Diagnostic procedures for assessing the severity of alloimmune fetal anemia

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Summary

Introduction *(chapter 1)*

Before or during pregnancy, the mother, who types negative for a specific red cell antigen, might develop antibodies against the offending antigen. This so-called alloimmunization can occur after a blood transfusion, after organ transplantation or after a fetomaternal transfusion. The maternal antibodies can pass through the placenta into the fetal circulation. In case the fetus is positive for the antigen, the red cells will be destructed and anemia may develop. In serious cases this can lead to massive hydrops and ultimately to intrauterine death. In the Netherlands, there are annually approximately 200 pregnancies in which potentially dangerous maternal antibodies against fetal erythrocytes are diagnosed (mainly anti-D, anti-c and anti-K antibodies). Pregnant women with a high concentration of such antibodies are at high risk for fetal complications. In approximately 30% of these high-risk pregnancies intrauterine transfusion is warranted. Severe fetal anemia can already occur at 16 weeks of gestation.

Diagnostic procedures are focussed on timely detecting severe fetal anemia, before hydrops or death occur. Early recognition and treatment of fetal anemia before hydrops fetalis develops, leads to a better outcome. However, treatment of a fetus in utero that has only moderate anemia carries several risks, such as procedure-related fetal loss rates, boostering of existing antibodies and the development of new antibodies. In differentiating between moderate and severe fetal anemia, several techniques are used. A first diagnostic tool is the history of the woman. The obstetrical history is a part of this anamnesis. Important is also the perception of fetal movements by the mother. A reduction of the fetal movements felt by the mother is an alarming signal and raises the suspicion of severe fetal anemia. The second diagnostic technique is to follow the concentration of maternal antibodies. When these are rising, the fetus may be in danger for anemia. The third diagnostic technique is measurement of bilirubin concentration in amniotic fluid. This method was described by Liley in 1961 and is based on the increase of optical density at a wavelength of 450 nanometres, the Δ OD 450. A fourth diagnostic technique is ultrasonography. On a B-mode image, discrete sign of hydrops can be seen. Fetal organs involved in the production

and destruction of red cells, such as liver and spleen, can be measured. These organs are often enlarged in severe anemia. Placental thickness can also be measured. With M-mode ultrasound, some measurements can be performed more accurately. With Doppler, the blood velocity in the fetal arteries and veins can be measured. A fifth diagnostic technique is cardiotocography. The fetal heart frequency is measured for 30 to 45 minutes. This technique is, however, inappropriate for diagnosing fetal anemia, because, even in very severe anemia, there is most often a normal pattern of the fetal heart frequency. A final diagnostic technique is fetal cordocentesis. At cordocentesis the exact hemoglobin concentration can be measured. However, the risk of severe complications for the fetus at cordocentesis is approximately 2 or 3 %.

In this thesis, the link between severe fetal anemia and on the one hand, the bilirubin concentration and on the other hand, some more recently used ultrasonographic measurements are studied. The treatment of fetal anemia is symptomatic and is aimed at keeping the fetus in a good condition by intrauterine transfusion until term thus avoiding life-threatening fetal anemia and iatrogenic prematurity at the same time.

The aim of the different studies in this thesis was to analyse the physiology of fetal anemia and the evaluation of diagnostic techniques to predict the optimal timing for fetal blood transfusion.
This thesis contains two parts: the chemical approach and the ultrasonographic approach.

Part 1: Chemical approach

The chemical techniques are based on the fact that destruction of fetal hemoglobin will increase the bilirubin concentration of amniotic fluid. Amniotic fluid is obtained by amniocenteses. The degree of yellowness of the amniotic fluid will be measured as described by William Liley in 1961. This yellowness is caused exclusively by bilirubin.

First, a systematic literature review was performed *(chapter 2)* on the diagnostic accuracy of bilirubin measurement in amniotic fluid and of middle cerebral artery peak systolic velocity in the prediction of severe fetal alloimmune anemia. The advantage of Doppler measurements is their non-invasiveness. The conclusion of this review, based on mainly retrospective studies, was that the sensitivity for both techniques is variable but quite similar. In case of equal sensitivity, it seems clear that the non-invasive technique is preferable. A new large multicenter study in which both techniques are prospectively tested in the same patients has meanwhile been performed.

In *chapter 3* we studied how accurately the bilirubin concentration in amniotic fluid predicts severe fetal anemia in the second and third trimester in D-alloimmunized pregnancies. Seventy-nine non-hydropic singleton pregnancies were included where amniocentesis was performed within 4 days of first fetal blood sampling. Amniotic fluid Δ OD 450 values were plotted on a Liley's chart. In 1961 William Liley described a chart with on the x-as gestational age and on the y-as the Δ OD 450. In this chart, 3 zones were defined: zone 1 (non-anemic), zone 2 (moderate anemia) and zone 3 (severe anemia). The original Liley chart is from 27 to 36 weeks, the extrapolated Liley curve extends from 18 to 36 weeks. Accuracy, sensitivity and specificity were calculated for two commonly used cut-off levels on the Liley chart. Sensitivity of Δ OD 450 values in Liley's zone 3 or the upper third of Liley's zone 2 was 95% before and 98% after 27 weeks. We concluded that Liley's extrapolated curve predicts severe fetal anemia with high sensitivity and reasonable specificity.

In *chapter 4* we investigated the pathways of bilirubin from fetal blood to amniotic fluid. Therefore, we studied the relation between bilirubin concentration in blood and in amniotic fluid in 68 non-hydropic rhesus D-alloimmunized anemic fetuses at first intrauterine transfusion. In these alloimmunized fetuses, the amniotic fluid/fetal blood ratio for bilirubin decreased from 0.09 at 28 weeks to 0.05 at 33 weeks. In normal, non-anemic fetussen, amniotic fluid/fetal blood ratios for bilirubin, and for albumin, are in the same range and show a similar decrease during gestation. On the basis of these findings, we hypothesised that amniotic fluid bilirubin concentration is determined, firstly, by fetal blood concentration and, secondly, by the amniotic fluid/fetal blood ratio of albumin.

Among five possible pathways bilirubin could take to build up a concentration in amniotic fluid (fetal kidneys, lungs, skin, bowel, membranes), the intramembranous pathway appears to be the only remaining possibility. During fetal life, bilirubin in amniotic fluid is mainly unconjugated. Unconjugated bilirubin is bound to albumin almost completely. It is very improbable, therefore, that urine or alveolar fluid contribute substantially to the bilirubin concentration in amniotic fluid, because the albumin concentration in these body fluids is 100 to 200 times lower than in fetal plasma. The albumin concentration in amniotic fluid on the other hand, is only 10 to 20 times lower than in fetal plasma. Because of the very low albumin concentrations in urine and alveolar fluid, these fluids act as a barrier for unconjugated bilirubin leaving the plasma and entering the amniotic fluid compartment. A meconium origin of amniotic fluid bilirubin is inconsistent with a clinically relevant correlation between amniotic fluid and fetal blood bilirubin concentration. The fetal skin probably serves as a major pathway for solute and water exchange, including unconjugated bilirubin, between amniotic fluid and fetus till 16 weeks of gestation. In fetuses of more than 25 weeks, however, the fetal skin is completely keratinized. The fetal membranes, on the other hand, retain a high permeability until term. Therefore, bilirubin exchange between fetal blood and amniotic fluid most probably occurs through the intramembraneous pathway.

Part 2: Ultrasonographic approach

Since 1995 several publications have shown that middle cerebral artery blood velocities during systole of the fetal heart are increased in case of fetal anemia. It is still unclear if this increased blood flow velocity in anemia is the result of changes in blood viscosity, in contractility of the heart or in peripheral brain resistance.

In order to study the effect of blood viscosity on the systolic blood flow velocities in the middle cerebral artery, blood flow velocities were measured before and after intrauterine transfusion. After all blood viscosity increases during intrauterine transfusion. In *chapter 5* we describe the effect of a large increase of the heamatocrit on middle cerebral artery peak and umbilical vein maximum velocities in anemic fetuses. Therefore, middle cerebral artery peak flow velocities and umbilical vein maximum flow velocities were measured before, immediately after and 12-24 hours after 60 intrauterine transfusion. The middle cerebral artery peak flow velocity decreased immediately after transfusion in 59 of the 60 cases. There was a rise in umbilical vein maximum flow velocity immediately after intrauterine transfusion in 37 of the 60 cases. The conclusion was that an acute large increase of the fetal hematocrit significantly decreases middle cerebral artery peak flow velocity. The effect on umbilical vein maximum velocity was, however, unpredictable. The fact that the umbilical vein maximum flow velocity in several cases increases after intrauterine transfusion is of course in contradiction with the saying that the viscosity of fetal blood is the most important factor determining blood flow velocity. Also the wide range in arterial blood flow velocities in fetuses with the same hematocrit points to the fact that, besides hematocrit, other factors such as cardiac output and peripheral resistance must play an important role.

In *chapter 6* we studied the diagnostic accuracy of cardiac ventricular wall thickness and cardio-thoracic ratio in the prediction of severe fetal anemia. The thickness of cardiac wall of the left and the right ventricle and the inter-ventricular septum were measured in diastole using M-mode

ultrasound. The cardio-thoracic circumference ratio was measured on the B-screen. The measurements were obtained in alloimmunized fetuses. Then, two by two tables were constructed to compare the frequency of abnormal cardiac ultrasound measurements in severe and non-severe fetal alloimmune anemia. Complete measurements were obtained in 15 alloimmunized fetuses with severe anemia and in 16 alloimmunized fetuses without severe anemia. Sensitivities of cardiac ultrasound ranged between 0 and 47% and specificities between 77 and 97%. The conclusion was that diagnostic accuracy of ventricular wall thickness and cardio-thoracic ratio in the prediction of severe fetal alloimmune anemia was disappointing. More than 50% of measurements in severely anemic fetuses were within the normal reference ranges.

In *chapter 7,* we evaluated the effect of fetal anemia and intrauterine transfusion on ventricular shortening fraction. During systole, the ventricles of the heart decrease in size. The procentual decrease in size of the ventricles during systole can be measured with ultrasound and expressed as ventricular shortening fraction. The end-diastolic and end-systolic transverse dimensions of the left and the right ventricles were obtained in 23 fetuses before and after 49 intrauterine transfusions. The blood volume given at intrauterine transfusion was expressed as a percentage of estimated fetoplacental blood volume. Shortening fractions of the left and right ventricles differed significantly between three time points: before, immediately after and one day after intrauterine transfusion. Left ventricular shortening fraction decreased immediately after transfusion in 43 (88%) of the 49 procedures. Right ventricular shortening fraction decreased immediately after transfusion in 42 (86%) of the 49 procedures. At the first intrauterine transfusion, there appeared to be only a weak correlation between the decrease in shortening fraction of both ventricles and the transfused volume. The conclusion was that transfusion significantly decreases the shortening fraction of both ventricles of the fetal heart. There is, however, little correlation between the decrease in shortening fraction and the volume of red cells given at intrauterine transfusion.

We hope that the different studies in this thesis will contribute to improved understanding of the physiologic changes in fetal anemia and thus to a less invasive and timely diagnosis of severe fetal anemia. Finally, the studies described in this thesis leads to an improved insight into the hemodynamic changes, during and after treatment with intrauterine transfusion.