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Transfusion Medicine | GUIDELINES

BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn

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INTRODUCTION

Prior to the availability of anti-D immunoglobulin (anti-D Ig), the incidence of Rh D alloimmunisation in D negative women following two deliveries of D positive, ABO-compatible, infants was approximately 16%, and haemolytic disease of the fetus and newborn (HDN) due to anti-D was a significant cause of morbidity and mortality (Urbaniak & Greiss, 2000). Following routine post-partum administration of anti-D Ig, the rate of alloimmunisation dropped to approximately 2%. A further reduction in the sensitisation rate ranging from 0·17 to 0·28% was achieved by introducing routine antenatal prophylaxis during the third trimester of pregnancy (Tovey *et al.*, 1983a,b; Huchet *et al.*, 1987; Mayne *et al.*, 1997; MacKenzie *et al.*, 1999). Associated with this reduction in sensitisation is a reduction in mortality associated with HDN, from 46/100 000 births to 1·6/100 000 births (Pilgrim *et al.*, 2009).

These findings contributed to the National Institute for Clinical Excellence (NICE) recommendation that all D negative pregnant women who do not have immune anti-D, should be offered additional routine prophylaxis with anti-D Ig during the third trimester of pregnancy (NICE, 2002, 2008).

OBJECTIVES

The objective of this guideline is to provide healthcare professionals with practical guidance on the use of anti-D Ig as immunoprophylaxis to prevent sensitisation to the D antigen during pregnancy or at delivery for the prevention of HDN.

This guideline is an update of the 2006 BCSH guideline on the use of anti-D immunoglobulin for Rh prophylaxis (Parker *et al.*, 2006), and takes into account the updated NICE guidance for routine antenatal anti-D prophylaxis (NICE, 2008). This revision also aims to ensure concordance with other BCSH guidelines including guidelines for estimation of fetomaternal haemorrhage (BCSH, 2009), blood grouping and antibody testing in pregnancy (BCSH, 2007) and recently published compatibility procedures in blood transfusion laboratories (Milkins *et al.*, 2012) as well as professional guidelines produced by the Royal College of Obstetrics & Gynaecologists (RCOG Green Top N°22, updated 2011).

METHODS

This guideline was developed in accordance with the standard British Committee for Standards in Haematology (BCSH) methodology for producing BCSH guidelines. The guideline group was selected to be representative of medical and scientific UK-based experts. A search of published literature was undertaken using the Cochrane Library, Pubmed, MedLine, Embase and internet searches using the following key words and relevant MeSH terms: anti D, anti-D Ig immune globulin, pregnancy, antenatal, prophylaxis, rhesus, Rh D, Rh D haemolytic disease, erythroblastosis fetalis. This search covered the period 1999 to March 2013 and was limited to the English language and humans. The papers included were subjected to critical reading by the authors using the CASP appraisal tool (CASP, 2004) and were ranked according to the hierarchy of evidence. This approach took account of the NICE systematic review undertaken in 2000 (Chilcott *et al.*, 2003), and the NICE Health Technology Assessment report published in 2007. The writing group produced the draft guideline, which was subsequently revised by consensus by members of the Transfusion Task Force of the British Committee for Standards in Haematology. The guideline was reviewed by a sounding board of UK haematologists, the BCSH (British Committee for Standards in Haematology and the BSH Committee (British Society for Haematology) as well as representatives from the Royal College

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of Obstetrics and Gynaecology and the Royal College of Midwifery, with comments incorporated where appropriate. Criteria used to assign levels of evidence and grades of recommendations are as outlined by the Agency for Healthcare Research and Quality (AHRQ) at http:www.ahrq.gov (Appendix 1).

1. SUMMARY OF KEY RECOMMENDATIONS

1. Following potentially sensitising events, anti-D Ig should be administered as soon as possible and always within 72 h of the event. If, exceptionally, this deadline has not been met some protection may be offered if anti-D Ig is given up to 10 days after the sensitising event (Grade 1C).
2. In pregnancies <12 weeks gestation, anti-D Ig prophylaxis is only indicated following ectopic pregnancy, molar pregnancy, therapeutic termination of pregnancy and in cases of uterine bleeding where this is repeated, heavy or associated with abdominal pain. The minimum dose should be 250 IU. A test for fetomaternal haemorrhage (FMH) is not required (Grade 2C).
3. For potentially sensitising events between 12 and 20 weeks gestation, a minimum dose of 250 IU should be administered within 72 h of the event. A test for FMH is not required (Grade 2C).
4. For potentially sensitising events after 20 weeks gestation, a minimum anti-D Ig dose of 500 IU should be administered within 72 h of the event. A test for FMH is required (Grade 2C).
5. Appropriate tests for FMH should be carried out for all D negative, previously non-sensitised, pregnant women who have had a potentially sensitising event after 20 weeks of gestation, and additional dose(s) of anti-D Ig should be administered as necessary (Grade 1C).
6. All D negative pregnant women who have not been previously sensitised should be offered routine antenatal prophylaxis with anti-D Ig (RAADP) either with a single dose regimen at around 28 weeks, or two-dose regimen given at 28 and 34 weeks (Grade 1B).
7. It is important that the 28-week sample for blood group and antibody screen is taken prior to the first routine prophylactic anti-D Ig injection being given. This forms the second screen required in pregnancy as stated in the BCSH Guidelines for Blood Grouping and Red Cell Antibody Testing during pregnancy (BCSH c, 2007; NICE CG62, 2008) (Grade 2C).
8. Routine Antenatal Anti-D Ig Prophylaxis (RAADP) should be regarded as a separate entity and administered regardless of, and in addition to, any anti-D Ig that may have been given for a potentially sensitising event (Grade 2C).
9. Following birth, ABO and Rh D typing should be performed on cord blood and if the baby is confirmed to be D positive, all D negative, previously non-sensitised, women should be offered at least 500 IU of anti-D Ig within 72 h following delivery. Maternal samples should be tested for FMH and additional dose(s) given as guided by FMH tests (Grade 1B).
10. In the event of an intrauterine death (IUD), where no sample can be obtained from the baby, an appropriate dose of prophylactic anti-D Ig should be administered to D negative, previously non-sensitised women within 72 h of the *diagnosis of IUD*, irrespective of the time of subsequent delivery (Grade 1C).
11. Where intra-operative cell salvage (ICS) is used during Caesarean section in D negative, previously non-sensitised women, and where cord blood group is confirmed as D positive (or unknown), a minimum dose of 1500 IU anti-D Ig should be administered following the re-infusion of salvaged red cells, and a maternal sample should be taken for estimation of FMH 30–45 min after reinfusion in case more anti-D Ig is indicated. It is important that clinicians inform the transfusion laboratory if ICS has been used to ensure that correct dose of anti-D Ig is issued (Grade 2C).
12. Auditable records of issue and administration should be maintained to allow full traceability of anti-D immunoglobulin (Good Practice Point, Grade 2C).
13. Where anti-D is detected in a blood sample from a pregnant woman, further history should be taken and investigation undertaken to establish whether this is immune or passive. The outcome will inform clinical decisions regarding Anti-D prophylaxis and antenatal follow-up (Grade 2C). If no clear conclusion can be reached as to the origin of the anti-D, then prophylaxis should continue to be administered in accordance with guidelines for D negative women who have not formed immune anti-D (grade 2C).

2. POTENTIALLY SENSITISING EVENTS REQUIRING ANTI-D IG PROPHYLAXIS

Pregnant D negative women with no immune anti-D should be offered prophylactic anti-D Ig for potentially sensitising events listed in Table 1. A dose of anti-D Ig appropriate to the gestation (see 3–5) should be administered within 72 h of a potentially sensitising event. However if, exceptionally, this deadline cannot be met, some protection may still be offered if anti-D Ig is given up to 10 days after the sensitising event (Lee *et al.*, 1999; RCOG, 2011).

Recommendation:

Following potentially sensitising events, anti-D Ig should be administered as soon as possible and always within 72 h of the event. If, exceptionally, this deadline has not been met some protection may be offered if anti-D Ig is given up to 10 days after the sensitising event (Grade 1C).

Table 1. Potentially sensitising events in pregnancy

Amniocentesis, chorionic villus biopsy and cordocentesis
Antepartum haemorrhage/Uterine (PV) bleeding in pregnancy
External cephalic version
Abdominal trauma (sharp/blunt, open/closed)
Ectopic pregnancy
Evacuation of molar pregnancy
Intrauterine death and stillbirth
In-utero therapeutic interventions (transfusion, surgery, insertion of shunts, laser)
Miscarriage, threatened miscarriage
Therapeutic termination of pregnancy
Delivery – normal, instrumental or Caesarean section
Intra-operative cell salvage

3. POTENTIALLY SENSITISING EVENTS IN PREGNANCIES OF LESS THAN 12 WEEKS OF GESTATION

3.1. Laboratory tests required

A maternal blood group and antibody screen should be performed to determine or confirm the Rh D group and check for the presence of immune anti-D. The reagents used should conform to BCSH guidelines for pre-transfusion compatibility procedures (Milkins *et al.*, 2012).

Women with anomalous Rh D typing results should be treated as D negative until confirmatory testing is completed.

A test for fetomaternal haemorrhage (FMH) is NOT required.

3.2. Administration of anti-D Ig

In cases of spontaneous complete miscarriage confirmed by scan where the uterus is not instrumented, or where mild painless vaginal (PV) bleeding occurs before 12 weeks, prophylactic anti-D immunoglobulin is not necessary because the risk of FMH and hence maternal exposure to the D antigen is negligible.

In cases of therapeutic termination of pregnancy, whether by surgical or medical methods, and regardless of gestational age, previously non-sensitised D negative women should receive a minimum dose of 250 IU prophylactic anti-D Ig within 72 h of the event (RCOG, 2011).

There is a significant potential for sensitisation in cases of ectopic pregnancy (Hartwell, 1998). A minimum dose of 250 IU anti-D Ig should be administered to all cases of ectopic pregnancy in previously non-sensitised, D negative women regardless of the mode of management (RCOG, 2011). The authors note that the recent NICE guidance on the management of ectopic pregnancy and miscarriage (NICE, 2012) specifically recommends against offering anti-D Ig if ectopic pregnancy is solely managed medically but without any clear evidence to support this. The authors feel that this has potential for causing confusion resulting from inconsistency with established current practice based upon RCOG green top and BCSH guidelines and introduces complexity into decision making without strong evidence to support any such change.

There is significant potential for sensitisation in cases of molar pregnancy. A minimum dose of 250 IU anti-D Ig should be administered to all cases of molar pregnancy in previously non-sensitised, D negative women (RCOG, 2010).

Evidence that women are sensitised after uterine bleeding in the first 12 weeks of pregnancy, where the fetus is viable and the pregnancy continues, is scant (Ghosh & Murphy, 1994). Therefore anti-D Ig is not necessary in women with threatened miscarriage with a viable fetus where bleeding completely stops before 12 weeks gestation. However, 250 IU anti-D Immunoglobulin should be administered where bleeding is heavy or repeated or where there is associated abdominal pain particularly if these events occur as gestation approaches 12 weeks (Grade 2C recommendation). Gestational age should be confirmed by ultrasound.

Recommendation

In pregnancies <12 weeks gestation, Anti-D Ig prophylaxis (minimum dose 250 IU) is only indicated following an ectopic pregnancy, molar pregnancy, therapeutic termination of pregnancy and in some cases of uterine bleeding where this is repeated, heavy or associated with abdominal pain. A test for FMH is not required (Grade 2C)

4. POTENTIALLY SENSITISING EVENTS IN PREGNANCIES OF 12 WEEKS TO LESS THAN 20 WEEKS OF GESTATION

4.1. Laboratory tests required

A maternal blood group and antibody screen should be performed to determine or confirm the Rh D group and check for the presence of anti-D. The reagents used should conform to BCSH guidelines for pre-transfusion compatibility procedures (Milkins *et al.*, 2012).

If anti-D is identified, further history should be obtained and investigation undertaken to determine whether this is immune or passive (as a result of previous injection of anti-D Ig).

If no clear conclusion can be reached as to the origin of the anti-D detected, then the woman should continue to be offered anti-D prophylaxis on the assumption that it may be passive.

Women with indeterminate Rh D typing results should be treated as *D negative* until confirmatory testing is completed.

A test for FMH is NOT required before 20 weeks gestation.

4.2. Administration of anti-D Ig

For any potentially sensitising event listed in Table 1, confirmed D negative, previously non-sensitised, women should receive a minimum dose of 250 IU anti-D Ig within 72 h of the event (RCOG, 2011).

D negative women presenting with continual uterine bleeding between 12 and 20 weeks gestation should be given at least 250 IU anti-D Ig, at a minimum of 6 weekly intervals (Grade 2C).

Recommendation

For potentially sensitising events between 12 and 20 weeks gestation a minimum dose of 250 IU should be administered within 72 h of the event. A test for FMH is not required (Grade 2C).

5. POTENTIALLY SENSITISING EVENTS IN PREGNANCIES OF 20 WEEKS OF GESTATION TO TERM

5.1. Laboratory tests required

A maternal blood group and antibody screen should be performed to determine or confirm the Rh D group and check for the presence of immune anti-D. The reagents used should conform to BCSH guidelines for pre-transfusion compatibility procedures (Milkins *et al.*, 2012).

If anti-D is identified, further history should be obtained and investigation undertaken to determine whether this is immune or passive (as a result of previous injection of anti-D Ig).

If no clear conclusion can be reached as to the origin of the anti-D detected, then the woman should continue to be offered Anti-D Ig prophylaxis on the assumption that it may be passive.

Women with anomalous or indeterminate Rh D typing results should be treated as D negative until confirmatory testing is completed.

A FMH test is required to detect fetal cells in the maternal circulation and, if present, to estimate the volume of FMH to allow calculation of additional anti-D doses required to clear the fetal cells. This should be performed according to BCSH guidelines for estimation of FMH (2009).

If FMH >4 mL is detected, follow-up samples are required at 48 h following an intravenous (IV) dose of anti-D or 72 h following an intramuscular (IM) dose to check for clearance of fetal cells (BCSH, 2009).

5.2. Administration of anti-D Ig

For any potentially sensitising event listed in Table 1, D negative, previously non-sensitised, women should receive a minimum dose of 500 IU anti-D Ig within 72 h of the event (RCOG, 2011).

A minimum of 500 IU anti-D Ig should be administered within 72 h for any potentially sensitising events regardless of whether the woman has already received RAADP at 28 weeks (BCSH, 2009).

Additional dose(s) of anti-D Ig will be necessary if the volume of FMH exceeds that covered by the standard anti-D Ig dose in use (BCSH a, 2009; RCOG, 2011). A follow-up blood sample should be taken at 48 h following each IV dose of anti-D and 72 h following each IM dose of anti-D to check if fetal cells have cleared.

In the event of continual uterine bleeding which is clinically judged to represent the same sensitising event, with no features suggestive of a new presentation or a significant change in the pattern or severity of bleeding, such as the presence of abdominal pain or another clinical presentation, a minimum dose of 500 IU anti-D Ig should be given at six weekly intervals. In the event of further intermittent uterine bleeding, estimation of FMH should be carried out at two weekly intervals. In this situation non-invasive fetal *RHD* typing using maternal plasma could be considered to reduce hospital attendance, blood sampling and avoid repeated administration of doses of anti-D, balanced against the small risk of false negativity (0·08–0·16%, Clausen *et al.*, 2002; Finning *et al.*, 2008) of fetal D typing by this technique.

If the two weekly FMH test shows the presence of fetal cells, additional anti-D Ig should be administered to cover the volume of FMH. The additional dose should be calculated as 125 IU if administered IM or 100 IU if administered IV for each mL of fetal red cells detected (minimum 500IU).

The additional dose should be offered regardless of the presence or absence of passive anti-D in maternal plasma, and FMH should be retested after 48 h if anti-D Ig has been given IV, or 72 h if given IM (Grade 2C).

If new symptoms develop suggestive of a sensitising event in addition to continual uterine bleeding (e.g. abdominal pain associated with a significant change in the pattern or severity of bleeding) then it should be managed as an additional sensitising event with an appropriate additional dose of anti-D and estimation of FMH. Each new sensitising event should be managed with an appropriate additional dose of anti-D Ig regardless of the timing or dose of anti-D Ig administered for a previous event.

Recommendation

For potentially sensitising events after 20 weeks gestation a minimum Anti D Ig dose of 500 IU should be administered within 72 h of the event (Grade 2C).

Recommendation

Appropriate tests for FMH should be carried out for all D negative, previously non-sensitised, pregnant women who have had a potentially sensitising event after 20 weeks of gestation, and additional dose(s) of anti-D Ig should be administered as necessary (Grade 1C).

6. ROUTINE ANTENATAL ANTI-D PROPHYLAXIS (RAADP)

This section takes account of the publication of the NICE guidance, which recommends that RAADP be offered to all D negative, non-sensitised, pregnant women (NICE, 2002, 2008).

6.1. Laboratory tests required

A sample should be taken for the routine antenatal 28-week blood group and antibody screen as described in the BCSH guidelines for blood group and red cell antibody testing in pregnancy (2006), before RAADP is given.

If anti-D is identified in this sample, further investigations should be undertaken to determine whether this is immune or passive (i.e. previous administration of anti-D Ig).

If no clear conclusion can be reached as to the origin of the anti-D detected, then the woman should continue to be offered anti-D Ig prophylaxis, and should continue to be monitored monthly until 28 weeks gestation and fortnightly thereafter.

6.2. Administration of routine antenatal anti-D Ig prophylaxis (RAADP)

There is good evidence that antenatal anti-D Ig prophylaxis using either a single large dose at 28 weeks gestation, (Bowman *et al.*, 1978; Trolle *et al.*, 1989; MacKenzie *et al.*, 2004), or two doses, given at around 28 and 34 weeks, respectively (Tovey *et al.*, 1983a,b; Mayne *et al.*, 1997; MacKenzie *et al.*, 1999), achieves a significant reduction in the incidence of maternal sensitisation to D. However, no direct comparative data is available to allow an evaluation of the relative efficacy of single dose vs two-dose regimen. NICE guidelines (NICE, 2008) recommend that the preparation with the lowest associated cost should be used. This cost should take into account the lowest acquisition cost available locally and costs associated with administration.

If using the two-dose regimen, a minimum dose of anti-D Ig 500 IU is recommended at 28 and 34 weeks.

Alternatively, a single dose of anti-D Ig, 1500 IU should be administered between 28 and 30 weeks. The single dose regimen may be more cost effective (Pilgrim *et al.*, 2009), potentially enabling better compliance and providing logistic benefits.

Use of routine *antenatal* anti-D Ig prophylaxis should not be affected by previous anti-D Ig prophylaxis administered for a sensitising event earlier in the same pregnancy.

Recommendation

All D negative pregnant women who have not been previously sensitised should be offered routine antenatal prophylaxis with anti-D Ig (RAADP) either with a single dose regimen given around 28 weeks, or two dose regimen given at around 28 and 34 weeks (Grade 1B).

Recommendation

It is important that the 28-week sample for blood group and antibody screen is taken prior to the first routine prophylactic anti-D Ig injection being given. This forms the second screen required in pregnancy as stated in the BCSH Guidelines for Blood Grouping and Red Cell Antibody Testing during pregnancy (BCSH c, 2007; NICE CG62, 2008) (Grade 2C).

Recommendation

The RAADP scheme should be regarded as supplementary to any anti-D Ig administered for sensitising episodes listed in Table 1 (Grade 2C).

6.3. Management of RAADP scheme

Information regarding the administration of RAADP must reach the transfusion laboratory promptly so this is available should a pregnant woman require pre-transfusion testing. This is essential because if anti-D is detected a record of anti-D Ig administration will help in the process of determining whether this is immune or passive, the outcome of which will influence management of the pregnancy.

Identification of women eligible for RAADP involves training and regular retraining of personnel responsible. This training must be carried out to ensure that all eligible women are correctly identified and their informed consent obtained.

Information leaflets should be made available to pregnant women to help with the informed consent process (RCOG, 2011).

Written requests for the injections, with suitable identification of the recipient, should be forwarded in a timely manner to the unit responsible for issuing the injection.

7. PROPHYLAXIS FOLLOWING BIRTH OF AN D POSITIVE CHILD OR INTRAUTERINE DEATH

7.1. Laboratory tests required

Following birth, a cord blood sample should be tested to obtain the ABO and Rh D type of the baby. If a cord blood sample is not collected for any reason, a heel prick sample from the baby should be obtained as soon as possible (BCSH, 2007). The reagents used should conform to the BCSH pre-transfusion compatibility procedures (Milkins *et al.*, 2012). Anomalous or indeterminate cord Rh D groups should be treated as D positive until confirmatory testing is completed. If a sample cannot be obtained, the baby should be assumed to be D positive for the purpose of administration of anti-D Ig.

A direct antiglobulin test (DAT) on the cord blood sample is not routinely performed since it may be positive in a proportion of cases because of antenatal prophylaxis with anti-D Ig. However, a DAT should be performed if haemolytic disease of the newborn is suspected or anticipated because of a low cord blood haemoglobin concentration &/or the presence of maternal immune red cell antibodies.

Maternal samples for confirmatory ABO and Rh D type and FMH testing should be collected after sufficient time has elapsed for any FMH to be dispersed in the maternal circulation. A period of 30–45 min is considered adequate (Mollison *et al.*, 1997) and the samples should ideally be taken within 2 h of delivery primarily to ensure that the sample is taken prior to woman's discharge from the hospital (RCOG, 2011).

FMH testing should be undertaken on all D negative women delivering D positive infants to determine if additional doses of anti-D Ig are required.

If an FMH >4 mL is detected, follow-up samples are required at 48 h following an IV dose of anti-D or 72 h following an IM dose to check for the clearance of fetal cells (BCSH a, 2009).

7.2. Administration of anti-D Ig

If the baby's blood group is D positive, a minimum of 500 IU anti-D Ig should be administered to previously non-sensitised D negative women, within 72 h of the delivery (Crowther & Middleton, 1997).

Administration of *postpartum* anti-D Ig prophylaxis should not be affected by previous routine antenatal anti-D Ig prophylaxis or by antenatal anti-D Ig given for a potentially sensitising event.

A dose of 500 IU, IM is considered sufficient to treat a FMH of up to 4 mL fetal red cells (WHO Technical Report 468, 1971). Where it is necessary to give additional doses of anti-D Ig, as guided by tests for FMH, the dose calculation is traditionally based on 125 IU anti-D Ig/mL fetal red cells for IM administration. However, healthcare professionals should refer to manufacturer's guidance depending on which product is used (see section 11·2).

In cases of large FMH, and particularly if FMH is in excess of 100 mL, a suitable preparation of IV anti-D Ig should be considered. *Preparations licensed for IM injection only*, *must never be given IV*. Consideration should be given to limiting batch exposure, but this should not delay the timely provision of anti-D Ig.

When intra-operative cell salvage (ICS) is used for Caesarean section, reinfused blood may contain fetal red cells. Published literature using different cell salvage apparatus, techniques and volume of blood reinfused suggests that the volume of fetal red cells in re-infused blood varies from 1 to 20 mL (Fong *et al.*, 1999; Catling *et al.*, 1999; Allam *et al.*, 2008). Since the volume of fetal red cells in ICS blood is variable and can be relatively large, it is recommended that a minimum anti-D Ig dose of 1500 IU be administered after reinfusion of salvaged red cells if the cord blood group is D positive (or if the cord group cannot be established for whatever reason). Maternal samples should be taken for estimation of FMH 30–45 min after the re-infusion of salvaged red cells, and additional dose(s) of anti-D administered if necessary, and appropriate follow-up FMH testing performed. It is important that clinicians inform the transfusion laboratory if ICS has been used to ensure that correct dose of anti-D Ig is issued.

Recommendation

Following birth, ABO and Rh D typing should be performed on cord blood sample and if the baby is confirmed to be D positive, all D negative, previously non-sensitised, women should receive at least 500 IU of anti-D Ig within 72 h following delivery. Maternal samples should be tested for FMH and additional dose(s) given as guided by FMH tests (Grade 1B).

Recommendation

If there is an intrauterine death (IUD) and hence no sample can be obtained from the baby, prophylactic anti-D Ig should be administered to D-negative, previously non-sensitised women. A minimum of 500 IU of anti-D Ig should be administered within 72 h following the diagnosis of IUD. Maternal samples should be tested for FMH and additional dose(s) given as guided by FMH tests. It should be noted that the diagnosis of IUD is the sensitising event rather than delivery and hence anti-D Ig should be administered within 72 h of diagnosis (Grade 2C).

Recommendation

If cord blood sample cannot be obtained or if cord blood group cannot be established for any reason, at least 500 IU anti-D Ig should be administered to D negative, previously non-sensitised women (Grade 2C).

Recommendation

Where intra-operative cell salvage (ICS) is used during Caesarean section in D negative, previously non-sensitised women, and where cord blood group is confirmed as D positive (or unknown), a minimum dose of 1500 IU anti-D Ig should be administered following the re-infusion of salvaged red cells, and a maternal sample should be taken for estimation of FMH 30–45 min after reinfusion in case more anti-D Ig is indicated. It is important that clinicians inform the transfusion laboratory if ICS has been used to ensure that correct dose of anti-D Ig is issued (Grade 2C).

8. MANAGEMENT OF TRANSFUSION OF D POSITIVE BLOOD COMPONENTS TO D NEGATIVE GIRLS OR WOMEN OF CHILDBEARING POTENTIAL

8.1. D positive platelet transfusions

Whenever possible, D negative platelets should be transfused to D negative girls or women of child bearing potential, who need a platelet transfusion. Occasionally, if the appropriate product is not available or its availability would cause unacceptable delay, it may be necessary to transfuse D positive platelets. In these circumstances, prophylaxis against possible sensitisation to the D antigen by red cells contaminating the platelet product should be given (Menitove, 2002).

A dose of 250 IU anti-D immunoglobulin should be sufficient to cover up to five adult therapeutic doses of D positive platelets given within a 6-week period (BCSH b, 2003) (Grade 2B). In severely thrombocytopenic patients with platelet count of $\leq 30 \times 10^9$/L, anti-D Ig should be given subcutaneously, or IV if a preparation suitable or IV route is available, to avoid the risk of IM bleed following IM injection.

It is not necessary to administer anti-D Ig to D negative females without childbearing potential, or males who receive D positive platelets (BCSH b, 2003; Menitove, 2002).

8.2. Inadvertent transfusion of D positive blood to D negative women of childbearing potential

When less than 15 mL have been transfused, the appropriate dose of IM anti-D Ig may be given (see Section 11·2). When more than 15 mL have been transfused, it is preferable to use the larger anti-D immunoglobulin preparation (1500 or 2500 IU); however, IV anti-D immunoglobulin is the preparation of choice, achieving adequate plasma levels immediately. *IM only preparations of anti-D immunoglobulin must not be given IV*.

The quantitation of D positive red cells should be performed by flow cytometry (FC) after 48 h if an IV dose of anti-D has been given or 72 h if an IM dose has been given (Grade 2C), and further anti-D Ig given until there are no detectable D positive red cells in circulation.

When more than one unit of D positive blood has been transfused, a red cell exchange transfusion should be considered to reduce the load of D positive red cells in the circulation and the dose of anti-D Ig required to prevent immunisation. In this situation advice should be sought from a specialist in Transfusion Medicine, and the patient should be counselled regarding the implications of both non-intervention (for future pregnancies) and of treatment, including any hazards from receiving donated blood, the exchange procedure itself and of larger doses of anti-D Ig, including IV anti-D Ig (RCOG, 2002).

A single blood-volume red cell exchange transfusion will achieve a 65–70% reduction in D positive red cells; a double volume exchange will achieve an 85–90% reduction. Shortly after the exchange transfusion, the residual volume of D positive red cells should be estimated using FC.

Passive anti-D Ig given in large doses may remain detectable and tests for immune anti-D may not be conclusive for several months.

9. FUTURE DEVELOPMENTS INCLUDING THE ROLE OF CELL FREE FETAL DNA TESTING

At the time of writing this guideline, the recommendation in the UK is that all previously non-sensitised, D negative, pregnant women are offered RAADP. However, the disadvantage of this approach is that approximately 40% of D negative women who are carrying an D negative child will be given routine prophylactic anti-D Ig unnecessarily. This equates to approximately 40 000 women in the UK who are receiving prophylaxis unnecessarily. In recent years, advancements in fetal blood group genotyping using cell free fetal DNA (cffDNA) from maternal blood samples taken at 16–20 week gestation, have made it possible to determine fetal D type with a diagnostic accuracy of around 96% (Geifman-Holzman *et al.*, 2006; Finning *et al.*, 2008; Daniels *et al.*, 2009). The risk of a false negative result (i.e. missing an D positive fetal blood group) by this technique, is small and currently estimated to be around 0·08 to 0·16% (Finning *et al.*, 2008; Clausen *et al.*, 2012). Fetal blood group genotype can also be determined for Rh C, c, E and Kell (K) status using cffDNA from maternal plasma.

Routine fetal *RHD* typing for all D negative pregnant women has been introduced in Denmark and The Netherlands to allow selective use of RAADP though this has not yet been recommended in the UK (Clausen *et al.*, 2012).

10. KEY STANDARDS FOR CLINICAL AUDIT

Audits of practice should to be undertaken on a continuing basis to ensure compliance with these guidelines and, where identified, variance or concerns in relation to compliance should be addressed (DH a, 1997; DH b, 1998).

Suggested audit standards:

- All non-sensitised D negative pregnant women are offered anti-D for sensitising events during pregnancy and at delivery of an D positive baby.
- All non-sensitised D negative pregnant women are offered RAADP and that the provision of adequate information (anti-D Ig leaflets), choice and consent are documented.
- All non-sensitised D negative women undergoing therapeutic termination of pregnancy are offered anti-D Ig regardless of the method of termination or gestational age.
- All D negative pregnant women have an FMH test for sensitising events after 20 weeks of gestation and at delivery of an D positive baby.
- All non-sensitised D negative pregnant women with FMH volumes exceeding the volume covered by the standard dose of anti-D Ig in use, are given sufficient additional anti-D Ig and that a follow-up sample is taken to check clearance of fetal cells.
- Where anti-D Ig is indicated for potentially sensitising events, it is administered within 72 h of a sensitising event.
- Evidence of appropriate documentation of traceability of anti-D Ig injections to their recipients.

The UK haemovigilance scheme, Serious Hazards of Transfusion (SHOT) is conducting a study looking at women who have produced an immune anti-D that is detectable for the first time in the current pregnancy, whether detected at booking, 28 weeks, delivery or at any other time within the pregnancy. For any woman identified, there will be supplementary questions about previous pregnancies, recorded sensitising events, anti-D prophylaxis and outcome. This study began in January 2013. Transfusion laboratories are encouraged to report any new cases of immune anti-D.

11. PRINCIPLES, SAFETY, AVAILABILITY, DOSAGE AND ADMINISTRATION OF ANTI-D Ig

11.1. Safety of anti-D Immunoglobulin

Polyclonal anti-D Immunoglobulin (anti-D Ig) used in the UK is prepared from pooled plasma from non-UK blood donors who have high levels of anti-D Ig either due to previous sensitisation

or intentional immunisation. Plasma is screened for HBsAg, anti-HIV and HCV RNA. In addition, the manufacturing process includes viral inactivation steps in order to further reduce the risk of viral transmission. The theoretical risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) remains unquantifiable, though is likely to be extremely small.

Product safety data submitted by manufacturers to inform National Institute of Health and Clinical Excellence technical appraisal guidance 156 (NICE, 2002, 2008) indicates a very low rate of reporting a probable or possible adverse event (Pilgrim *et al.*, 2009), estimated to be less than one event per 80 000 doses of anti-D Ig. The majority of reported adverse events were not considered serious. There is no evidence to suggest that anti-D Ig administered to women during pregnancy is harmful to the fetus.

Allergic reactions are very rare but severe hypersensitivity including anaphylaxis may occur. Anti-D preparations may contain trace amounts of IgA (less than $5\,\mu g\,mL^{-1}$) and hence patients with known antibodies to IgA have a greater risk of developing potentially severe hypersensitivity and anaphylactic reactions. If symptoms of allergic or early signs of hypersensitivity reactions (including generalised urticaria, tightness of the chest, wheezing, hypotension, and anaphylaxis) develop, administration of anti-D must be discontinued immediately and appropriate treatment instituted. Manufacturer's prescribing information for Rhophylac® recommends that medication such as adrenaline should be available for immediate treatment of acute severe hypersensitivity reactions (http://www.rhophylac.com/includes/PDF/RhophylacPI2.pdf).

The precise mechanism by which anti-D Ig prevents alloimmunisation is unknown. Possible mechanisms include rapid clearance of anti-D coated D positive red cells by macrophages and down-regulation of antigen-specific B cells (Kumpel, 2006; Bichler *et al.*, 2004).

11.2. Preparations available in the UK, dosage and route of administration of anti-D Ig

The following anti-D Ig preparations are currently available in the UK at the time of writing this guideline:

D-GAM® (Bio Products Laboratory, Elstree, UK): available as 250, 500, 1500 and 2500 IU vials, for IM use only.

Rhophylac® (CSL Behring, Haywards Heath, UK): available as 1500 IU prefilled syringe, for IM or IV use.

It should be noted that due to the nature of the manufacturing process, D-GAM® preparations are suitable for IM USE ONLY and therefore *must not* be administered IV because of the risk of severe hypersensitivity reactions due to the presence of trace amounts of IgA and other plasma proteins. However, Rhophylac®, prepared by ion exchange chromatography, is a purer product and is suitable for IV and IM administration.

Where a specific dose of anti-D Ig is mentioned in this guideline, it is intended as the minimum recommended dose for a specific clinical situation. The actual dose given will depend on the type and size of anti-D Ig preparations available in individual centres and thus may be higher than is clinically necessary. The dose of anti-D Ig is specified as international units (IU), and 1 μg of anti-D Ig is equivalent of 5 IU.

A dose of 500 IU, IM is considered sufficient to treat a FMH of up to 4 mL fetal red cells (WHO Technical Report 468, 1971). Where it is necessary to give additional doses of anti-D Ig, as guided by tests for FMH, the dose calculation is traditionally based on 125 IU Anti-D Ig/mL fetal or inadvertently transfused D positive red cells, for IM administration. However, healthcare professionals should refer to manufacturer's guidance depending on the product used. The datasheet for Rhophylac, based upon pharmacokinetic data, suggests administering at least 100 IU mL^{-1} of fetal or transfused D positive cells either IV or IM.

There is some evidence from pharmacokinetic studies (Bichler *et al.*, 2003; Woelfer *et al.*, 2004) that high Body Mass Index (BMI) is associated with lower serum peak levels of anti-D Ig following IM administration. However, it is unknown whether this observation translates to a higher sensitisation rate in overweight women. On the basis of the available evidence, a firm recommendation cannot be made regarding a higher dose or IV route of administration in women with high BMI.

When large or multiple doses of anti-D Ig are necessary, consideration should be given to limiting batch exposure whenever possible, for example, reserving the same anti-D Ig batch for RAADP and the postnatal dose, but this should not in any way delay the timely provision of anti-D Ig.

The deltoid muscle is an appropriate and safe site for IM administration of anti-D Ig (Smith *et al.*, 1972). If the gluteal region is used, particular care should be taken to ensure that the injection is given into muscle, as absorption may be delayed if it only reaches the subcutaneous tissues. In women with severe thrombocytopenia (platelet count $\leq 30 \times 10^9$/L) or a history of a bleeding disorder such as severe Von Willebrand disease, anti-D Ig should be administered IV or subcutaneously depending on whether a preparation suitable for IV use is available. Women with significant bleeding disorders such as Von Willebrand disease should be managed jointly with a haemophilia centre.

11.3. Documentation and audit trail of the issue and administration of anti-D Ig immunoglobulin

The EU guide on good manufacturing practice recommends that there is clear documentation and record keeping to ensure traceability of all blood products (including anti-D Ig) from donors to recipients (European Commission, 2000). The Health Service Circular, Better Blood Transfusion (HSC 2007/001; DH 2007) also specifies the requirement for maintaining adequate traceability records for anti-D Ig.

There are distinct advantages in the hospital transfusion laboratory being involved in the issue and administration process for anti-D Ig, as the information will be stored automatically in the laboratory computer system. However, it is recognised that local arrangements may vary and other departments may be responsible for the storage and issue of anti-D Ig. In any case it is recommended that complete records of issue and

administration are maintained in order to allow traceability of anti-D Ig to recipients.

Documentation accompanying the anti-D Ig injection should include a report containing the following details:

- Woman's identity including surname, forename, date of birth and a unique ID number
- The date when the injection is to be given.
- Name and address of the woman's GP whenever possible.
- Name of woman's Obstetrician/Midwife whenever possible.
- Hospital/antenatal clinic administering anti-D Ig.

Details of the injection including the product description and batch number, the dosage and route (IM or IV), site, date and time of administration should be recorded in the woman's maternity record (both hospital clinical and handheld notes).

It is also important that these details are centrally recorded in the hospital transfusion laboratory computer so that this information is readily available should pre-transfusion testing be required.

Recommendation

Adequate records of issue and administration should be maintained to allow full traceability of anti-D immunoglobulin (Grade 2C).

11.4. Informed consent

All pregnant women must be offered written and verbal information about anti-D Ig to inform their decision about receiving anti-D Ig. Maternal consent must be obtained prior to giving anti-D Ig, and the woman's decision to either accept or decline the injection should be clearly recorded by the healthcare professional, both in the woman's 'hand held' and hospital records (RCOG, 2011). NICE guidance recommends that choice is offered at the time of recording blood group in her antenatal healthcare records.

11.5. Assessment of the volume of fetomaternal haemorrhage

This is required when an D negative woman experiences a potentially sensitising event after 20 weeks gestation, and after the birth of an D positive baby, to determine whether the standard dose of anti-D Ig administered has been sufficient to remove all fetal red cells from the maternal circulation (RCOG, 2011; BCSH a, 2009). FMH testing of initial and follow-up samples should be undertaken according to BCSH guidelines for the estimation of FMH (BCSH a, 2009).

Where the FMH result shows that insufficient anti-D Ig has been given to cover the bleed volume an additional dose of anti-D Ig should be administered within 72 hours of the sensitising event. The additional dose of anti-D Ig should be calculated as 125IU for every 1mL fetal red cells (but see section 11·2) and should take into account any postnatal anti-D Ig dose, if already given. but not any dose given as RAADP.

A follow-up FMH sample should be tested (after 72 hours if the additional anti-D Ig is given intramuscularly or after 48 hours if given intravenously) to check for clearance of fetal cells. Further dose(s) of anti-D Ig and continued follow-up will be necessary if fetal cells remain detectable. A FMH confirmed by flow cytometry as > or = to 4mL is considered to be significant, and even if such a bleed is covered by the standard anti-D dose administered, a follow-up FMH sample is still required to confirm that anti-D Ig has been administered and to check for clearance of fetal cells. (BCSH a, 2009)

The presence of free anti-D in maternal plasma does not necessarily indicate adequate prophylaxis and additional doses of anti-D Ig should be continued until D positive red cells are no longer detectable (BCSH a, 2009; RCOG, 2002).

Anti-D detectable in samples from pregnant D negative women

Antibody screens on maternal pre-transfusion samples may be positive following injection of anti-D Ig. Detectable anti-D may be passive or immune and there is no serological method for distinguishing between the two. Even if measured at the pharmacokinetic peak, passive anti-D rarely exceeds 0·4 IU mL^{-1} unless more than 1500 IU has been given IV. The pharmacokinetic peak level is lower and later following IM anti-D and would rarely exceed 0·2 IU mL^{-1} following 1500 IU. The level will fall with time (Bichler *et al.*, 2003; MacKenzie *et al.*, 2006).

There have been several cases in the UK where immune anti-D has been mistakenly assumed to be prophylactic without a validated method of measuring the strength of serological reaction or taking into account an accurate history (2010 SHOT Annual Report). It is important therefore that regardless of any prior administration of anti-D Ig, any anti-D detected at 28 weeks should be quantified and the results made available in the woman's hand-held and hospital records.

Recommendation

Where anti-D is detected in a sample from a pregnant woman, further history should be obtained and investigations undertaken to establish whether this is immune or passive. If no clear conclusion can be reached as to the origin of anti-D, then prophylaxis should continue to be administered in accordance with guidelines for D negative women who have not formed immune anti-D (Grade 2C).

CONFLICT OF INTEREST

None of the authors have declared a conflict of interest.

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Appendix 1

Levels of evidence and grade of recommendations

Grading of Recommendations Assessment, Development and Evaluation (GRADE)

Strength of recommendations:

Strong (grade 1): Strong recommendations (grade 1) are made when there is confidence that the benefits do or do not outweigh harm and burden. Grade 1 recommendations can be applied uniformly to most patients. Regard as 'recommend'.

Weak (grade 2): Where the magnitude of benefit or no0074 is less certain a weaker grade 2 recommendation is made. Grade 2 recommendations require judicious application to individual patients. Regard as 'suggest'.

Quality of evidence

The quality of evidence is graded as high (A), moderate (B) or low (C). To put this in context it is useful to consider the uncertainty of knowledge and whether further research could change what we know or our certainty.

(A) High: Further research is very unlikely to change confidence in the estimate of effect. Current evidence derived from randomised clinical trials without important limitations.

(B) Moderate: Further research may well have an important impact on confidence in the estimate of effect and may change the estimate. Current evidence derived from randomised clinical trials with important limitations (e.g. inconsistent results, imprecision - wide confidence intervals or methodological flaws - e.g. lack of blinding, large losses to follow up, failure to adhere to intention to treat analysis), or very strong evidence from observational studies or case series (e.g. large or very large and consistent estimates of the magnitude of a treatment effect or demonstration of a dose–response gradient).

(C) Low: Further research is likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate. Current evidence from observational studies, case series or just opinion.

Appendix 2

SHOT flowchart to guide the appropriate administration of anti-D Ig (www.shotuk.org)

Anti-D Administration Checklist

Always confirm

- the woman's identity
- that the woman is RhD Negative using the latest laboratory report
- that the woman does not have immune anti-D using the latest laboratory report
- that informed consent for administration of anti-D Ig is recorded in notes

Potentially Sensitising Events (PSEs) during pregnancy

Gestation LESS than 12 weeks

Vaginal bleeding associated with severe pain	Administer at least **250 IU** anti-D Ig within 72 hours of event. Confirm product / dose / expiry and patient ID pre administration
ERPC / Instrumentation of uterus	
Medical or surgical termination of pregnancy	
Ectopic / Molar Pregnancy	

Gestation 12 to 20 weeks

For any Potentially Sensitising Event (PSE)	Administer at least **250 IU** anti-D Ig within 72 hours of event. Confirm product / dose / expiry and patient ID pre administration

Gestation 20 weeks to term

For any Potentially Sensitising Event (PSE) (**Irrespective** of whether RAADP has been given)	Request a Kleihauer Test (FMH Test) and immediately administer at least **500 IU** anti-D Ig within 72 hours of event. Confirm product / dose / expiry and patient ID pre administration
Does the Kleihauer / FMH test indicate that further anti-D Ig is required ?	Administer more anti-D Ig following discussion with laboratory

For continuous vaginal bleeding at least **500iu** anti-D Ig should be administered at a minimum of 6-weekly intervals, irrespective of the presence of detectable anti-D, and a Kleihauer / FMH Test requested every two weeks in case more anti-D is needed

Routine Antenatal Anti-D Prophylaxis (RAADP)

For Routine Antenatal Anti-D Prophylaxis (**Irrespective** of whether anti-D Ig already given for PSE)	Take a blood sample to confirm group & check antibody screen – do not wait for results before administering anti-D Ig
	Administer **1500 IU** anti-D Ig at **28 – 30** weeks
	OR
	Administer at least **500 IU** anti-D Ig at **28** weeks and then administer at least **500 IU** anti-D at **34** weeks
	Confirm product / dose / expiry and patient ID pre administration

At Delivery (or on diagnosis of Intra Uterine Death >20 weeks)

Is the baby's group confirmed as RhD positive ? OR Are cord samples not available ?	Request a Kleihauer Test (FMH Test)
	Administer at least **500 IU** anti-D Ig within 72 hours of delivery Confirm product / dose / expiry and patient ID pre administration
Does the Kleihauer / FMH test indicate that further anti-D Ig is required ?	Administer more anti-D following discussion with laboratory

SHOT anti-D Ig Administration Flowchart v7 October 2012