7, DR1, DR17, DR52, DP3	99
			Total cRF for consideration for re-transplantation	**99**

Conclusion

When assessing a single sample for a patient, only a snapshot can be determined. Individual test results can paint a widely different picture when compared to consideration of the whole profile and patient history. Inclusion of the H&I team in patient discussions, MDTs and assessments is recommended for complete shared understanding of requirements, risks and treatment plan.