Refresher Course

Non-Invasive Assessment of Fetal Haemolytic Anaemia

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Immune hydrops, secondary to fetal anaemia is caused by the presence of circulating antibodies against fetal red blood cell antigens (red cell-alloimmunisation). These antibodies are transferred from maternal blood to the fetal circulation by active transportation across the placenta, adsorbed to the D-positive erythrocytes and exist unbound in fetal serum. They act as a haemolysin which leads to red cell destruction. If the process is mild, the baby may be normal at birth and not require treatment but if haemolysis is severe, the fetus may become hydropic and die in utero.[1] The incidence of severe haemolytic disease of the fetus and newborn (HDFN) caused by red cell-alloimmunisation has been dramatically reduced due to the widespread use of prophylactic anti-D immunoglobulin.[2,3]

In order to bring forward the diagnosis of fetal haemolytic anaemia, and thus the need for transfusion, the only technique available to assess the actual degree of fetal anaemia is cordocentesis in order to obtain a fetal blood sample and measure fetal haemoglobin.[4] When the fetal haemoglobin is within 2 g/dl of the mean for gestational age and the Coomb's test is positive, the fetus is only mildly affected and therefore not in need of intrauterine therapy or early delivery. Fetuses with a hemoglobin deficit > 2 g/dl require blood transfusions.

However, both cordocentesis and intravascular transfusion are associated with procedure-related perinatal morbidity and mortality.[5,6] Furthermore, these invasive techniques carry the risk of feto-maternal haemorrhage, thereby increasing maternal antibody levels.[7] To improve the outcome, the number of invasive techniques should be kept to a minimum and non-invasive assessment to predict the degree of fetal anaemia has played an important role. Expectant management is indicated only in the presence of stable serial maternal antibody titres, a falling delta OD 450, normal antepartum fetal surveillance and in the absence of sonographic signs of fetal hydrops.

Currently many ultrasonographic parameters such as fetal liver length,[8,9] fetal spleen perimeter,[10] and Doppler measurements of flow velocities in the fetal aorta[11,12] have been present to have efficient values in the management of the fetuses. Serial measurements of maternal serum red cell antibody levels have also been an important part of monitoring because of the well-established association between maternal antibody concentration and pregnancy outcome.[13] The aim of management is to reach a safe gestational age for the fetus to be delivered, in a non-hydropic state. Non-invasive assessment has been first performed in many centres in the UK.

Real Time Ultrasound Assessments

Ultrasound assessment of the at-risk fetus is important for detecting fetal hydrops. Weekly ultrasound is appropriate in high-risk cases. The sonographic features of hydrops fetalis include ascites, pericardial and pleural effusions, subcutaneous and scalp oedema, polyhydramnios and placentomegaly.[14] There is no standard definition of hydrops and classification in terms of mild, moderate and severe is subjective. The earliest indicators of hydrops include the first signs of fetal ascites, such as the "bowel halo" sign of free fluid within the

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peritoneal cavity or the ability to visualise both sides of the fetal bowel, and the presence of fluid within the pericardial space[15] (Figure 1).

Increasing amniotic fluid volume is often seen before the development of hydrops without any explanation of its pathophysiology. Sonographic measurement of parameters such as extrahepatic and intrahepatic umbilical vein diameter, placental thickness, abdominal circumference, intraperitoneal volume and the ratio between the head and abdominal circumference have been investigated as to their usefulness in diagnosing anaemia but have now been shown to be unreliable in predicting fetal haematocrit in the absence of hydrops.[16-18]

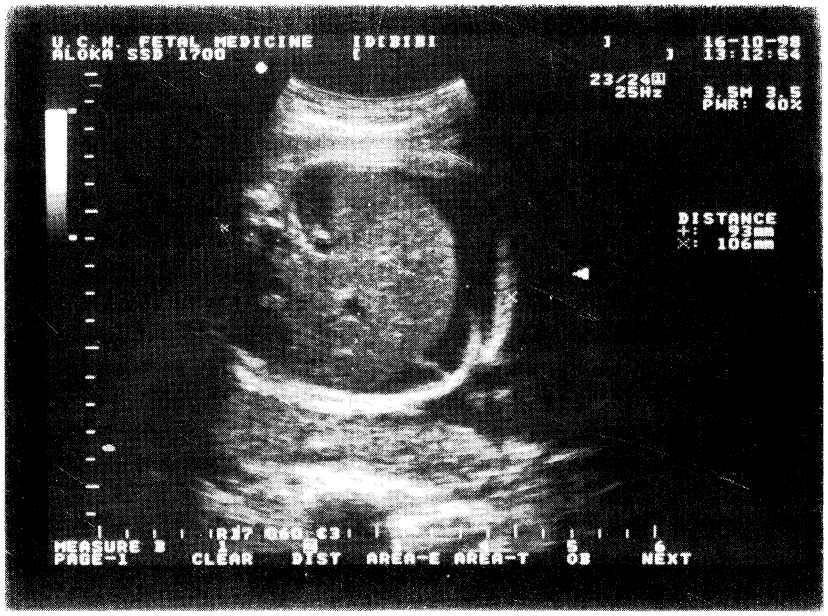

Figure 1. Hydropic fetus showing one of the sonographic indicators of hydrops (ascites). This can appear as "bowel halo", a sign of free fluid within the peritoneal cavity.

However, measurement of fetal liver length (as measured in a parasagittal plane)[19] (Figure 2) and fetal spleen perimeter (measured by tracing round the spleen in the same cross-sectional plane as the fetal stomach)[10] (Figure 3a, b) appear to show potential in evaluating fetal anaemia, enlargement of these dimensions reflecting the increased extramedullary erythropoietic mass.[9,10,19]

Fetal Spleen Perimeter

In early embryonic life, the yolk sac is the first site of erythropoiesis. From 8 weeks of gestation onwards, the liver is the main erythropoietic site. In the third trimester, the bone marrow takes over this function. Haematopoietic precursor cells can be found in embryonic spleens at 6-7 weeks gestation.[20] Fetal spleens produce up to one third of the red blood cells from 18 weeks gestation until term in a normal pregnancy.[21] The pathological mechanisms that produce splenomegaly include increased haematopoiesis, accelerated red blood cell destruction and congestion from raised venous pressure which may correlate with splenic enlargement. In severe Rhesus disease, rapid destruction of the antibody-coated red blood cells and, trapping and accumulation of reticulocytes and erythroblasts which occurs in the spleen causes splenomegaly.[22] The spleen was visualised behind the fetal stomach in a transverse scan of the fetal abdomen (Figure 3a). In this plane, the spleen

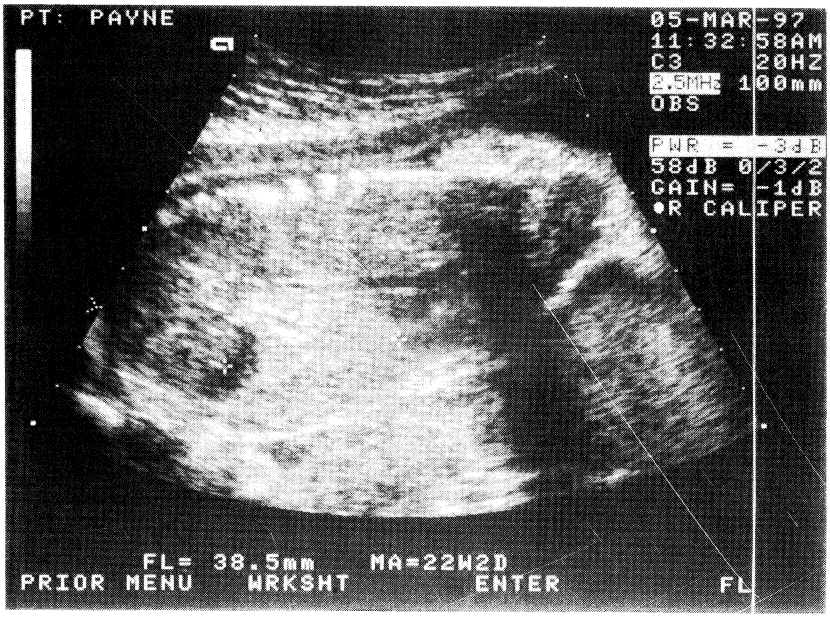

Figure 2. Fetal liver length measured in a parasagittal scan view of the fetal abdomen from the diaphragm to the lower edge of the right lobe of liver.

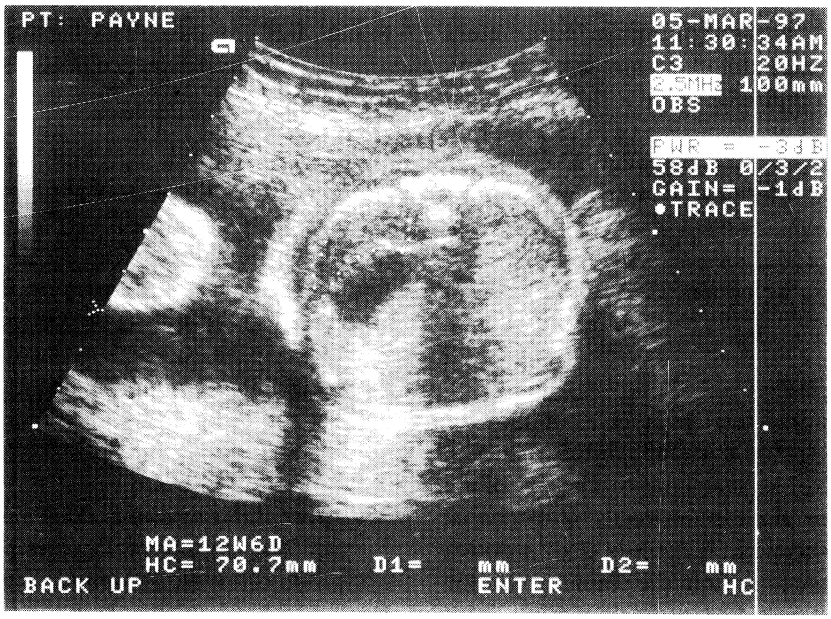

Figure 3a. The fetal spleen perimeter was measured by the trace method in the same cross-sectional plane as that used for the fetal stomach.

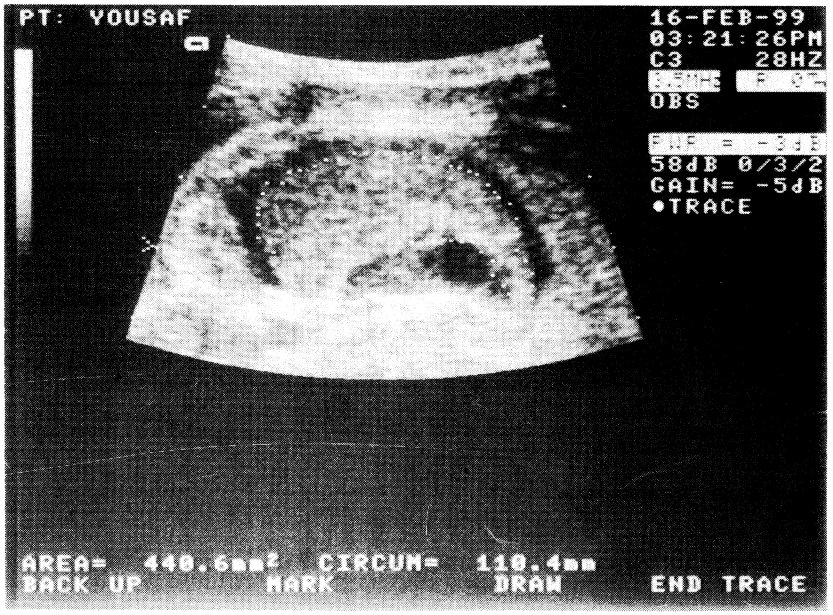

Figure 3b. The maximised of fetal spleen perimeter is shown.

perimeter was measured by tracing the caliper during freeze-frame on the screen.

Several ultrasonographic parameters including abdominal circumference measurement, were initially reported to be useful in detecting hepatosplenomegaly as an early sign of fetal anaemia[23] but the results have been disappointing.[17,24]

The fetal spleen can be visualised from 16 weeks gestation by ultrasound scanning in a traverse cross section of the fetal abdomen. Adequate measurements can always be obtained regardless of fetal position.

Fetal Liver Length

An enlarged fetal liver in isoimmunised pregnancies has proved a useful subjective sign of increased fetal liver haematopoiesis. The size of the fetal liver has been measured indirectly by abdominal circumference or diameter. Vintzileos et al[19] reported that direct measurement from the diaphragm to the lower edge of the right lobe of fetal liver was more useful than abdominal circumference. Fetal liver length seems to be more useful before the first transfusion than subsequently. This may be partly gestation dependent. The liver length measurement may be more useful before 30 weeks due to the switch to increased extramedullary haematopoiesis.

The fetal liver length is determined by an established technique[19]. First, the aorta is identified in a longitudinal plane; the transducer is moved parallel to this plane until both the right hemidiaphragm and the tip of the right lobe are visualised. The fetal liver size is measured from the right hemidiaphragm to the tip of right lobe (Figure 2). The fetal position sometimes makes it difficult to measure the right lobe, i.e. back up or right side down, but no particular position of the fetus is excluded. A freeze-frame capability is available and on-screen calipers are used for measurements.

Doppler Ultrasound Assessments

The use of fetal Doppler ultrasound in the evaluation of severe alloimmunisation is appealing in view of the correlation between fetal anaemia and elevated cardiac output and increased blood velocity measurements from fetal vessels such as the umbilical vein, descending thoracic aorta, middle cerebral

artery and common carotid artery.[12,25-28] Kirkinen et al[25] first reported a close correlation between umbilical venous blood velocities and postpartum haematocrits. It was postulated that the increased flow was the result of a reduced haematocrit.

Intracardiac Doppler evaluation and demonstration of increased outflow tract velocities provide evidence of increased cardiac work with worsening anaemia.[27] The accurate prediction of fetal anaemia using such non-invasive assessment has not been demonstrated. Iskaros et al[29] have suggested that pregnancies with a mild or no history of fetal anaemia may be monitored by a combination of serial antibody quantification and Doppler monitoring of umbilical vein maximal flow velocity (UVVmax). UVV max was recorded in a cross-section of the fetal abdomen during apnoea with an angle of insonation of 30°. (Figure 4)

In red cell alloimmunised pregnancies, there are significant correlations between the degree of fetal anaemia and Doppler parameters of increased velocity in the descending thoracic aorta and common carotid artery and increased umbilical venous flow.[30] The highest correlation is found in previously untransfused fetuses. These Doppler measurements are useful in identifying the anaemic fetus and therefore the timing of the first cordocentesis. Serial underlying Doppler studies can identify the transfused fetus that is developing unexpectedly rapid anaemia.

Umbilical Vein Maximum Flow Velocity (UVV max) Measurements

Volume flow can be calculated from the product of the cross-sectional area of the vessel (A) and the time-averaged mean velocity (V) measured by pulsed Doppler. This can be expressed relative to estimated fetal weight (wt) :
[volume flow (ml/min/kg) = VXA/wt].[31]

The ultrasonographer adjusts the plane of the scan to obtain a longitudinal image of the umbilical vein within the fetal trunk. The transducer is placed in the umbilical vein proper, and in neither the left portal vein nor the ductus venosus. Caliper markers are placed on the inner edges of the vessel wall to determine both the lumen diameter and the spatial orientation of the vessel. The transducer is directed automatically toward the point of measurement and range gated. The machine is able to compute and display flow values on a continuous basis. The flow trace can be frozen at any time to allow detailed measurements to be made.

Since the blood flow was angle dependent and angles between the ultrasound beam and the estimated direction of blood flow are often more than 30°, the actual velocities can not be obtained. Therefore, the most accurate measurement of volume flow is recorded in a cross-section of the fetal abdomen with an angle of insonation < 30° (Figure 4).

The rate of UVV max steadily increased with gestational age in the second half of pregnancy, reaching a maximum at approximately 37 weeks of gestation followed by a slight fall-off to term.[32] The flow per kilogram of fetal weight varies much less with gestational age. It is virtually constant until 35 weeks, with an average value of 120 ml/min/kg. Beyond 35 weeks a gradual decrease occurs, with the average value at 40 weeks being 90 ml/min/kg.[33]

Antibody Levels

Maternal Rh (D) Antibodies

It is known that Rhesus antibodies may develop during a first pregnancy, most frequently after the 28th week of gestation.[34] This led to the rationale, first proposed by Zipursky and Israelsin[35], for anti-D prophylaxis in pregnancy to reduce the incidence of the Rhesus alloimmunisation. The risk of Rh (D) alloimmunisation during or immediately after a first pregnancy is about 1.5%. Administration of 100 μg (500 IU) anti-D at 28 and 34 weeks gestation to women in their first pregnancy can reduce this risk to about 0.2% without, to date, adverse effects.[36] Serial measurements of maternal serum red cell antibody levels have traditionally been an important part of monitoring because of the well-established association between maternal antibody concentration and pregnancy outcome.

Routine antibody testing is by the indirect Coombs method. Many laboratories now use an automated system that quantifies the concentration of anti-Rh (D). Antibody levels do not always correlate very well with the severity of fetal disease,[37] probably reflecting the fact that not only the quantity but also

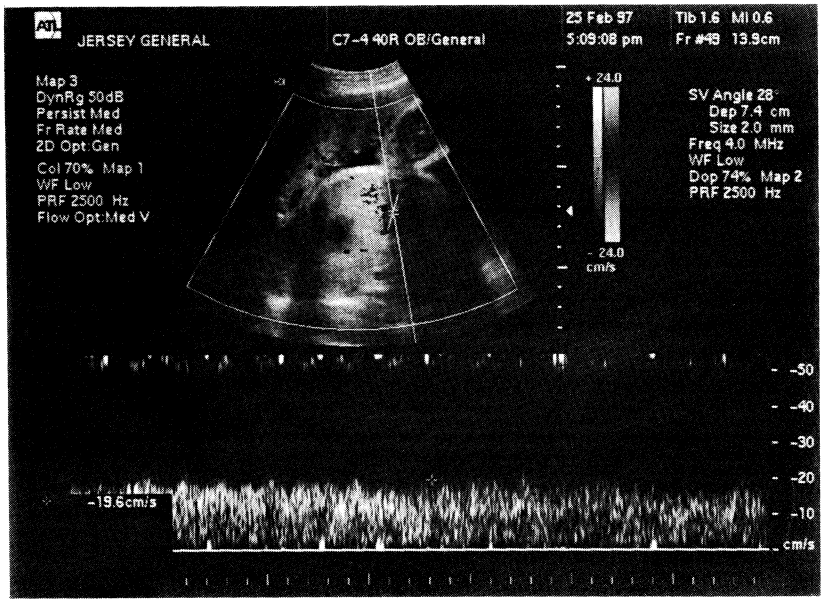

Figure 4. The umbilical vein maximum flow velocity (UVV max) is recorded in a cross-section of the fetal abdomen during apnoea with an angle of insonation < 30°.

the quality of antibodies may be important in predicting the severity, i.e. the immunoglobulin-G (IgG) subclass, Gm allotypes and number of IgG bound molecule is important[38-40]. A sudden rise in antibodies is more significant than the absolute value, particularly in more severely affected pregnancies, where antibody levels do not distinguish between hydropic and non-hydropic fetuses.

However, in the first affected pregnancy, or with only a previous mildly affected pregnancy, maternal antibody levels are reasonably reliable and should be measured at 2-4 weekly intervals.[41]

Nicolaides and Rodeck[13] have reported that as long as the maternal anti-D concentration was <15 IU/ml the fetus was at the most, mildly anaemic (Hb deficit <3g/dl) on cordocentesis. Therefore, invasive testing should be performed in those pregnancies where the antibody levels are above 15 IU/ml. A sudden rise in antibody levels often indicates worsening disease which may require intervention. A critical titre between 1:8 and 1:32 dilutions is correlated with a significant risk of fetal hydrops.[42,43]

Antibodies Other Than Anti-D

Alloimmunisation may develop to antigens other than D. Most atypical alloimmunisation results from blood transfusion, since cross-matched blood is only compatible with ABO blood system and for D in the Rh blood group system. About 1-2% of individuals have developed atypical alloimmunisation following blood transfusion.[44] C and Kell antibodies are able to cause severe haemolysis in the fetus[45] and are second only to anti-D as a cause of fetal death[2] while many of the antibodies that develop are of little or no clinical significance. Several aspects must be considered even though the management is similar to that in Rh-D haemolytic disease.

Anti-c is a Rhesus antibody developing in a Rh (D) positive woman and can lead to confusion. Haemolytic disease from anti-c is less severe than that due to anti-D. If there is no appropriate management, hydrops in utero may occur due to severe fetal anaemia.

Anti-Kell can also cause severe HDFN and in some cases the percentage of reticulocytes in the

fetal blood is much lower than expected.[22] Hydrops fetalis may develop very quickly in cases where amniotic fluid delta OD 450 indicates mild to moderate disease.[46] This can be explained by the fact that the antibody causes suppression of effective erythropoiesis in the fetus rather than haemolysis. Therefore the bilirubin level does not increase significantly even in the presence of very severe anaemia. Management should induce both early fetal blood sampling (FBS) for Kell grouping and evaluation of anaemia. Serial ultrasound scanning should be performed.

Other Non-Invasive Assessments

Previous Obstetric History

In the first sensitised pregnancy, the risk of fetal disease is low and significant fetal anaemia and hydrops is unlikely to occur. The severity of the fetal disease is likely to be worse, with onset of fetal anaemia at an earlier gestational age in a subsequent pregnancy. Fetal death or hydrops due to alloimmunisation before 18 weeks is rare.[40]

Fetal Behaviour

Monitoring of fetal movements needs further investigation. A reduction in fetal movements is associated with the ultrasound findings of hydrops. It indicates a severely distressed fetus, although fetal movements have been present until the time of death.[47,48] Sporadic or absent breathing movements may also be an ominous sign.

Fetal behaviour is assessed using the biophysical profile scores as a framework. Parameters observed include fetal tone, gross body and limb movements, breathing movements, amniotic fluid volume, and fetal heart rate variability and reactivity.[49] In an alloimmunised pregnancy, a fetal behavioural study is useful to evaluate fetal status, and timing of transfusions. Lack of spontaneous fetal movements, muscle tone or breathing movements on ultrasound and an abnormal fetal heart rate pattern (FHR) are a common finding in moribund hydropic fetuses and indicate the need for immediate intravascular fetal transfusion.[50]

Fetal Heart Rate Patterns

When fetal anaemia is severe enough to compromise the oxygen carrying capacity of blood, a cardiotocograph may show an abnormal FHR patterns, such as decreased reactivity and baseline variability, tachycardias, spontaneous decelerations or sinusoidal patterns.[51] A decreased in FHR reactivity and baseline variability may be the first cardiotocographic (CTG) indicators of anaemia and CTG patterns do not allow for accurate prediction of mild-to moderate fetal anaemia.[52,53]

CONCLUSION

The use of ultrasound in the diagnosis of fetal anaemia is relatively limited until overt hydrops is present. Serial ultrasound measurements have failed to identify anaemia in Rhesus (D) alloimmunisation affected pregnancies and there is a poor correlation between a number of ultrasound markers with fetal haematocrit as shown by Nicolaides et al.[24] Nicolaides and Rodeck[13] have shown that maternal serum anti-D level are correlated with the degree of fetal anaemia and can be studied as an indicator of the severity of Rhesus alloimmunisation, particularly in the absence or mild history of fetal haemolytic anaemia. However, fetal anaemia will lead to extramedullary haematopoiesis in which the liver and spleen play an important role and there can be studied in order to predict fetal anaemia.[10,19] An increase in their measurements indicate the risk of fetal anaemia.

Another line of investigation for the non-invasive prediction of fetal anaemia is based on animal data indicating that fetal blood velocities in venous circulating beds increase secondary to an elevated cardiac output and a decrease in blood viscosity. Iskaros et al[29] have found that serial antibody quantification and Doppler monitoring of the UVV max can be used to predict fetal anaemia in cases with mild or no history of fetal haemolytic pregnancies. A previous history can indicate the onset and severity of fetal anaemia which is very useful and important for the management of Rhesus (D) alloimmunised pregnancies. The combination of previous history, anti-D levels and ultrasonographic parameters including UVV max, fetal liver length and fetal spleen perimeter can give rise to greater accuracy in the prediction of fetal anaemia and defer the first invasive procedure performed.

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