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Clinical Management of Hemolytic Disease of the Newborn and Fetus

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1. Introduction

Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloantibodies directed against antigens present in fetal red cells. Paternally inherited antigens of the Rh system, which differ to those from the mother, are present on fetal red cells and when the maternal immune system makes contact with a significant number of these cells create an immune response with antibodies against these antigens. This may happen because of fetomaternal transplacental bleeding (in traumatic events during pregnancy, obstetric procedures, labor, cesarean section) or by events unrelated with pregnancy, such as transfusion, contamination by needle use, etc. Maternal antibodies (IgG) can cross the placenta and activate macrophages in the fetal spleen which cause fetal red cell destruction with subsequent hemolytic anemia. This leads to jaundice and kernicterus in the newborn or hydrops and death in the fetus.

Before the 70's, HDFN was a major obstetric problem, that had a large impact on fetal and neonatal morbidity and mortality. Today, without an appropriate programme, up to 50% of untreated HDFN will result in death or severe brain damage. In developing countries, especially those lacking an efficient prophylactics programme, this causes an important public health problem. In fact, it has been estimated that more than 50 thousand fetuses could be affected by this condition every year in India (Zipursky and Paul, 2010). With the established use of post-natal anti-D prophylaxis for rhesus (Rh) negative women, together with its increasing use for routine antenatal prophylaxis, the incidence of Rh-D sensitization has dramatically fallen (Hughes RG et al., 1994). Nevertheless, 15-17% of the Caucasian population in Europe and North America is D negative (Ubarkian S, 2002). With the sensitization against other red cell antigens such as Kell RhC/c, RhE/e, this pathology could still affect a large number of pregnancies every year, with significant health and financial implications (Abdel-Fattah SA et al., 2002; Illanes S and Soothill P, 2009). In England and Wales, about 520 fetuses develop HDFN each year, of which about 37 would die in the fetal or neonatal period and 28 would present developmental problems (Daniels G et al., 2004, NICE 2008).

On the other hand, in fetus affected by HDFN, survival rates can exceed 90 percent if anemia is diagnosed and treated with intrauterine blood transfusions in a timely manner (Van Kamp IL et al., 2001). Women with rising red cell antibody levels are usually referred to tertiary fetal medicine units for specialized management. The main challenge facing fetal medicine

specialists today is not the skill required for invasive therapy, but rather the non-invasive monitoring of the disease so that its progress can be predicted to guide the need and timing of intrauterine transfusions to minimize unnecessary invasive testing (Ubarkian S, 2002).

2. Non-invasive management

2.1 Use of cell-free fetal DNA for the determination of fetal RhD genotype

The identification of blood group genes and subsequent detection of the molecular bases of blood group polymorphisms has made it possible to predict blood group phenotypes (Avent ND et al., 2000). The source of DNA used to predict fetal blood groups was initially done invasively by sampling amniotic fluid or chorionic villi (Finning KM et al., 2002). However, the related risk of the obstetric procedures (0.5–1% for fetal loss) (Nanal R et al., 2003) and risk of fetomaternal hemorrhage (amniocentesis 17%) (Tabor A et al., 1987) was associated with an unwanted increase in gestational maternal immunization (Murray JC et al., 1983).

The fact that cell-free fetal DNA (ffDNA) is present in the plasma of pregnant women in sufficient quantities for the determination of fetal RhD genotype (Lo YM, 1999), leads to the possibility of fetal D typing using a non-invasive approach. If the rhesus sequence is present in a D-negative women's blood it is indicative that the fetus is D-positive (Lo YM et al., 1997). Initially, cell-free DNA was studied as a tumor marker (Lo YM et al., 1998), but the presence of Y signals in pregnant women carrying a male fetus was the first evidence that this technique could be used to assess the fetus condition as well as for prenatal diagnosis (Lo YM et al., 1997). In a normal pregnancy, the placental tissue goes through a physiological remodeling via apoptosis and necrosis in the chorionic villus. As a consequence, ffDNA is released to the maternal plasma in increasing amounts as gestation progresses (Wataganara T and Bianchi DW, 2004; Alberry MS et al., 2009; Huppertz B et al., 2006; Fomigli L et al., 2000; Arnholdt H et al., 1991; Illanes S et al., 2009).

Non-invasive prenatal diagnosis using ffDNA is the focus of intense research nowadays because of its many potential uses. It's being evaluated for inherited diseases and genetic disorders such as trisomy 21 (Ehrich M et al., 2011; Deng YH et al., 2011; Sehnert AJ et al., 2011), trisomy 18 (Sehnert AJ et al., 2011), β -thalassaemia (Li Y et al., 2009; Hahn S et al., 2011), hemophilia (Tsui NB et al., 2011), X-linked genetic disorders (Miura K et al., 2011) and achondroplasia (Chitty LS et al., 2011). Genome-wide scanning may be implemented for fetal genetic prenatal non-invasive diagnosis (Lo YM et al., 2010) and quantitative changes in ffDNA blood levels have been proposed as a potential marker for preeclampsia (Hahn S et al., 2011). Finally, the combination of real-time PCR with improved rhesus D (RhD) typing enables a highly accurate prediction of fetal D status from maternal plasma (Finning KM et al., 2002). Moreover, this is now available as a world-wide service (Daniels G et al., 2004; Finning KM et al., 2002; Finning KM et al., 2004; Legler TJ et al., 2002; Rouillac-Le Sciellour C et al., 2004; Van der Schoot CE et al., 2006; Tynan JA et al., 2011; Tounta G et al., 2011).

A recent meta-analysis has been performed to evaluate the diagnostic sensitivity and specificity of fetal Rh genotyping using ffDNA (Geifman-Holtzman O et al., 2006). A total of 3261 maternal plasma samples were analyzed in 37 publications and approximately 500 study protocols in order to assess fetal RhD status. Results showed total accuracy of 91.4% (94.8% if studies with small numbers of samples were excluded), with a wide variation, from 31.8 to 100 percent, depending on which protocol, gestational age at testing and study

design was applied. Two recent studies have evaluated the feasibility of this testing in the first trimester of pregnancy. Akolekar et al tested patients at 11-13 weeks using a high-throughput robotic technique. They concluded that it was an accurate method with a positive predictive value of 100% and a negative predictive value of 96,5% (Akolekar R et al., 2011). The second study, reported a sensitivity of 100% and a specificity of 93%, with a 97% diagnostic accuracy for RhD genotyping in the first trimester of pregnancy using a quantitative PCR method (Cardo et al., 2010)

Non-invasive fetal RhD genotyping was compared to traditional postnatal serologic assay in a large scale validation study (Müller SP et al., 2008). The authors studied over one thousand samples of RH negative women who gave whole blood specimens at a gestational age of 25 weeks. Tests were drawn up using an innovative automated DNA extraction method using magnetic tips and spin columns that have been recently developed by members of Special Non-Invasive Advances in Fetal and Neonatal Evaluation Network of Excellence (SAFE NoE) (Chitty LS et al., 2008; Legler TJ et al., 2007). The sensitivity of fetal *RHD* genotyping was 99.7% for spin columns and 99.8% for magnetic tips, and these results were comparable to conventional serology (99.5%). In the case of specificity, the serology was slightly better (99.7% versus 99.2% for spin columns and 98.1% for magnetic tips). It has also been established that it is an accurate method in multi-ethnic populations such as Brazil, by using two or three exons for *RHD* gene (Amaral DR et al, 2011; Chinen PA et al., 2010). This new approach has significantly reduced the number of invasive procedures carried out in different fetal medicine units for fetal D grouping (Finning KM et al., 2004) and has proved that the automated DNA extraction method can be used in a clinical setting.

Non-invasive studies for other blood group antigens have also been flourishing, including Kell antigen, the second most important cause of hemolytic disease (Li Y et al., 2008), RhC/c and RhE/e (Li Y et al., 2008; Van der Schoot CE et al., 2003; Finning K et al., 2007). The International Blood Group Reference Laboratory, at Bristol (Finning K et al., 2007) has developed and tested allele-specific primers for detecting the K allele of KEL and alleles of RhC/c RhE/e (Van der Schoot CE et al., 2003), with great accuracy for each allele. The matrix assisted laser desorption/ionization time-of-flight mass spectrometry or MALDITOF MS (Li Y et al., 2008), is able to detect the fetal KEL1 allele in KEL negative mothers with an accuracy of 94%. In a recent meta-analysis, collective reported diagnostic accuracy of fetal RhCE genotyping, with a combined accuracy for fetal genotyping of 96.3% for RhC/c and 98.2% for RhE/e (Geifman-Holtzman O et al., 2009) was estimated. A recent Dutch report, after 7 years of non-invasive fetal blood group genotyping from maternal blood samples for D, K, c, and E groups, revealed that diagnosis could be achieved in 97% of cases in a medium gestational age of 17 weeks, with no false-positive or false-negative results, implying that it is an accurate and applicable diagnostic tool in clinic (Scheffer P et al., 2011). The use of cell-free fetal DNA in maternal plasma for fetal RhD genotype could eventually enable the screening of all D negative pregnant women, thereby confining the administration of prophylactic anti-D only to those pregnancies in which it is needed (Bianchi DW et al., 2005). Since the accuracy of the actual test is not 100%, there is an ongoing debate about the advantage of introducing such a policy. Some researchers propose that guided prophylaxis should have a lower cost than the routine prophylaxis to all RhD negative women (Daniels G et al., 2009). However, a recent cost benefits study evaluated the implementation of this strategy in England and Wales, and concluded that is unlikely to be sufficiently cost-effective for a large scale introduction. They estimated that only minor

savings would be gained and that an increase in maternal sensitization may be unacceptably high due to test inaccuracies in different ethnic minority populations (Szczepura A et al., 2011). It is expected that new technologies should alter this picture. Nevertheless, any policy for the prevention of unnecessary administration of human-derived products, such as prophylactic anti-D, should be taken into account because of the potential contamination of blood products that at the present time cannot be tested, as unidentified viruses or prions (Avent ND. 2008, Avent ND. 2009).

3. Detection of fetal anemia non-invasively by ultrasonography

3.1 Ultrasound findings

Severe anemia causes tissue hypoxia (Soothill PW et al., 1987), with endothelial damage and increased capillary permeability. This may lead to protein loss into the interstitial space, hypoproteinaemia and consequently ascites (Nicolaidis KH et al., 1985). Moreover, in response to red cell haemolysis and fetal anemia, extramedullary haematopoiesis occurs, increasing portal and umbilical venous pressures. This would impair hepatic function and protein synthesis, resulting in worsening hypoproteinaemia which would further deteriorate the hydrops process (Bowman JM, 1978; Socol ML et al., 1987). The ultrasonographic features of hydrops include ascites (the earliest sign), pleural effusions, pericardial effusions, scalp edema, subcutaneous edema and polyhydramnios. These findings are an indication of a hemoglobin deficit of more than 6 standard deviations below the normal mean for gestational age, and will need urgent intrauterine fetal transfusion (Nicolaidis KH et al., 1988).

The many attempts to identify sonographic fetal anemia features which occur before the development of fetal hydrops have been unsuccessful (Queenan JT, 1982, De Vore GR et al., 1981, Nicolaidis KH et al., 1988), because of their failure to quantify the real degree of the fetal anemia (Nicolaidis KH et al., 1988). Moreover, these ultrasound findings, including the evaluation of the liver and spleen, have been abandoned, because when high quality Doppler measurements are used to predict fetal anemia, these anatomic evaluations add little useful independent information. In our practice, we don't usually look for any structural measurement or appearance, save for the early signs of fetal ascites.

3.2 Fetal Doppler ultrasonography

Doppler ultrasonography is a non-invasive method used for studying fetal hemodynamic changes in vessels that supply fetal organs responding to pathological conditions. In anemic fetuses, the Doppler measurement that describes the hemodynamic changes occurring in response to this pathological condition has been attempted in several vessels. However, because of the rapid hemodynamic changes observed in the middle cerebral artery (MCA) (Mari G et al., 2000), have transformed the measurement of its peak systolic velocity (the maximum Doppler shift at the peak of the spectral curve) in the gold standard for anemia fetal prediction. (Campbell S et al., 1995). After Vyas et al in 1990 (Vyas et al., 1990) described an increase in the average MCA time for mean blood velocity in fetal anemia cases, Mari and colleagues reported that the degree of fetal anemia could be accurately detected by Doppler measurement of blood-flow velocity in the MCA, with an inverse relationship between the MCA peak velocity and the fetal hematocrit, with no false negative results for anemic fetuses (Mari G et al., 2000). The statistically significant increase in fetal hematocrit, following intrauterine transfusion, also resulted in a rapid reduction in the

middle cerebral artery peak velocity. These results confirm that the traditional management of pregnancies complicated by Rh alloimmunization with serial invasive amniocentesis to determine bilirubin levels is no longer required. Even more, a recent study has shown that Doppler measurement of the peak velocity of systolic blood flow in the MCA can safely replace invasive testing in the management of Rh-alloimmunized pregnancies, avoiding all the complications related with the traditional invasive approach (Oepkes et al., 2006).

Several studies have used the MCA Dopplers in a clinical basis for the prediction of fetal anemia with at-risk cases, without ultrasound evidence of fetal hydrops. These have shown that there is a good correlation with fetal Hemoglobin (Abdel-Fattah SA et al., 2002). This non-invasive investigation can be reliable in predicting anemia in cases in which the need to sample fetal blood is not certain, therefore delaying invasive testing until treatment is likely to be required. The neonatal outcome where invasive testing has been avoided (based on reassuring MCA Doppler velocity results) did not result in life-threatening fetal or neonatal morbidities (Abdel-Fattah SA et al., 2005). Therefore, the routine use of MCA Doppler's can avoid unnecessary invasive procedures on at-risk fetuses. There are several normal reference ranges of fetal blood flow velocity in the middle cerebral artery. However, when compared in terms of discriminatory power, sensitivity and specificity, Mari's curve and its given cut-offs perform better when fetal anemia is predicted. (Mari G et al., 2000; Bartha JL et al., 2005).

4. Invasive approach

Intrauterine blood transfusion of anemic fetuses represents one of the great successes of fetal therapy. After the first approach with intraperitoneal blood transfusion introduced in 1963 by Liley (Liley AW, 1963), Rodeck (Rodeck CH et al., 1981) described intravascular fetal blood transfusion (IVT) by the needling of the chorionic plate or umbilical cord vessels via fetoscopy direct vision. In 1982, Bang in Denmark started IVT by umbilical cord puncture under ultrasound guidance. This is now the gold standart (Bang et al., 1982). IVT has produced a marked improvement in the survival rate of the anemic hydropic fetus. This in turn can also prevent complications from developing by treating anemic non-hydropic fetuses, where moderate or severe anemia is detected non-invasively by Doppler ultrasonography, by increased peak velocity of systolic blood flow or time-averaged mean velocity in the MCA in fetuses at risk (Abdel-Fattah SA et al., 2002; Mari G et al., 2000). It is estimated that between 10 and 12% of fetuses of sensitized RHD negative women will require IVT (NICE 2008) with the survival rate exceeding 95% in experienced centers, particularly when opportune IVT treatment is established in a timely manner (Van Kamp IL et al., 2001).

When possible the umbilical vein is sampled because artery puncture may pose a risk for bradycardia (Weiner CP et al., 1991). The hemoglobin concentration (Hb) is measured and interpreted according to gestational age, with the severity classified on the fetal hemoglobin deviation from the normal mean for gestation into mild (hemoglobin deficit less than 2 g/dl), moderate (deficit 2-7 g/dl), and severe (deficit greater than 7 g/dl) (Nicolaidis KH et al., 1988). A blood transfusion will be attempted in cases were a moderate or severe anemia is detected. For IVT to be realized, the blood volume required to correct the fetal Hb needs to be calculated, using pre-transfusion fetal Hb, the donor blood Hb (adult blood usually packed to a hematocrit of about 70-80%) and the gestational age (Nicolaidis KH et al., 1986). The volume required is given as fast as possible without causing changes to the fetal heart

rate and it seems that the feto-placental unit is able to handle the blood volume expansion much more easily than when transfusing neonates without the benefit of a placenta. Infusion of packed blood through a 15-cm long, 20-gauge needle at rates of 1–10 ml/min does not result in significant hemolysis (Nicolaidis KH et al., 1986). After the volume calculated to correct the Hb deficit has been given a post-transfusion, Hb is measured to help time the subsequent transfusion. After two or three transfusions, fetal blood production is suppressed and instead adult blood cells become more dominant. The fall of Hb becomes very predictable at about 1% haematocrit point per day (Thein AT and Soothill P, 1998). We aim to complete the last transfusion at 35–36 weeks and then to induce labor at 37 weeks to allow maturation of both the pulmonary and hepatic enzyme systems. With this programme, we hope to avoid neonatal exchange transfusions.

As this management of anemic fetuses is increasing, and the number of cordocentesis and transfusions are decreasing, the problem of maintaining the skills needed is rising too. It has been suggested that operators should perform at least 10 procedures per year to keep competence. (Lindenburg IT et al., 2011). Complications associated with intrauterine procedures such as cord hematoma, hemorrhage, fetal bradycardia and intrauterine death could increase in the future (Illanes S and Soothill PW, 2006). A possible solution would be to introduce a health policy that gave transfusions, via some centers, to all those cases that needed one. This could potentially avoid any complications such as lack of operator training.

5. Neonatal outcome

For the neonate, the consequences of HDFN are anemia and hiperbilirrubinemia. Postnatal treatment options include top-up red blood cells transfusions for the former, and phototherapy and exchange transfusions for the latter. Top-up transfusions, even with a minimal risk, carry a theoretical possibility of anaphylactic reaction and transmission of viral disease. In contrast, exchange transfusion carries a high morbidity and mortality rate (5% and <0.3% respectively), but the number of neonates requiring exchange transfusions has reduced due to advances in phototherapy.

Few studies specifically investigate the short-term neonatal outcomes for pregnancies affected by hemolytic red cell alloimmunisation. Two recent retrospective studies have assessed this question. De Boer *et al.* (De Boer *et al* 2008) investigated the short-term morbidity for neonates treated for Rhesus disease with or without IVT. Those treated with IVTs were found to require a higher number of top-up red blood cell transfusions and had less need of phototherapy. However, both groups had a similar need for exchange transfusion. The second study, is a Scottish report of postnatal outcomes following intrauterine transfusion, and showed that 20% of newborn needed exchange transfusion, 50% had top-up transfusion, and most of them needed phototherapy (McGlone L et al., 2009). More studies are needed, to evaluate the neonatal outcomes and associated morbi mortality, related to the number of transfusions, the gestational age at first and last transfusion, and the hemoglobin level at first IVT.

6. Conclusion

The management of the HDFN represents one of the genuine successes of fetal therapy. The current aspects of this clinical management have shifted from a long-established invasive

approach to a non-invasive one. This applies to the detection of fetuses at risk of HDFN with the use of cell-free fetal DNA in the plasma of pregnant women to determine fetal RhD genotype. If the fetus is D negative, then it is not at risk and no further procedures are required; if it is D positive the appropriate management of the pregnancy can be arranged. On the other hand, maternal plasma testing for fetal RhD genotype could eventually enable the screening of all D negative pregnant women, thereby confining the administration of prophylactic anti-D only to those pregnancies in which it is needed. In addition, when a fetus is antigen positive, the follow up of these fetuses is for the detection of moderate or severe anemia non-invasively by Doppler ultrasonography on the basis of an increase in the peak velocity of systolic blood in the middle cerebral artery. When anemia is suspected, an invasive approach is required in order to perform an intrauterine blood transfusion which should only be attempted when the fetus needs transfusion.

7. Summary

Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloantibodies directed against paternally inherited antigens on fetal red blood cells. It was also a significant cause of fetal and neonatal morbidity and mortality until the introduction of anti-D immunoglobulin during pregnancy and shortly after delivery. However, it is still a major problem in affected pregnancies. The emphasis of current clinical management of HDFN is a non-invasive approach. This work is carried out on fetuses at risk with HDFN, with the use of cell-free fetal DNA in the plasma of pregnant women, in order to determine the fetal RhD genotype, or to see if the fetus is antigen positive. If the mother is sensitized, for the follow up and detection of moderate or severe anemia – this is done, primarily, non-invasively by Doppler ultrasonography of the middle cerebral artery. If anemia is suspected, an invasive approach is required in order to perform an intrauterine blood transfusion. This management represents one of the genuine successes of fetal therapy.

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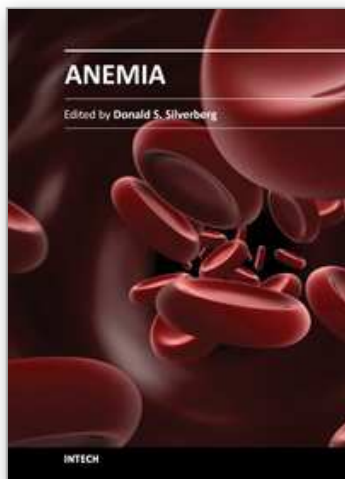
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This book provides an up- to- date summary of many advances in our understanding of anemia, including its causes and pathogenesis, methods of diagnosis, and the morbidity and mortality associated with it. Special attention is paid to the anemia of chronic disease. Nutritional causes of anemia, especially in developing countries, are discussed. Also presented are anemias related to pregnancy, the fetus and the newborn infant. Two common infections that cause anemia in developing countries, malaria and trypanosomiasis are discussed. The genetic diseases sickle cell disease and thalassemia are reviewed as are Paroxysmal Nocturnal Hemoglobinuria, Fanconi anemia and some anemias caused by toxins. Thus this book provides a wide coverage of anemia which should be useful to those involved in many fields of anemia from basic researchers to epidemiologists to clinical practitioners.

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