Diagnosis and management of non-anti-D red cell antibodies in pregnancy

Authors Ketan Gajjar / Chris Spencer

Key content:
• Anti-D prophylaxis has reduced the incidence of haemolytic disease of the newborn.
• A variety of non-anti-D red cell antibodies can cause a degree of neonatal haemolysis.
• The frequency of antibody testing should be individualised.
• Management of non-anti-D alloimmunisation should be aimed at minimising perinatal morbidity.

Learning objectives:
• To understand the causes and risk factors for maternal non-anti-D antibodies.
• To learn when to initiate invasive testing in the antenatal period.
• To know when to deliver the baby to maximise perinatal outcome.
• To learn about which antibodies can cause fetal hydrops and intrauterine haemolysis.

Ethical issues:
• When is it necessary to perform paternal blood tests to determine red cell antibody status?
• When should the mother be delivered in cases where the red cell antibody detected has a weak association with neonatal haemolysis?

Keywords: amniocentesis / fetal blood sampling / fetal genotype / intrauterine infusion / middle cerebral artery peak systolic velocity (MCA-PSV)

Please cite this article as: Gajjar K, Spencer C. Diagnosis and management of non-anti-D red cell antibodies in pregnancy. The Obstetrician & Gynaecologist 2009;11:89–95.

Author details
Ketan Gajjar MRCOG
Specialist Registrar
Department of Obstetrics and Gynaecology, St. John's Hospital, Chelmsford, Essex, UK
Email: gajjarkb@hotmail.com (corresponding author)

Chris Spencer MRCOG
Consultant Obstetrician/Gynaecologist
Department of Obstetrics and Gynaecology, St. John's Hospital, Chelmsford, Essex, UK
Introduction
Red blood cell alloimmunisation is a recognised cause of haemolytic disease of the fetus and the newborn (HDFN). It occurs when a mother’s immune system is sensitised to foreign red cell surface antigens, stimulating the production of IgG antibodies. These antibodies can cross the placenta and if the fetus is positive for the surface antigen this results in haemolysis of fetal red cells and subsequent anaemia.

The International Society of Blood Transfusion now recognises 302 blood group antigens, most of which belong to one of 29 genetically discrete blood group systems. Antibodies to many of these 302 antigens have the potential to cause HDFN and they are, therefore, clinically significant.

The three most common alloantibodies that cause significant HDFN are anti-D, anti-c and anti-Kell. Of lesser importance, but still with the potential to cause HDFN, are anti-e, -Ce, -Fya, -Jka and -Cw. Anti-Lea, -Leb, -LuA, -P, -N, -Xga and high-titre low-avidity antibodies, such as anti-Kna, are not associated with HDFN. Anti-C, -E and -G have all caused HDFN, but this is rare and the outcome is seldom severe. Box 1 gives a summary of non-anti-D antibodies and their clinical significance.

The introduction of anti-D prophylaxis has greatly reduced the frequency of HDFN due to anti-D; this antibody still remains the most important cause of HDFN. The number of irregular antibodies reported during pregnancy has, however, increased, in part because of greater use of blood transfusion in the obstetric population. The prevalence of HDFN is now approximately 1 in 21 000 births; in England and Wales, approximately 500 fetuses develop HDFN annually.

In the West, the incidence of irregular antibodies is reported to be about 1–2% in pregnant women. This figure is reduced to about 0.09–0.24% when only those that are clinically significant are taken into consideration. In a recent population study conducted in the Netherlands, the prevalence of positive antibody screens was 1.80, with a 1:300 prevalence of clinically relevant alloantibodies other than anti-D. Overall, severe HDFN (7–8 cases per 100 000 pregnancies) requiring intrauterine or postnatal (exchange) transfusions occurred in 3.7% of fetuses at risk; this was 11.6% for anti-K, 8.5% for anti-c and 1.1% for anti-E.

Pathophysiology
It was previously thought that the pathogenesis of HDFN was similar for all antibodies; however, the anti-Kell antibody is an exception. Although HDFN due to anti-Kell is characterised by fetal anaemia, amniotic fluid and/or cord bilirubin levels are not generally reported to be elevated. In addition, maternal antibody levels and amniotic fluid spectrophotometric estimation (\(\Delta OD_{450}\)) do not correlate well with fetal anaemia. Anti-Kell antibodies appear to cause significant suppression of erythropoiesis by inhibition of Kell positive erythroid early progenitor cells rather than by red cell destruction and, unlike rhesus disease, outcome is not affected by previous obstetric history. The routes of maternal sensitisation are via blood transfusion or fetomaternal haemorrhage (i.e. transplacental passage of fetal erythrocytes) associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy or invasive obstetric procedures. These antibodies can cross the placenta during pregnancy in alloimmunised women and, if the fetus is positive for the erythrocyte surface antigens, this can result in haemolysis of fetal erythrocytes and subsequent anaemia.

In England and Wales, about 500 fetuses develop haemolytic disease annually and they require close monitoring. Nevertheless, each year about 25–30 babies die from HDFN. In addition, it is believed that fetal loss due to haemolytic disease before 28 weeks of gestation accounts for about 20 spontaneous abortions each year. As the majority of these alloimmunisations in the non-anti-D group occur following unmatched blood transfusions, it has been suggested that prevention is possible by routinely screening and matching donor blood for Rhc and Kell type for subsequent use in women of childbearing age or younger. Furthermore, because prophylaxis is not available for atypical antibodies, alloimmunisation during pregnancy will continue to occur.

Diagnosis of non-anti-D antibodies

Maternal history
A history of a previously affected pregnancy or blood transfusion is not always accurate in predicting the severity of fetal disease and should not be relied upon; however, it may help in risk assessment. It is recommended that women who

---

Box 1

<table>
<thead>
<tr>
<th>Common</th>
<th>Anti-Kell, -c, -E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncommon</td>
<td>Anti-e, -C, -Ce, -Cw, -Kpa</td>
</tr>
<tr>
<td></td>
<td>-Kpb, -k, -Jka, -Wha, -Fya, -G, -ce</td>
</tr>
<tr>
<td></td>
<td>-Ew, -H, -Gz, -Be, -Evans, -Tar</td>
</tr>
<tr>
<td></td>
<td>-Sec, -JAL, -Jsa, -Jsib, -Ul, -Ku</td>
</tr>
<tr>
<td></td>
<td>-K22, -Vel</td>
</tr>
<tr>
<td>Rare</td>
<td>Anti-M, -Jba, -Ge3, -Vel, -JFP</td>
</tr>
<tr>
<td></td>
<td>-Kg, -JONES, -HJK, -REIT, -Aia</td>
</tr>
<tr>
<td></td>
<td>-MAM, -Lan, -Jra, -Emm, -AnWj</td>
</tr>
<tr>
<td></td>
<td>-FEL, -Fyb, -MINS, -Diego</td>
</tr>
<tr>
<td></td>
<td>-Gartch</td>
</tr>
<tr>
<td>No documented cases</td>
<td>Anti-P1PPK, -Lua, -Lub, -Yla, -Xg</td>
</tr>
<tr>
<td></td>
<td>-Sciana, -Don, -Dob, -Cob, -LW</td>
</tr>
<tr>
<td></td>
<td>-Lutheran, -ChRg. -Cromet</td>
</tr>
<tr>
<td></td>
<td>-Knoops-COST, -Indian, -Ok</td>
</tr>
<tr>
<td></td>
<td>-JMH, -I, -GIL, -Er, -LKE, -Lewis</td>
</tr>
</tbody>
</table>
have previously had an infant affected by HDFN should be referred before 20 weeks of gestation to a specialist unit for advice and for assessment of fetal haemolysis, irrespective of antibody level.¹²

### Antibody screening

The current antibody screening protocol is depicted in Table 1. All pregnant women have samples taken early in pregnancy, ideally at 8–12 weeks of gestation, to screen for the presence of red cell antibodies, along with ABO and D typing. If the antibody screen is positive, further testing of maternal blood is done to determine the antibody specificity, concentration, origin and level of antibody or antibodies and the likelihood of HDFN. This may mean sending the blood samples to a reference laboratory.

The clinician responsible for the woman’s antenatal care must be informed of the likely significance with respect to both the development of HDFN and potential maternal transfusion problems. The management of pregnancies in which red cell antibodies are detected varies depending upon the clinical significance and titre of the antibody. A referral to a tertiary fetal medicine unit will, in certain cases, be necessary.

Irrespective of the mother being RhD positive or negative, a further blood sample is taken at 28 weeks of gestation to recheck the ABO and D group and to screen further for red cell antibodies.¹³ RhD positive women are just as likely as RhD negative women to form antibodies other than anti-D late in pregnancy.¹⁴

### Paternal antigen status

A fetus can only be affected by maternal red cell alloantibodies when its red cells express the antigen. When it is certain that the father of the fetus is negative for the antigen, the fetus must be negative and, therefore, not at risk. The complexities of paternal testing and the potential for misidentification of the father need to be acknowledged.¹⁵ If the father of the fetus is positive, the paternal genotype should be analysed for homozygosity or heterozygosity. If homozygous, most babies are affected and thus they are potentially at risk of developing HDFN. Fifty percent of babies are at risk of developing HDFN when the father is heterozygous.

### Anti-Kell and other Kell system antibodies

The Kell red cell antigen system includes 24 different members and at least eight different antigens have been associated with HDFN. The most common of these is Kell (K or KEL 1) and cellulano (k or KEL 2). The incidence of anti-Kell immunisation is low in the obstetric population, at 0.1–0.2%.¹⁶ The Kell antigen is present on the red cells of 9% of white and 2% of black people, with antigen positive individuals being heterozygous in 97.8% of white people and 100% of black people. Consequently, proportionately fewer Kell negative women are likely to carry a Kell positive fetus than RhD negative women are to carry an RhD positive fetus.¹⁷

Most anti-Kell antibodies appear to be induced by unmatched blood transfusions and it is becoming common practice for girls and women of childbearing age to be transfused only with Kell-negative red cells, although anti-Kell antibodies stimulated by transfusion seem to cause a less severe disease than those stimulated by previous pregnancy.¹⁸ Transplacental haemorrhage, however, may also be responsible for Kell alloimmunisation when there is no history of blood transfusion. It is important to note that a previous obstetric history of Kell alloimmunisation does not reliably predict outcome in subsequent pregnancies, unlike the situation with anti-D antibodies.

When a positive antibody screen for Kell is found, a baseline titre should be obtained. This should be followed by determination of paternal Kell antigen status by peripheral blood assay, although in more than 80% of cases the partner is Kell negative. If the father is Kell negative and a confidential enquiry establishes paternity, no further samples are required until 28 weeks, when the antibody should be titrated and further antibodies excluded. If the father is homozygous, the fetus will carry the Kell antigen and be at risk. Fifty percent of fetuses of heterozygous fathers can carry the Kell antigen on their red cells. In these situations, the mother should be referred to a specialist unit and titration of samples should be performed at monthly intervals until 28 weeks and at fortnightly intervals thereafter. Whilst it has been stated that the severity of HDFN due to anti-Kell is not associated with antibody titres, several reports¹⁹ of significantly affected pregnancies have correlated with antibody titres of at least 1 in 32. When the critical titre is reached, it is necessary to establish the degree and severity of fetal anaemia. Amniocentesis for spectral analysis of amniotic fluid is not reliable and cordocentesis is associated with significant risks to the fetus. The controversy about invasive testing and which test to use has now been resolved by the use of Doppler assessment of the fetal middle
In addition to anti-Kell, as described above, it is possible that antibodies of all Kell-system specificities have the potential to cause HDFN. This is usually, but not invariably, mild. Haemolytic disease of the fetus and the newborn requiring at least fetal transfusion has been reported for anti-k, -Kpa, -Kpb, -Jsa, -Jsb, -Ula, -Ku and -K22.1

Anti-c antibodies
The anti-c antibody belongs to the rhesus blood group system and all antibodies to rhesus system antigens should be considered capable of causing severe HDFN. In a series of studies20,21 14–21% of c-positive babies born to mothers with anti-c required exchange transfusion. Women with anti-c should be retested at monthly intervals up to 28 weeks of gestation and then every 2 weeks. Samples from women with anti-c should be quantified and any additional antibodies identified. Paternal testing should be done to help determine the presence or absence of the c-antigen and to plan the frequency of maternal antibody testing. Approximately 80% of males are positive for the c-antigen, with 60% being heterozygous. If the father is antigen negative, the fetus will be c-antigen negative and unaffected by anti-c antibodies. If he is homozygous, the fetus will be affected, but if he is heterozygous, the fetus has a 50% chance of being affected. The clinical management of affected pregnancies is similar to that of any pregnancy where there are clinically significant antibodies, although it is important to note that anti-c may cause delayed anaemia in the neonate. Consequently, the neonatal team should be informed of mothers with anti-c antibodies before delivery.

Table 2 summarises the management of raised anti-c levels.21,22

<table>
<thead>
<tr>
<th>Anti-c level</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7.5 iu/ml</td>
<td>Continue to monitor</td>
</tr>
<tr>
<td>7.5–20 iu/ml or the ≥1:32</td>
<td>Risk of moderate HDFN – refer to specialist unit</td>
</tr>
<tr>
<td>&gt;20 iu/ml</td>
<td>Risk of severe HDFN – refer to specialist unit</td>
</tr>
</tbody>
</table>

Anti-E antibodies
The E antigen belongs to one of the 49 antigens of the rhesus system and is capable of causing HDFN. Approximately 40% of serum samples with anti-c also contain anti-E.23 Anti-E can occur either as a natural IgM antibody without immune stimulation or as an IgG antibody in women with a history of blood transfusion or previous pregnancy. A survey of anti-E in pregnancy in the UK demonstrated that HDFN was mild in 77% of cases (48/62), moderate in 13% of cases (8/62) and severe (6/62) in 10% of cases.24 A larger study25 reviewed 283 pregnancies affected by anti-E antibodies and concluded that an antibody titre ≥1:32 is critical and should prompt further fetal evaluation with Doppler assessment of the fetal middle cerebral artery.

Fya and Fyb (Duffy) antibodies
The Duffy antigen system consists of two antigens: Fya and Fyb. In a study26 of 68 pregnancies in which anti-Fya was detected, 10 babies had mild disease and 3 babies were severely affected, with 2 requiring intrauterine transfusion. Overall, antibody titre levels did not correlate well with fetal outcome. Only one case of HDFN due to anti-Fyb has been reported.1

Jkb (Kidd) antibodies
The Kidd antigen system consists of two antigens: Jka and Jkb. Anti-Jka has been reported to be a cause of mild haemolytic disease of the newborn but there is one report of severe HDFN caused by anti-Jka.1 There is a recent case report27 of a newborn who presented with severe HDFN associated with anti-Jkb, which resulted in neonatal death.

ABO antibodies
ABO antibodies rarely cause HDFN and when they do it is generally mild, although minor symptoms involving a small degree of red cell destruction may be relatively common. Haemolytic disease of the fetus and newborn caused by ABO is restricted almost exclusively to the fetuses of group O mothers and it is thought that the IgG antibodies anti-A and anti-B are responsible.1

Other red cell antibodies
Many other atypical antibodies apart from those mentioned above have been reported as the cause of HDFN; a summary is given in Box 1. In all these cases, retesting at 28 weeks generally provides sufficient information to determine management of the pregnancy. A medical decision should be made regarding the more frequent testing of women with a previous history of children with HDFN. In general, a titre of ≥1:32 is likely to cause HDFN, although a clear-cut association between titre and HDFN has not been established. The presence of any further antibodies should be determined and any clinically significant antibodies titrated as above.

Determination of fetal genotype
When a clinically significant antibody of high concentration is present and/or the woman has a
history of HDFN and the father is heterozygous for the relevant antigen, it may be clinically relevant to determine the genotype of the fetus. The main purpose is to identify women with antigen positive fetuses so that they can be informed and prepared for further monitoring during their pregnancy. It will also identify those pregnant women who have antigen negative fetuses and who are not at risk of developing HDFN, so that further invasive procedures can be avoided.

Fetal DNA for genotyping by PCR assay can be obtained by amniocentesis or chorionic villus sampling. These invasive techniques carry a risk of fetal loss and may boost maternal antibody levels. Consequently, methods of using maternal plasma to identify the blood group of the fetus have been evaluated and this technique has been validated for RhD, Rhc, RhE and Kell typing. In the first trimester of pregnancy, the amount of fetal DNA in maternal plasma may be too low to detect but as pregnancy progresses these levels increase. It is thus advisable that the test is used only for pregnancies that have progressed beyond 16 weeks for RhD, Rhc and RhE and 20 weeks for the Kell antigen. The accuracy of fetal RhC/c and RhE/e genotype estimations from maternal plasma is thought to be between 96–98%, which compares well with the reported accuracy of fetal RhD (95%). Use of free fetal DNA seems to be superior to fetal DNA extracted from fetal nucleated red cells found in maternal blood. Cell-free DNA isolated from maternal plasma contains approximately 3–6% fetal DNA, the rest being maternal in origin. The benefits of this technology are that even if a significant rise in non-anti-D antibodies is found in pregnancy and the fetal genotype is negative for the antigen isolated, no further surveillance is necessary. In women whose fetus is positive for the antigen, monitoring for haemolysis in the fetus will be required at regular intervals throughout the rest of the pregnancy. Whether the finding of fetal cells from a previous pregnancy is a potential problem in fetal genotyping remains to be seen but could possibly be minimised by analysing paternal and sibling serum samples.

The role of MCA-PSV Doppler assessment
The anaemic fetus attempts to enhance oxygenation by increasing cardiac output, thus increasing the velocity of blood flow. Anaemia also results in decreased blood viscosity, which, in turn, results in increased velocity of blood flow. Middle cerebral artery peak systolic velocity >1.5 multiples of the median for the specific gestation is predictive of moderate or severe fetal anaemia, with 100% sensitivity and a false positive rate of 12%. Pregnancies at risk should be monitored on a weekly basis. Reliable MCA-PSV values can be obtained as early as 18 weeks of gestation but care should be taken after 35 weeks, after which time the false positive rate increases and consideration should be given to converting to amniocentesis for surveillance or delivery of the baby. Using the correct technique is a critical factor when determining MCA-PSV with Doppler ultrasonography. Consequently, this procedure should only be performed by those with adequate training and experience.

Invasive monitoring

Amniocentesis
Amniotic fluid containing high levels of bilirubin, such as that found in fetuses with severe haemolytic disease, is yellowish-brown. Analysis allows detection of the presence and severity of fetal haemolysis and anaemia. Using spectral analysis of amniotic fluid at a wavelength of 450 nm at a given gestation, the amount of bilirubin present in affected pregnancies can be compared with unaffected pregnancies. Fetal status can be determined by plotting the Δ OD450 measurement on either a Liley graph in the late second or third trimesters or on a Queenan curve for earlier gestations (19–25 weeks). Fetal blood sampling should be considered when Δ OD450 readings reach Liley’s zone 3, or if they are rising in the upper third of zone 2. Serial amniocentesis is often necessary every 1–4 weeks.

There are two major concerns with this investigation. One is the accuracy of detecting fetal anaemia before 27 weeks in cases of anti-Kell alloimmunisation, where the amniotic fluid bilirubin levels can be normal. The second is the risks associated with this invasive procedure, which include preterm rupture of membranes, preterm labour, infection, haemorrhage, fetal death and a rise in antibody levels caused by fetomaternal haemorrhage. Fetal loss rates relating to amniocentesis are reported to be 0.25–1% per procedure. Fetomaternal haemorrhage, which can significantly increase the severity of the disease, can occur in 2.3–17% of procedures. A recent randomised trial established the measurement of MCA-PSV as being superior to amniocentesis in predicting fetal anaemia and, thus, in current practice, amniocentesis is rarely performed for the assessment of fetal anaemia.

Fetal blood sampling and intrauterine transfusion
These are indicated if monitoring of the middle cerebral artery indicates fetal anaemia or if Δ OD450 readings reach Liley’s zone 3 or they are rising in the upper third of zone 2. Fetal blood sampling helps provide direct and accurate diagnosis of anaemia and fetal acidosis, especially in cases of anti-Kell alloimmunisation and before 26–28 weeks of gestation, when Δ OD450 values of amniotic fluid are unreliable. An additional
advantage of fetal blood sampling is that, by providing direct access to the fetal circulation, the same procedure can be used to transfuse the fetus.

The fetal loss rate is dependent on gestational age, site of sampling and underlying pathology. The risk is 1–3% in uncomplicated cases, but if the fetus is affected by hydrops, the fetal loss rate can be as high as 20%.³⁷

Fetal blood can be taken from either the placental cord insertion site or the intrahepatic vein. Complications of fetal blood sampling include: fetal bradycardia; haemorrhage; cord haematoma and tamponade; and fetal death.

Maternal and cord blood tests at the time of delivery

A direct agglutinin test (DAT) should be done on the cord sample whenever maternal serum has been found to contain red cell antibodies. A positive DAT is not in itself diagnostic of HDFN. If it is positive, however, the infant’s haemoglobin and bilirubin levels should be checked to diagnose or exclude HDFN. In situations when the DAT is positive and the infant shows signs of HDFN, further tests are recommended to confirm the red cell antibody specificity.

Timing of delivery

Based on the analysis by Klumper et al.,³⁸ it appears beneficial to perform the final intrauterine transfusion at up to 35 weeks of gestation, with delivery anticipated at 37–38 weeks. Prior intrauterine transfusion is not an indication for an elective caesarean section in a non-anaemic transfused fetus. With careful monitoring and appropriate timing of transfusions, vaginal delivery should be considered at 37–38 weeks of gestation. If complications occur during intrauterine transfusion after viability is achieved, immediate delivery should be performed. As preterm delivery is a possibility with fetal transfusions, antenatal steroids should be administered to the mother once viability has been achieved. At the delivery,
cord blood should be collected for analyses of haemoglobin, packed cell volume and bilirubin and for a direct antiglobulin test.

Conclusion

Non-anti-D red cell antibodies associated with HDFN are rare but, partly because of the introduction of anti-D prophylaxis, have been increasing in frequency. The main non-anti-D antibodies that are likely to cause fetal anaemia are anti-c, anti-K and anti-E. Furthermore, it is important to understand the unpredictable nature of Kell alloimmunisation and its subsequent effects on the fetus. Figure 1 provides a clinical algorithm for the management of non-anti-D antibodies from booking until delivery and Figure 2 provides a clinical algorithm for the management of significantly raised titres of non-anti-D antibodies in pregnancy.

Increased use and availability of noninvasive approaches, such as testing of fetal DNA in maternal plasma and MCA-PSV Doppler assessment, should reduce the need for invasive procedures (Figure 2). MCA-PSV Doppler assessment has now almost completely replaced amnioncensis in the prediction of the anaemic fetus in alloimmunisation and is the primary surveillance test for these at-risk pregnancies.

References