

Objective

To ensure a uniform RCI Clinical Policy for the management of large D positive fetomaternal haemorrhage, red cell transfusion, platelet transfusion and bone grafts in D negative females of childbearing potential

Changes in this version

CR32205 – Prefix FMH added to document title.

CR48585 – Update SPN216 to acknowledge that the RCI investigation of D positive SOT/Tx into D neg individual is not UKAS accredited

CR48586 – Update SPN216 to include a section related to D positive SOT into D negative individuals

Requirements

The Kleihauer test is reliable for identifying feto-maternal haemorrhages (FMHs) that are less than 2mL. BSH guidelines recommend that when the size of the FMH is >2mL, the volume should be rechecked using a different technology. The second technology used within NHSBT is Flow Cytometry (FC) for D positive red cells. An advantage of FC is that it is a reliable method for measuring large volumes of D positive red cells accurately.

More than 99% women have FMHs <4mL. Therefore in most hospitals, the cord sample is tested shortly after delivery and the standard dose of anti-D immunoglobulin [Ig] (500IU or 1500IU) is given if the baby is D positive and mother and baby discharged before the results of the Kleihauer test are available. The Kleihauer tests are usually undertaken as a batch during the next 24 hours, and sometimes as late as 48h later over weekends and holidays.

When the Kleihauer Test shows a FMH >2mL, hospitals should be advised to establish the volume of the FMH by FC, using the same post-delivery maternal sample used for the Kleihauer test. FC should be advised even when 1500IU anti-D Ig has been given because of the limitations of the Kleihauer test to measure accurately volumes >2mL¹.

NHS Blood and Transplant (NHSBT) provides a 24h FC service and this service is available from the following Red Cell Immunohaematology (RCI) laboratories; Colindale, Filton, Liverpool and Barnsley.

FC testing outside normal hours should be undertaken **only** when there is a clinical indication, i.e. to enable prophylaxis to be given within 72 hours of a FMH or following an inadvertent transfusion of D positive blood. When time permits FC should be undertaken on the next working day. When urgent FC is required, the local RCI BMS will advise of the nearest laboratory providing a 24h service. It is suggested as guidance that the result should be reported within 60 hours of the sensitising event. There may of course be some flexibility in this time frame if for example the mother is still in hospital or lives nearby and the team looking after her can act on a result provided closer to 72 hours to facilitate testing in routine hours.

- a) If FMH confirmed by FC is less than 4mL: no further testing or follow-up required. NB Uncertainty of measurement must be considered in assessing confidence that the bleed is less than 4 mL.
- b) If FMH confirmed by FC is greater than or equal to 4mL but less than is covered by the standard anti-D Ig in use: This is considered to be a 'significant' bleed and follow-up is required to check for clearance of fetal cells, to confirm that the anti-D Ig has been given.
- c) If FMH confirmed by FC is greater than the FMH volume that would be covered by the anti-D in use: Additional anti-D required and follow-up to check for clearance of fetal cells¹.

The preparation and route of administration should take into account the required dose of anti-D and the number of injections required. Some preparations of anti-D are only manufactured and licensed for intramuscular (IM) use².

D-Gam (BPL) 250IU/500IU/1500IU anti-D Ig vials – IM only

(ZLBBehring) Rhophylac 1500IU anti-D Ig prefilled syringe for use IM or IV(Intravenous)

Dosage

Intramuscular (IM) anti-D:125IU is recommended per millilitre of D positive fetal red cells. Maximum recommended dose for IM anti-D Ig is 10,000IU, which will neutralise 80mL of D positive cells.

Intravenous (IV) anti-D: 100IU is recommended per millilitre of D positive fetal red cells.

When should the intravenous route be used?

- IV anti-D is likely to clear D + fetal cells more rapidly. In theory it can be given at appropriate dose for any bleed or sensitising event if appropriate facilities and trained staff are available.
- For FMH > 80 mL consider giving IV anti-D Ig or combination of IV and IM anti-D Ig IV Rhophylac can be administered by slow IV injection (over a period of 5 to 10 minutes). Maximum bolus IV dose for Rhophylac is 15,000IU³, which will only neutralise 150 mL D pos cells.
- For massive FMH, greater than 150 mL: administer 15,000IU of Rhophylac anti-D Ig IV with which to neutralise 150mL D positive RBC. For the remaining FMH, give IM Rhophylac anti-D Ig. Calculate how much additional IM anti-D is required (based upon IM 125IU Rhophylac anti-D Ig neutralising 1mL of D positive red cells).

Timing of follow-up samples

Follow-up sample should be taken:

- 72 hours after IM anti-D Ig is given
- 48 hours after IV anti-D Ig is given

A Consultant must be contacted by the on call BMS to discuss cases where a follow up FMH sample is taken before or after the recommended time frame and if the result suggests further anti-D is required.

When follow-up testing shows residual D positive cells, this could mean that the dose of anti-D Ig was inadequate, clearance of coated D positive cells is slow due to RE blockade or anti-D Ig was not administered. In these rare instances, further anti-D Ig should be given as it has been shown that anti-D Ig given up to 9 days later can prevent sensitisation, although it may not be 100% effective. In these cases, further follow-up should be undertaken 72h later if given IM and 48h later if given IV.

While D positive red cells are still detectable in the circulation, the presence of free passive anti-D must not be taken as proof that the dose of anti-D Ig is adequate. It has been shown that in spite of free anti-D being present, circulating D positive cells can result in sensitisation.

Monitoring the Newborn

The blood volume of the newborn is approx 80mL/kg and the red cell volume approx 60mL/kg. When the FMH is large, the infant's Hb should be checked. When the infant's Hb is disproportionately high compared to the volume of the FMH, chronic feto-maternal haemorrhage over a long period should be suspected and the woman counselled that prophylaxis may not be effective.

INADVERTENT TRANSFUSION OF D POSITIVE BLOOD

Anti-D is required only for females with the potential for childbearing.

The consultant responsible for the patient at the hospital should be informed. The risks and benefits of utilising different therapies to suppress sensitisation should be discussed. Exchange transfusion reduces the D positive red cell load but entails exposure to a relatively large number of additional donor blood components and red cell antigens other than D on the packs exchanged. In addition, there may be vascular access, haemodynamic and citrate toxicity risks to be considered. The woman should be counselled about the planned treatment; including exchange transfusion with several units of D negative blood, if this is being considered, the potential for sensitisation, the risk of being sensitised and its consequences.

- a) When more than 2 units of D positive blood have been transfused, consideration should be given to exchange transfusion.^{4,5}

One total blood volume exchange will reduce the number of D+ RBC by 65% to 70% and a double volume exchange reduces the number of D positive red cells by 85% to 90%.¹

- b) Following 1-2 volume exchange, the residual volume of D positive RBC should be estimated using flow cytometry. Hospital to provide patient's body weight in kg. (Table 1 Appendix 1) calculate how much intravenous anti-D Ig is required (based upon IV 100IU Rhophylac anti-D Ig neutralising 1mL of D positive red cells).
- c) The maximum bolus dose for IV Rhophylac is 15,000IU (administered by slow IV injection, over a period of 5 to 10 minutes). If the recipient of the D positive blood has more than 150 mL of circulating D positive RBC the patient will require further doses of intravenous anti-D. Allow 24 hrs before giving additional IV anti-D injection (this will give time to neutralise 150mL of D pos RBC in the first instance over 24 hrs). Following this give residual calculated anti-D dose (a maximum of 3000IU every 8 hrs).⁵

As repeat high dose IV anti-D is to be given, it is advisable to give IV hydrocortisone/IV piriton as premedication before each IV anti-D.

The presence of residual D positive red cells should be assessed by flow cytometry 48 hrs after administering the last IV anti-D dose.

If D positive cells are still present further anti-D should be administered based upon the number of residual D positive red cells. Calculate the dose IV or IM accordingly.

When doses in excess of 2500IU are given passive anti-D may be detectable by IAT up to 6 months later.

A negative antibody test is not 100% proof that immunisation has been prevented. It is possible that the subject has been immunised, but anti-D is below the level of detectability by standard tests. Under these circumstances, following a further stimulus, such as a future pregnancy with a D positive fetus, there may be a brisk increase in the anti-D level (secondary immune response).^{6,7}

- d) There were 2 cases reported in 1970 and 1971 using intramuscular (IM) anti-D Ig. Both were inadvertently transfused with 1000mL D positive blood and both received IM anti-D Ig over 7 and 8 days. IM anti-D (5mL) was given every 12 hrs, monitored for evidence of haemolysis and measured the clearance. If the patient is not well or fit enough for exchange transfusion an option for providing a combination of IM/IV anti-D should be discussed.^{8,9}
- e) Remind hospital to report to SHOT/MHRA.

TRANSFUSION OF D POSITIVE PLATELETS

See British Committee for Standards in Haematology (BCSH) Guidelines¹⁰. 50IU of anti-D is sufficient to cover one adult transfusion dose of platelets over a 6 week period so 250IU should be sufficient to cover 5 adult therapeutic doses.

D POSITIVE SOLID ORGAN TRANSPLANT

Solid organ transplant (SOT) of a D negative recipient can be received from a D positive donor^{11,12}. When transplanted, the organ has the potential to release residual donor RBCs into the recipient's circulation¹³. This is minimised by organ flush processes, which are standardised in the NHS^{13, 14, 15}. Sensitisation can occur from residual D positive RBCs from the donor organ^{1, 12, 16, 17, 18}. An investigation by flow cytometry should be performed 24 hours post-transplant to determine the volume of D positive RBCs and prophylactic anti-D should be administered within 72 hours post-transplant¹³. Follow up sample requirement and prophylactic anti-D dose recommendations should follow earlier guidance in this document relating to FMH investigation.

Current methodology used in the RCI laboratory is not UKAS accredited for the investigation of samples from D negative recipients of D positive solid organ transplant¹³. The RCI methodology does not consider the reduced size of residual adult D positive RBCs from the donor organ, or the packed RBC volume of the recipient¹³. Consultant approval is required for testing to take place under concession¹³.

ANTI-D PROPHYLAXIS FOR D POSITIVE BONE GRAFTS

Typically donated femoral heads are used. Normal bone marrow cellularity is defined as 30-70%. Cellularity varies with sex and age: higher in neonates and reduces with age. Average cellularity is around 50% in donor population aged between 40yrs and 70yrs. Of this, Myeloid: erythroid ratio varies between 2:1 to 4:1. Hence, if considered the scenario of BM containing 30 mL marrow – 15 mL will be cell contents described as “red marrow” of which the erythroid content will be around 3mL to 5mL. Femoral heads contain on average 10-15mL of bone marrow (20mL in the worst-case scenario) with a considerable donor to donor variation in the proportion of red and yellow marrow. There is also a small amount (maximum 2mL) of normal blood on the outside of the bone (personal communication: A Chandrasekar, R Lomas). With fresh frozen and frozen irradiated femoral heads, the entirety of the bone, marrow, stroma and blood could be transplanted. Some surgeons will wash the bone in theatre prior to implantation, but not all. There is also the possibility of multiple femoral heads (up to 4) being used in the same procedure. Taking all this into account, the standard dose of anti-D 500IU should cover the number of D positive red cells in a D positive bone graft, equivalent of one femoral head. There is no role of assessing residual D positive cells by flow cytometry in the setting of frozen bone graft (RBCs won't be intact and won't be detectable for investigation).

Recommendation: 500IU anti-D IgG (IM) prophylaxis for one femoral head for D negative recipients of female childbearing potential. If multiple femoral heads are used, the dose should be calculated based on 500IU anti-D IgG per femoral head.

Related Documents / References

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Appendix 1

Table 1: Average whole blood volume (mL/Kg) of a Newborn, Child, Adult (male/female)

	Average Whole Blood Volume (mL/kg Body Weight)
Newborn, 15-30 minutes old	76.5
Newborn, 24 hours	83.3
Children, 3 months	87.0
Children, 6 months	86.0
Children, 1 year	80.0
Children, 6 years	80.0
Children, 10 years	75.0
Children, 15 years	71.0
Men	71.0
Women	70.0

Reference: Geigy Scientific Tables, 7th Ed; 1971.

Note: Red cells average 45% of whole blood volume in adults, 75% in newborns.