Fetal anaemia

Alec McEwan

Abstract
Fetal anaemia is a rare condition which can result from a failure of production of fetal red blood cells, accelerated haemolysis, or fetal bleeding. The three most common causes are parvovirus B19 infection, red cell isoimmunisation and fetomaternal haemorrhage. All obstetricians are likely to encounter these clinical scenarios at some point, even if management is predominantly by fetal medicine specialists. All three topics have been covered in previous articles of this journal, but here are presented three real cases illustrating these causes of fetal anaemia, and emphasising again the key points.

Keywords anti-D antibodies; fetal anaemia; fetomaternal haemorrhage; hydrops; Kleihauer; middle cerebral artery peak systolic velocity; parvovirus B19; red cell isoimmunisation

Introduction
Fetal anaemia is a relatively rare occurrence, usually managed by fetal medicine specialists and neonatologists. Red cell isoimmunisation is one of the most common causes, and this topic was covered in detail in Volume 20 of this journal (issue 2). This article describes three case histories which illustrate the causes and presentations of fetal anaemia and emphasises the need for all obstetricians to have knowledge of this uncommon condition.

Anaemia develops when red cell production is inadequate, or when breakdown of erythrocytes is accelerated, or when red cells are lost through bleeding. All three mechanisms can also be seen to cause anaemia in the fetus (Table 1).

Diagnosing anaemia in a child or adult is simple to do by measuring haemoglobin values on venous blood. Red cell size and haemoglobin concentration, and a blood film, go a long way towards isolating a cause when combined with the full clinical picture. Fetal blood sampling is possible, but very much more technically challenging and hazardous, requiring specialist skills and usually only when the development of the fetal anaemia has occurred over a prolonged time frame i.e. not with acute fetal bleeding.

The breakthrough with screening for fetal anaemia came with the development of Doppler sonography. Blood in an anaemic fetus is less viscous and the velocity of blood flow in certain fetal vessels can be measured and be seen to be elevated above the normal range. Cardiac output may also be elevated somewhat in these fetuses, further contributing (although to a much lesser extent) to the increase in peak systolic blood flow velocities. A group led by Mari are usually credited for bringing the use of middle cerebral artery peak systolic velocity (MCA PSV) measurements into widespread mainstream practice for the non-invasive assessment of fetal anaemia. The middle cerebral artery is usually readily accessible for Doppler measurements, and the use of angle correction means that absolute velocities can be recorded (unlike when assessing a growth restricted fetus when pulsatility index i.e. a ratio, is used). The fetus must be quiescent, and a few measurements are usually taken. The Doppler gate should be placed at the proximal part of the near field MCA, just as it emerges from the Circle of Willis. The value is plotted on a chart, and significant anaemia is highly unlikely with values which lie below 1.5 multiples of the median for the gestation in question. As values exceed this threshold, the likelihood of significant fetal anaemia increases.

It must be recognised that the use of MCA PSVs is only a screening test for fetal anaemia, and there is a risk of both false positive and negative results. The overall accuracy of this test for predicting moderate and severe fetal anaemia has been quoted as 85%, which is 9% better than the use of serial amniocentesis and ΔOD450 estimations and also clearly avoids the risk and unpleasantness of multiple needle insertions. Furthermore, it is useful for detecting fetal anaemia from any cause, not just those causing haemolysis. However, the false positive rate is 12% and, although less common, false negatives do also occur. Nevertheless, it is now considered the gold standard for screening for fetal anaemia.

Table 1

<table>
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<th>Causes of fetal anaemia</th>
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<tr>
<td>Failure of red cell production</td>
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In some circumstances, the fetal anaemia may only be recognised after birth. However, there is treatment available for prenatally diagnosed fetal anaemia distant from term. The first ever fetal intrauterine transfusions (IUTs) were performed into

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the fetal peritoneal cavity, from where, incredibly, the red cells were absorbed across the bowel wall to reach the fetal circulation. Alternatively, intracardiac transfusions were performed. With very significant improvements in real-time ultrasound scanning, these routes are only utilised now in severe cases at extremely preterm gestations. More usually, at the time of fetal blood sampling from the umbilical vein, blood is transfused directly into the intravascular space, the volume determined by fetal size and haemoglobin. The quoted risk of complications (2%) increases to 5–20% in an hydropic fetus.

Case 1

A woman in her first pregnancy, with a previously straightforward antenatal course, presented at 38 weeks gestation with a three-day history of reduced fetal movements. She had experienced no pain or vaginal bleeding, and her BP was normal and urine clear. Her uterus was soft and non tender on examination. A CTG was performed (Figure 1) and the registrar raised the possibility that the trace was sinusoidal and performed a vaginal examination with ARM. The patient was 3 cm dilated and the liquor was clear. Fifteen minutes later the decision was made to perform an emergency caesarean section. The baby was born 35 min later and was noted to be pale and floppy at delivery but required minimal resuscitation and was given Apgar scores of 8 at 1 min, 8 at 5 min and 9 at 10 min. The venous cord pH was 7.27 (BE -6.4) and the arterial pH 7.19 (BE -8.0). A review of the baby at 1 h of life was reassuring. However, 30 min later the baby was admitted to the neonatal unit pale and floppy and went on to develop seizures and required ventilation for 5 days. The haemoglobin on admission to the NNU was found to be 4.9 g/dl and a blood transfusion was given. A direct Coombs test was negative, however a maternal Kleihauer test was found to be strongly positive. A subsequent newborn MRI showed widespread ischaemic changes in the cortical deep and periventricular white matter, and the thalamocapsular region. These changes were indicative of an injury occurring a few days before birth. Limb stiffness was detectable by discharge and the parents were warned of a high chance of their child developing cerebral palsy. The obstetric and neonatal team concluded that the fetus had suffered a massive fetomaternal haemorrhage (FMH) at some point at least three days prior to delivery and that this probably occurred over a relatively short duration.

Fetomaternal haemorrhage (FMH)

FMH can be defined as the passage of fetal red blood cells across the trophoblast layer and into the maternal circulation, but also includes the movement of maternal erythrocytes in the opposite direction. Loss of fetal red cells into the maternal circulation occurs in most pregnancies but the volume of blood in the majority of cases is less than 0.025 ml. In less than 1% of pregnancies is the volume 15 ml or more. Massive FMH has been variably defined as a loss of >80 ml or >150 ml, and this occurs in approximately 1 in 1000 and 1 in 5000 births respectively.

There are two well-established methods of measuring the size of a fetomaternal bleed. The Kleihauer–Betke screen relies on the fact that adult haemoglobin can be eluted from erythrocytes by acid, whereas fetal Hb is resistant to this. A maternal blood smear can be treated with acid and then stained with erythrosin B. Maternal erythrocytes appear as ‘ghosts’ on microscopy, whereas the fetal red blood cells are stained cherry red. The fetal Hb containing cells can be counted manually, and a volume calculated using a simple formula. This test is labour intensive and very imprecise, but nevertheless widely available. Flow cytometry is the second method. Fluorescently labelled monoclonal antibodies against HbF are mixed with the maternal blood sample and fluorescent cells (those containing HbF) are sorted and counted separately. This test is fast, and more accurate, but is not available universally, and often not out-of-hours.
Both tests can overestimate the size of an FMH if the mother has high levels of persistent HbF herself, and can underestimate if ABO incompatibility or isoimmunisation against other red cell antigens means that fetal red blood cells are cleared very quickly from the circulation. Some fetal red blood cells may survive for a number of weeks in the maternal blood, so these tests do not allow estimates of the timing or duration of the FMH.

Even if the quantity of blood could be very accurately measured, the impact on the fetus would depend on the gestation and the time frame over which the bleeding occurred. The fetoplacental blood volume expands from approximately 30 ml at 20 weeks gestation to 80–90 ml/kg by term. Larger bleeds will be less well tolerated at earlier gestations. Slow haemorrhage over a number of weeks will be tolerated better than the same total volume of blood loss occurring over a few minutes, which is likely to be associated with fetal hypotension and acute acidosis. It is not uncommon for a fetus to drop its haemoglobin below 50 g/l in the setting of fetal haemolytic disease or parvovirus infection. However, these babies usually do very well in the long run, following transfusions. They develop their anaemia gradually, and there is no haemodynamic compromise associated with it, as opposed to the hypotension occurring with a sudden massive FMH.

The exact pathophysiology of FMH is not well understood, although there are a number of situations associated with it (see Table 2). The most common time for FMH is at delivery, but if the bleed occurs after the cord is clamped then there will be no consequence for the fetus/newborn. The estimates of the incidence of FMH judged by positive Kleihauer tests taken following delivery therefore grossly overestimate the incidence of fetomaternal bleeds which occur antenatally. Furthermore, it is not known if birth therefore grossly overestimate the incidence of fetomaternal dence of FMH judged by positive Kleihauer tests taken following delivery, but if the fetus is ‘topped-up’ with blood, there can be no reassurance that another sudden and possibly heavier bleed won’t occur. A number of small case series attest to the relative safety of transfusing the fetus in this situation, but an occasional stillbirth is to be expected because of the unpredictability of the bleeding. Women who are Rhesus D negative will of course need sufficient exogenous anti-D to protect themselves against isoimmunisation. Unfortunately, if the bleed has been occurring, or has occurred, a few days prior to presentation then the administration of the anti-D may be too late to prevent this happening. If an FMH has been treated then it would seem appropriate to deliver the baby after 34 weeks gestation has been reached.

Managing subsequent pregnancies is no less daunting. Although the recurrence risk might be expected to be low (and probably is) there are cases on record of repeated FMH causing problems in subsequent pregnancies. It is tempting to offer serial Kleihauer tests and MCA Dopplers to women who have experienced FMH in previous gestations, but there is no good evidence to support this, and the risk of false positive MCA peak systolic velocities is a real one. Despite this, most women will feel the need for some form of surveillance and indeed we have seen in our department a woman who had a stillbirth with her first pregnancy, presumed secondary to a large FMH, who went on to have repeated silent bleeds in her subsequent pregnancy, detected by serial Kleihauer’s, requiring two intrauterine transfusions followed by elective preterm delivery.

Case 2

A 39 year old RhD negative woman presented at 37 weeks in her first pregnancy with a three day history of reduced fetal movements. Ultrasound scanning confirmed intrauterine fetal death. A Kleihauer was performed, along with many other investigations, and this showed a massive fetal maternal haemorrhage of 105 ml. A similar volume was estimated using flow cytometry. A silent FMH was felt to be the cause of the fetal death and her husband was found to be homozygous RhD positive. 7500 IU of Anti-D were given by slow intravenous injection, in an attempt to prevent isoimmunisation, however tests at 4 and 6 months postpartum showed maternal Anti-D levels of 22 and 9 IU/ml, confirming isoimmunisation against RhD. She was counselled that any future pregnancies with her husband would be complicated by haemolytic disease of the fetus and newborn, but reassured that first affected pregnancies were usually only mildly affected, and that treatments were available.

A year later, she conceived again. Figure 2a shows the anti-D levels over the course of the earlier part of the pregnancy. Regular MCA PSV Dopplers were performed and these remained reassuring until 28 weeks gestation at which point they became severely abnormal (Figure 2b). This was associated with a marked increase in the level of Anti-D antibodies. These changes

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### Table 2

<table>
<thead>
<tr>
<th>Fetomaternal Haemorrhage (FMH) and its associations</th>
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<tbody>
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<td><strong>Causes of FMH</strong></td>
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<tr>
<td>Delivery</td>
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<td>Abruption</td>
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<td>Decreased or absent fetal movements</td>
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<tr>
<td>Stillbirth</td>
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<tr>
<td>Hydrops fetalis</td>
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<tr>
<td>Caesarean section</td>
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<td>Non reassuring CTG</td>
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<td>Fetal growth restriction</td>
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<td>Neurological injury eg cerebral palsy</td>
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**Consequences of FMH**

- Delivery
- Neonatal anaemia
- Increased or absent fetal movements
- Stillbirth
- Hydrops fetalis
- Caesarean section
- Non reassuring CTG
- Sinusoidal CTG
- Fetal growth restriction
- Neurological injury eg cerebral palsy

Table 2
were detected on a Friday, and the plan made for fetal blood sampling and intrauterine transfusion on the following Monday. However, over the weekend she complained of reduced fetal movements and the CTG became abnormal (Figure 2c). An emergency IUT was performed on the Sunday, which brought the fetal Hb from 3.0 to 11.2 g/dl. Of note, there were no hydropic changes on ultrasound scanning. Ten days later, the second IUT was performed. The fetal Hb had slipped back down to 6.9 g/dl and was elevated to 14.8 g/dl by this second transfusion. A third transfusion was performed three weeks later, when the Hb rose from 13.2 to 17.0 g/dl. The Anti-D levels continued to rise throughout the pregnancy, reaching 1000 lu/ml by the end. Steroids were administered and the baby was delivered at 35 weeks gestation in good condition, with a normal cord Hb. Phototherapy was given, and immunoglobulin, to the neonate, however exchange transfusion was not required. Following discharge, the baby became very anaemic again (due to persistent haemolyzing maternal red cell antibodies and suppressed
neonatal bone marrow) and three top up blood transfusions were needed at 5, 10 and 15 weeks of age, along with erythropoietin injections.

Two years later, she presented with her third pregnancy. She had been counselled that any future pregnancies would be complicated by earlier and more severe fetal haemolytic disease, and that intrauterine transfusions at less than 24 weeks gestation were very likely, but also more hazardous. Free fetal DNA studies were performed to confirm that the fetus was in fact RhD positive (as would be expected from the paternal blood group). Thereafter, she received weekly injections of intravenous immunoglobulin (IVIG) from 14 weeks in an effort to ameliorate the haemolytic process in the fetus. Maternal Anti-D levels were relatively slow to rise, and the MCA PSV remained normal until 28 weeks, at which time successful IUTs began, resulting in the birth of a healthy baby at 36 weeks gestation. There was general agreement that the maternal IVIG therapy had reduced the severity of the disease process from what would have been expected.

Rhesus D isoimmunisation

Red cell alloimmunisation has been covered fully in this journal previously, and the intention of this article is only to illustrate key points with real cases. Although Rhesus D isoimmunisation is the focus in this case, fetal anaemia can also be caused by maternal antibodies generated against the c, E, Kell and Duffy antigens.

The woman in case 2 isoimmunised following a large silent fetomaternal haemorrhage, despite the use of large doses of IV Anti-D. She then developed a very aggressive immune response, generating large amounts of intensely haemolytic endogenous Anti-D. Her second pregnancy shows how a fetus can appear normal on scan (i.e. no hydrops) even when the fetal Hb is very low, and also that CTG abnormalities associated with severe fetal anaemia may not be the classic ‘sinusoidal’ pattern. Furthermore, this case emphasises the need for very close surveillance because changes to the MCA Doppler PSV may only occur suddenly and only with severe anaemia.

In Rhesus D haemolytic disease, haemoglobin values decline between transfusions, on average, by 0.4 g/dl per day, but this is quite variable. Subsequent IUTs are normally scheduled for approximately two week intervals, but this will depend on the Hb level following the previous transfusion and the rate of Hb decline does seem to slow down somewhat after the second IUT, probably because the transfused red cells are RhD negative and not at risk from the Anti-D antibodies. Although MCA Dopplers can be used to guide subsequent transfusions, they are said to become less reliable after the second IUT, and also after 35 weeks gestation. Somewhat paradoxically, babies born from severely isoimmunised pregnancies, where 3 or more IUTs have been performed, are less likely to become seriously jaundiced and require exchange transfusion. This is because their blood is almost exclusively RhD negative transfused blood by the time of delivery, and these cells are not at risk of rapid haemolysis from the maternal Anti-D which is only slowly cleared from the newborn circulation over the first three months post delivery. However, their own bone marrow erythropoiesis may have been suppressed and emerging RhD positive red cells are still at risk of chronic haemolysis whilst the maternal antibodies clear. Top up blood transfusions are often required a few weeks after birth.

It is usually the case that subsequent pregnancies with a RhD positive fetus will be affected from an earlier gestation, and to a greater degree. The immune response becomes more aggressive with subsequent exposures to the RhD antigen. Intrauterine transfusions are technically more challenging at earlier gestations. For some time, it has been known that administration of IVIG to newborn infants with ongoing haemolysis from maternal red cell antibodies become less jaundiced and are less likely to need exchange transfusion. The exact mechanism is unclear, but non-specific binding of the IVIG may ameliorate the process of immune haemolysis. There is no high quality evidence supporting the use of IVIG during pregnancy, but case reports and small case series of severely isoimmunised women suggest that it is a beneficial thing to do. In the case presented here, it is reasonable to expect that the first IUT would have been needed significantly earlier than 28 weeks gestation had the IVIG not been given. However, it is an expensive product, and not without risk, and it should only be considered in women with a severe history who have been fully counselled by a haematologist.

Case 3

A 33 year old woman with a significantly raised BMI presented in her first pregnancy at 18 weeks saying that she had had multiple exposures to parvovirus infection. She worked with children, and there had been a recent outbreak of ‘slapped cheek’ at the nursery where she worked. Serological testing showed IgM antibodies to parvovirus B19 and she was subsequently referred to a fetal medicine centre when the middle cerebral artery peak systolic velocities reached 1.5 multiples of the median.

Ultrasound scanning showed a lateral placenta with a more posterior cord insertion. Repeat MCA Dopplers confirmed values just above the threshold for intervention with fetal blood sampling. However, the fetus was active and there were no signs of hydrops. A fetal blood sampling was thought to be technically very difficult due to the relatively early gestation and maternal body habitus. The MCA Doppler was repeated four days later and was stable. It was unclear if fetal anaemia was resolving or developing, and a further scan was planned five days later (23 weeks gestation). At this point the MCA Doppler values fell mostly below 1.5 MoM and another scan was planned one week later. However, at this point, ultrasound scanning showed skin oedema, ascites (Figure 3a), a pericardial effusion and cardiomegaly (Figure 3b) and a very raised peak systolic velocity in the MCA (Figure 3c). The following day, a partial intrauterine transfusion was performed, elevating the fetal Hb from 1.8 to 9.8 g/dl. A further week later, the hydrops was still present, but the MCA PSV was normal. However, a second intrauterine transfusion was given at 25 weeks gestation, taking the fetal Hb from 7.7 to 13.6 g/dl. Thereafter, the MCA Dopplers remained normal and the hydrops resolved. Her care was returned to the referring unit, and she went on to have a term normal delivery of healthy baby boy, with a normal cord Hb value.

Parvovirus B19 infection

Parvovirus B19 is a DNA virus which most commonly causes a childhood coryzal illness known as erythema infectiosum, or
‘slapped cheek syndrome’ because of the characteristic facial rash it may cause. Infected adults are often asymptomatic, although an arthropathy has been reported. Serious illness is only found in immunocompromised patients, or those with rapid red cell turnover. The virus has a propensity to infect red cell precursors in the bone marrow, temporarily shutting down erythropoiesis. Individuals with haemolytic anaemia may suffer an ‘aplastic’ crisis during this time, and become much more anaemic than normal. Fetal red blood cells also have a shorter life span, and an infected fetus may develop severe anaemia. This may either resolve spontaneously, or lead to fetal hydrops and death. Direct cardiac and hepatic infection may also contribute, by causing cardiac failure secondary to myocarditis and hypoproteinaemia secondary to hepatitis.

50–60% of pregnant women are non-susceptible to infection, having been infected at some point in their life beforehand. They will show IgG antibodies against parvovirus on serological testing of a booking sample. Susceptible women (IgM and IgG negative) show a seroconversion rate of approximately 1% during their pregnancies, but this may be three fold higher during a parvovirus epidemic. There is a 50–90% risk of maternal infection if there is a household contact, and susceptible women who work with children have a 1 in 3 risk of infection if there is an outbreak.

The incubation period is 5–10 days, and the rash normally develops 16 days after exposure. The infected individual is most infectious in the 10 days prior to the rash appearing, and much less so thereafter. In a susceptible individual, serum IgM antibodies will be detectable within 10 days, and IgG antibodies shortly thereafter. If an exposed individual is found to be susceptible on serological testing they should have a blood sample repeated two weeks later to see if infection has actually occurred. IgM antibodies are usually a reliable sign of a recent infection, but can occasionally persist for many years. Testing the booking sample can be very helpful in determining if the IgM antibodies have arisen during the pregnancy, indicating a definite recent infection.

The risk of vertical transmission to the fetus is 15% at 5–16 weeks and 20–75% after that. The closer to term the pregnancy is the greater the risk of vertical transmission.

Parvovirus does not seem to have any obvious teratogenic effects but is said to cause spontaneous fetal loss in approximately 15% of cases before 20 weeks gestation and 1–2% after that. Hydrops and fetal anaemia is only evident in a small proportion of these fetal losses and it has been suggested that parvovirus is more often the cause of spontaneous abortion than is actually recognised. Following maternal seroconversion, most fetal medicine units recommend fetal surveillance for 10–12 weeks.
weeks with weekly to fortnightly MCA peak systolic velocities. If fetal anaemia does not develop during this time, the woman can be reassured and returned to normal care. The decision for intervention with fetal blood sampling is usually made on the MCA Dopplers exceeding the 1.5 MoM threshold. However, unlike fetal haemolytic disease caused by red cell alloimmunisation, the natural history of the anaemia in parvovirus infections is not necessarily progressive. Case 3 however illustrates well the potential hazards of a more conservative approach. The risk of complications with fetal blood sampling and intraterine transfusion is variably quoted at approximately 2%. However, the risks are significantly higher if the fetus is already hydropic and it is common practice to give a partial transfusion first, followed by a second one a week later if this is the case. The hydropic fetus is far less well equipped to cope with the volume load of a full transfusion. It is the case though that one or two transfusions will normally suffice. The bone marrow will recover spontaneously, and fetal red cell production does resume. The long term outcome for these children is extremely good, even those with severe fetal anaemia and hydrops, provided they survive the critical time in utero.

**Practice points**

- Fetal anaemia is rare but can be caused by failure of red cell production (e.g. parvovirus), accelerated red cell haemolysis (e.g. red cell isoimmunisation), or fetal bleeding (e.g. fetomaternal haemorrhage).
- Measuring the peak systolic velocity in the middle cerebral artery is a safe and sensitive way of detecting fetal anaemia, but there is a risk of false positive and negative results.
- Be aware that CTG changes and fetal hydrops only occur with severe fetal anaemia.
- The diagnosis can only be made with certainty by fetal blood sampling, which carries a 1—2% risk of complications.
- Consider FMH as a cause of sudden slowing or cessation of fetal movements, and investigate this possibility with a Kleihauer test, or MCA PSV.
- Once the maternal red cell antibody thresholds recommended by blood transfusion service have been reached, the care of an isoimmunised woman should be transferred to a fetal medicine specialist capable of IUTs.
- Immunoglobulin therapy is not strongly evidence based for use in ameliorating fetal haemolysis, but small case studies do lend support.
- Women exposed to parvovirus during pregnancy should have blood taken to see if they have pre-existing immunity. If they are non-immune, then they should avoid working with children if there is a parvovirus outbreak, and they should have repeated serological tests to detect if they have become infected.

**FURTHER READING**


