Prevention and management of RhD alloimmunisation in pregnancy

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ABSTRACT
Rhesus D alloimmunisation remains a major problem despite the use of anti-D prophylaxis. Immunisation during pregnancy commonly occurs in the absence of any overt sensitising event. Appropriate and timely intervention can significantly reduce the rate of immunisation (17% - 1%). In this respect, a simple algorithm for the management of RhD negative women in pregnancy is presented.
Traditionally, identifying and monitoring fetuses at risk of anaemia in RhD alloimmunised pregnancies required invasive testing such as serial amniocentesis. This procedure is associated with significant complications. Recent evidence favours a non-invasive approach by measuring the peak systolic velocity in the fetal middle cerebral artery. This Doppler method has the same accuracy in predicting fetuses at risk of anaemia when compared with amniocentesis, but without its complications. We therefore present new guidelines for the monitoring of RhD alloimmunised pregnancies.

Introduction
Despite the introduction and widespread use of Rhesus (Rh)D immunoglobulin for the prevention of haemolytic disease of the fetus/newborn (HDFN), Rh alloimmunisation remains a significant problem in perinatology. At least 600-700 new cases of Rh sensitization still occur each year in the UK, and the failure rate of prophylaxis worldwide is 1-2%. In KwaZulu-Natal, of the estimated 3600 RhD-negative pregnant women, 95 new cases of Rh sensitization occurred in the past year (personal communication - South African National Blood Service).

It is estimated that only about 16-17% of RhD negative women who deliver a Rh positive fetus will become alloimmunised if not administered anti-D immunoglobulin (Ig). Factors that affect the risk of alloimmunisation in a susceptible RhD negative woman include: (i) volume of fetal-maternal haemorrhage (FMH); (ii) degree of maternal immune response; and (iii) concurrent ABO incompatibility.

The most important cause of anti-D antibody stimulation is immunization during the antenatal period where there has been no overt sensitizing event. Late sensitization during pregnancy is responsible for 18-27% of cases. In second or subsequent pregnancies it may be impossible to distinguish late sensitization from failure of prophylaxis at the end of the last pregnancy.

While the prevention of Rh alloimmunisation is the responsibility of all health-care workers, the management of alloimmunised pregnancies requires specialized care. From a public health viewpoint, emphasis must be placed on prevention; we therefore present in this article an algorithm for the management of RhD negative women in pregnancy and the immediate puerperium. A newer non-invasive approach to the monitoring of the alloimmunised pregnancy is also discussed.

PREVENTION OF RH D ALLOIMMUNISATION
Pathophysiology
During pregnancy, small volumes of fetal red cells continually pass into the maternal circulation. This trafficking of red cells increases as gestation progresses. Bowmen et al showed at least 0.01ml of fetal cells in 3%, 12% and 46% in women in first, second and third trimesters respectively. In most women, this load of RhD antigen on fetal red cells and red cell precursors are rapidly cleared by the maternal reticulo-endothelial system and therefore do not stimulate the mother's immune system. However, when a large volume of fetal blood enters the maternal circulation, her immune system is stimulated, leading to the production of B lymphocyte clones that recognise the fetal red cell as a foreign antigen. Thus, an initial IgM anti-D response is established, but it is short-lived and a rapid switch to IgG production occurs. Unlike IgM, IgG crosses the placenta and destroys fetal red cells causing fetal anaemia. Haemolytic disease of the fetus/newborn however can range in severity from being detectable only in laboratory tests to severe fetal anaemia resulting in fetal hydrops, stillbirths or birth of babies with severe anaemia and jaundice.

ABO – Incompatibility
ABO blood group status affects the risk of alloimmunisation. With ABO-compatible fetus, the overall risk of alloimmunisation if not treated with anti-D is approximately 16%, however, if ABO-incompatibility exists, the risk is only 1-2%. The protec-
tive effect of ABO- incompatibility is thought to be due to de-
struction of the fetal ABO-incompatible red cells in the mater-
nal circulation, before Rh sensitization occurs.

Immunoprophylaxis
Antenatal and postnatal administration of anti-D Ig has been
established to prevent RhD alloimmunisation. However, for it
to work it must be given in a sufficient dose and before immu-
nization has occurred. Therefore, anti-D immunoglobulin is nor-
mally given to the mother as soon as possible, at most within 72
hours of a potentially sensitizing event, to bind to the fetal red
cell antigen in the maternal circulation thereby destroying the
fetal RhD positive cells and preventing antibody formation.

Anti-D should also be administrated following each poten-
tially sensitizing event. Such events include threatened miscar-
riage, ectopic pregnancy, any invasive prenatal procedure, an-
tepartum haemorrhage, external cephalic version and abdomi-
nal trauma. Since fetal Rh antigens may be present from as early
as the 30th day post-conception, anti-D should be given fol-
lowing ectopic pregnancies, therapeutic and spontaneous mis-
carriages. Feto- maternal haemorrhage (FMH) increases with
advancing gestation with the greatest amount occurring during
delivery. Anti-D given during the antenatal period should there-
fore be repeated postpartum in the unsensitised woman who has
delivered a Rh-positive baby.

Anti-D Immunoglobulin dosage
The dosage for anti-D immunoprophylaxis varies in the differ-
ent parts of the world. The UK MRC dosage trial showed that
100µg (500IU) anti-D given intramuscularly is capable of sup-
pressing immunization by 4-5mls of RhD positive cells, as ef-
effectively as both 200µg (1000IU) and 300µg (1500IU) dosages.6
In SA, the following intramuscular dosage is recommended:
(a) <20 weeks gestation -50µg (250IU); (b) ≥20 weeks gesta-
tion -100µg (500IU); and (c) Postpartum -100µg (500IU) - if
baby is Rh positive. Additional dose may be required in situa-
tions where a greater amount of feto-maternal haemorrhage is
suspected e.g. abruptio placenta, manual removal of placenta,
ruptured uterus, multiple pregnancy, placenta praevia, and
abdominal trauma. The Kleihauer-Betkey acid elution test that
detects fetal haemoglobin (HbF) in the maternal circulation is
used to calculate the additional dosage required using the for-

dula that 25µg (125 IU) of anti-D immunoglobulin (Ig) is ad-
ministered per additional 1 ml of red cells.1 A more specific but
more expensive test is flow cytometry that detects RhD posi-
tive cells making it particularly helpful in patients with high
HbF levels.7

Some women who had received anti-D Ig during the anten-
ntal period may have detectable anti-D antibody in their blood at
delivery. It is impossible to distinguish between passive anti-D
Ig and weak anti-D resulting from early immunization. Hence,
postnatal anti-D prophylaxis should be given to all women who
had antenatal anti-D Ig; and at delivery of an Rh positive baby,
have weak anti-D antibody levels detected.

Routine use of antenatal anti-D prophylaxis
The routine use of antenatal anti-D should not be affected by
whether the woman has already had anti-D prophylaxis for a
potentially sensitizing event earlier in pregnancy. Like-wise
postpartum anti-D prophylaxis should similarly not be affected
by whether she had routine antenatal anti-D prophylaxis and/or
anti-D prophylaxis for a potentially sensitizing event. Although
not a routine practice in all units in SA, it is recommended that
anti-D prophylaxis (100µg) be offered to all unsensitised RhD
negative pregnant women at 28 weeks and 34 weeks gestation.
The rationale being that the most important cause of anti-D an-
tibodies now is alloimmunisation during the antenatal period,
when no overt sensitising event has occurred.

The current British recommendations for immunoprophylaxis
from the Royal College of Obstetrics and Gynaecology (RCOG)3
and the National Institute of Clinical Excellence (NICE)4 guide-
lines are as follows:
- After delivery, irrespective of the dose of antenatally admin-
istered immunoglobulin, postnatal immunoprophylaxis must
be given and include a screening test to identify women with
a large FMH who need additional immunoglobulin;
- Anti-D immunoglobulin should be given after sensitising
events before delivery and after a miscarriage;
- Anti-D immunoglobulin is no longer necessary in women
with a threatened miscarriage with a viable fetus and cessa-
tion of bleeding before 12 weeks gestation.
- At least 500IU of anti-D immunoglobulin should be given to
non-sensitised RhD negative women at 28 weeks and 34
weeks pregnancy.

Red cell alloimmunisation
Haemolytic disease of fetus/newborn (HDFN) could also be
caused by other antigens of the Rh blood group systems (e.g.: c, E)
as well as from antigens of the non-Rh blood group sys-
tems (e.g.: Kell, Duffy, Kidd, Fy, etc). Some authors prefer to
use the term red cell alloimmunisation rather than Rh
alloimmunisation.1,9,10,13 Except for RhD alloimmunisation, the
other causes of red cell alloimmunisation are beyond the remit
of this discussion. This includes the transfusion of RhD posi-
tive blood components to Rh-negative women. Suffice to say
that all cases of red cell alloimmunisation should be referred to
an appropriate centre capable of managing such a condition.
Importantly, anti-D Ig does not protect against the develop-
ment of other antibodies that can cause HDFN. Nonetheless, the
principles of management are similar regardless of the type of anti-
body involved, although care needs to be taken with pregnan-
cies complicated by Kell alloimmunisation, where antibody con-
centrations does not correlate with disease severity.

Exceptions to the rule for anti-D prophylaxis
In a few situations anti-D prophylaxis may be neither neces-
sary nor cost-effective. Such circumstances may include those
women who: (i) opt to be sterilised after birth; (ii) are in a stable
relationship with the father of the child, and the father is known
or found to be RhD negative; and (iii) are certain that they will
not have another child after the current pregnancy. Anti-D pro-
phylaxis must be given in any situation of doubt in the unsensitised RhD negative pregnant women, following any poten-
tially sensitizing event.

Precautions at delivery in the RhD negative preg-
nant women
- Avoid episiotomy
- Clamp and cut the cord on fetal side
- Allow placental side to drain out
- Avoid manual removal of placenta
Failure of anti-D prophylaxis
Possible reasons for failure of prophylaxis include:
- Failure to check the maternal Rh status
- Failure to administer Rh Ig when indicated.
- Error in typing maternal, paternal and infants’ blood group.
- Unrecognized FMH
- Inadequate Rh Ig dosage for the volume of FMH

NB: Anti-D Ig is a blood product. Administering anti-D Ig poses a few risks while inadequate therapy will result in Rh alloimmunisation. It is important that the women are adequately counselled about Rh prophylaxis so that they can make an informed choice. Ideally, the paternal genotype should be ascertained in all cases. If heterozygous then there is a 50% chance that the fetus is RhD positive and therefore at risk. The possibility of fetal RhD genotyping by maternal serum analysis using polymerase chain reaction will be a significant advance in future clinical practice. This will avoid unnecessary administration of antenatal anti-D Ig in the case of a RhD negative fetus.

An algorithm for the management of RhD negative women in pregnancy is shown in Figure 1. As a precautionary measure, checking the father’s genotype has been omitted. However, if the father’s genotype is known and confirmed Rh negative, no further monitoring for Rh alloimmunisation is required and the pregnancy managed as normal.

MONITORING OF RhD ALLOIMMUNISED PREGNANCY
The key to the management of pregnancies complicated by red cell alloimmunisation is the detection of fetal anaemia and the timely intervention by either intrauterine transfusion or delivery, before fetal death. Detection of fetal anaemia starts with the less sensitive methods such as a good reproductive history (details of previously affected pregnancies – particularly in-utero transfusions, neonatal anaemia and the need for exchange transfusions and phototherapy should be obtained), and measuring maternal serum antibody levels and finally, the accurate but invasive method i.e. fetal blood sampling (FBS) or cordocentesis.

Traditionally the identification of anaemic fetuses involved invasive methods such as amniocentesis and FBS. Amniocentesis allows for quantification of bilirubin content in amniotic fluid that occurs as a result of fetal haemolysis to indicate severity of anaemia. Cordocentesis allows direct measurement of haemoglobin and haematocrit as well as to check fetal blood group and Rh status. Being invasive, both these procedures (amniocentesis / FBS) entail some risk to mother and fetus including fetal death, accidental rupture of membranes, chorioamnionitis, preterm labour, miscarriage, worsening of alloimmunisation, cord accidents, fetal bleeding and fetal bradycardia requiring emergency caesarean section. Given these problems, it is evident that a non-invasive method to detect fetal anaemia would prevent complications and improve the monitoring of fetuses at risk.

With advances in fetal medicine there has now been a shift towards non-invasive methods of assessing fetal anaemia. The middle cerebral artery peak systolic velocity (MCA-PSV) for the diagnosis of moderate anaemia (a haemoglobin concentration of < 0.65 times the median) to severe anaemia (a haemoglobin concentration of < 0.55 times the median) has emerged as one method of non-invasive identification of fetal anaemia. An anaemic fetus will have a raised MCA-PSV due to increased blood flow to the brain due to the high output state and the reduced viscosity. Mari et al. (2005) have shown that when the fetal anaemia is corrected by blood transfusion, the MCA-PSV normalises. The good correlation between MCA-PSV and fetal anaemia has been shown in several studies with sensitivity ranging from 73 – 100% and specificity 71 – 80%. An anaemic fetus will have a raised MCA-PSV due to increased blood flow to the brain due to the high output state and the reduced viscosity. Mari et al. (2005) have shown that when the fetal anaemia is corrected by blood transfusion, the MCA-PSV normalises. The good correlation between MCA-PSV and fetal anaemia has been shown in several studies with sensitivity ranging from 73 – 100% and specificity 71 – 80%.

A more recent study comparing the accuracy of MCA-PSV with amniotic fluid delta optical density (ΔOD450) for detection of fetal anaemia against the gold standard of fetal blood sampling showed that MCA-PSV and ΔOD450 have similar test accuracy in detecting fetal anaemia. Since MCA-PSV is non-invasive, it is thus the preferred method of screening for fetal anaemia. Even in Kell - immunized fetuses, van Dongen et al. (2005) showed that the use of MCA-PSV in the detection of fetal anaemia had a sensitivity of 89%.

At King Edward VIII Hospital, Durban, the policy of non-invasive assessment of fetal anaemia by measuring the peak systolic velocity of middle cerebral artery, according to the technique described by Mari, is adopted. The best cut-off reference range of fetal blood velocity in the MCA for the prediction of moderate to severe fetal anaemia is the Mari curve - that of 1.5 MoM. If monitoring of the MCA suggests anaemia, FBS and ± intrauterine transfusions are performed. The procedure for intrauterine transfusions and its sequelae is described in most standard textbooks. Briefly, the patient should be counselled that the fetal loss rate related to the procedure depends on the gestation, site of sampling and underlying pathology. The risk of an uncomplicated FBS is 1-3%, but if the fetus is
Table 1. Monitoring of RhD alloimmunisation in pregnancy

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<th>Antibody titre ≥ 32</th>
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<td>- No anti-cells</td>
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<td>- Monitor antibody levels 2 weekly</td>
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<td>- Check Doppler MCA-PSV</td>
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MCA-PSV normal (< 1.5 MoM for gestation) and no Hydrops
- 2 weekly scans
- Delivery at 37-38 weeks

Hydrops and Antibodies (irrespective of antibody level / MCA-PSV and GA 20-34 weeks)
- Check fetal Hb and transfusion
- Deliver if > 34 weeks

MCA-PSV raised (> 1.5 MoM for GA)
- High Risk (Hydrops and/or previous affected pregnancy)
  - < 34 weeks – check fetal Hb and transfusion
  - ≥ 34 weeks – consider delivery
- Low Risk (No Hydrops)
  - Repeat Doppler MCA-PSV in 2-3 days
  - If still raised and no Hydrops, repeat Doppler MCA-PSV 2-3 days later
  - Persistently raised – check fetal Hb and ± transfusion
  - ≥ 34 weeks – consider delivery

Once transfusion commenced
- No need to monitor maternal antibody levels
- Weekly Doppler MCA-PSV
- Serial transfusions 2-4 weekly
- Gestational age range for transfusion
  - >20 weeks – < 36 weeks (depending on accessibility of cord)
- For HIV positive women, ideally CD4 count should be > 200 for intrauterine transfusion
- Transfuse to Hb just > 95th centile for that gestation (or Hct 45% - 50%)

MCA-PSV = middle cerebral artery peak systolic velocity
MoM = multiples of median
GA = gestational age
Hb = haemoglobin
Hct = haematocrit

CONCLUSION AND RECOMMENDATIONS

The primary aim in caring for the RhD negative women is the prevention of alloimmunisation. Every woman should have her ABO blood group, Rh type and antibody screen if Rh negative, checked at the first antenatal visit. The unsensitised RhD negative women are candidates for anti-D prophylaxis, unless the Rh status of the father is negative and paternity is certain. Anti-D is not given if the fetus / neonate are tested RhD negative.

RhD immunization largely occurs in the absence of overt sensitizing events. It is recommended that following delivery, irrespective of the dose of anti-D Ig routinely administered, postnatal prophylaxis should include a screening test to identify women with suspected large PMH who may need additional anti-D. Anti-D should be given after all sensitizing events antenatally and after a miscarriage or ectopic pregnancy, in all unsensitised RhD negative women. In any situation of doubt, anti-D Ig should be administered. Withholding anti-D has more disastrous consequences than an unnecessary dose. The routine administration of antenatal anti-D prophylaxis at 28 weeks and 34 weeks is also recommended.

On confirming maternal RhD alloimmunisation, identifying the fetus at risk of anaemia is a priority. This includes obtaining a good obstetric history and regular monitoring of maternal antibody titre levels. A woman that previously had an affected pregnancy is likely to have recurrence in her subsequent pregnancies, if not treated. It is therefore recommended that monitoring for fetal anaemia in her next pregnancy should commence at least 10 weeks earlier from gestation of previous affected fetus / neonate. The non-invasive approach using the MCA-PSV is recommended. Prompt diagnosis and referral to an appropriate facility will certainly reduce perinatal morbidity and mortality due to Rh alloimmunisation.

References