Diagnostic procedures for assessing the severity of alloimmune fetal anemia

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General introduction

1

Alloimmune hemolytic anemia of the fetus and newborn

A woman, negative for a red cell antigen, may produce antibodies when she comes into contact with erythrocytes positive for the offending antigen either by blood transfusion, an organ transplant, or as a consequence of a fetomaternal transfusion. In a pregnant woman, IgG antibodies will cross the placenta leading to hemolysis in the fetus and more or less severe fetal anemia. This process is often called erythroblastosis fetalis and consequences include intrauterine demise and neonatal hyperbilirubinaemiae, eventually leading to kernicterus with its concomitant morbidity and mortality.

In 1940, Landsteiner and Weiner discovered the clinically most important red cell antigen, the "rhesus factor" through their research with Macacus rhesus, a monkey species.[1] In the following decades, doctors learned to understand the pathogenesis of alloimmune hemolytic anemia, found ways to diagnose and treat the condition and even discovered how to effectively prevent the disease. It was a success story in medicine and at the same time it was the start of a new subspecialty: fetal medicine.

Prevention
Hemolytic disease of the newborn was once a major contributor to perinatal morbidity and mortality. During the past 4 decades, there has been a remarkable improve of fetal outcome, due to the improved neonatal care, the introduction of intrauterine transfusion, and the introduction of prevention with anti-D. Administration of anti-D to a rhesus-negative mother after the birth of a rhesus-positive child leads to decreased immunization rates. However, in some cases, rhesus immunization will already have occurred (during pregnancy) or the amount of anti-D may not suffice in cases of large fetomaternal transfusions. Blood transfusions and organ (kidney, bone) transplant are other possible causes of alloimmunization. Finally, maternal non-rhesus D red blood cell antibodies may also lead to fetal alloimmune anemia.

Screening
In the Netherlands, there is a nationwide screening policy since 1998. All pregnant women are tested in the first trimester of pregnancy for the presence of red cell antibodies. Screen-positive women are categorized as no-risk, low-risk or high-risk for fetal anemia according to the type of antigen and the concentration of antibodies. In case of high antibody titers, the Antibody Dependent Cell Cytotoxicity (ADCC) test is performed.[2] Pregnancies at high risk for fetal anemia, for example in case of anti-D antibodies with an indirect Coombs titer of >1/32 and an ADCC test of >50% are referred to Leiden University Medical Center (LUMC).

Diagnosis
When a woman is referred to LUMC the obstetrical history is taken into account. Further, ADCC tests and antibody titers are followed. The women are instructed to pay special attention to fetal movements. Ultrasound examinations are performed: depending on the severity of the immunization, weekly or bi-weekly. The ultrasound examination is focussed on fetal movements, early signs of hydrops, fetal liver and spleen enlargement, cor-thorax ratio, umbilical vein maximum flow and middle cerebral artery peak flow measurement. In case of a severe immunization and inconclusive ultrasound findings, amniocentesis and bilirubin concentration measurement is performed. Bilirubin is the main degradation product of hemoglobin and therefore a measure of its destruction. Bilirubin concentration in amniotic fluid is measured colorimetrically by performing a spectral absorption curve. The bilirubin absorption, expressed as Δ OD 450 is calculated as the difference between the measured light absorption at 450 nm and the background absorption at 450 nm.[3] If then there is suspicion of severe anemia, fetal blood examination and an intrauterine transfusion is performed.

Therapy
In case of a less advanced pregnancy the therapy of choice of fetal anemia is intrauterine blood transfusion. At LUMC intrauterine transfusions are performed as early as 16 weeks of gestation and repeat transfusions are given every 2-4 weeks up to 35 weeks,[4;5] aiming that women give

birth between 36 and 38 weeks of gestation. After birth, phototherapy, transfusions and/or exchange transfusions may be necessary.

Despite its undoubted merits, an intrauterine transfusion is not without risk. Procedure-related fetal loss rates are in the range of 1-3% per procedure.[4;5] Furthermore, every intrauterine transfusion carries the risk of fetomaternal hemorrhage, which may increase the severity of alloimmunization. A significant increase in antibody concentration occurs in 50% of cases after intrauterine transfusion. [6]

National strategy in the Netherlands
In the Netherlands all pregnant women have a blood test taken for irregular antibodies in the first trimester of pregnancy. In case of a rhesus negative mother without irregular antibodies the blood test will be repeated at 30 weeks and the mother will receive anti-D. In the Netherlands we have approximately 200 pregnancies yearly with new cases of potentially dangerous red cell antibodies. (170 anti-D, approximately 30 anti-K and anti-c). Of these, approximately 100 pregnant women a year will have high antibody titers. In the Netherlands, care for fetal alloimmunization is centralised and all severely immunized pregnancies are referred to the LUMC. In the LUMC, pregnant women with high antibody titers are followed with ultrasound examinations and, if deemed necessary, amniocentesis, until, in approximately 1/3 of them, suspicion of severe fetal anemia leads to the performance of an intrauterine transfusion. Yearly, around 100 intrauterine transfusions in approximately 30 fetuses are performed.

Outline of the thesis

This thesis is about the physiological basis and the diagnostic accuracy of two methods to asses the severity of fetal hemolytic anemia: measurement of bilirubin concentration in amniotic fluid on the one hand and fetal ultrasound on the other hand.

Part 1: Chemical approach

Since the 1960s, Δ OD 450 measurements have been the main diagnostic procedure for prediction of fetal anemia and since the 1990s, the Doppler technique is upcoming. We performed a Medline search *(chapter 2)* to compare those two diagnostic tools.

The Δ OD 450 technique has been shown to have good diagnostic qualities from 27 to 36 weeks of pregnancy. In *chapter 3,* we assessed the diagnostic accuracy of amniotic fluid Δ OD 450 in our patient population from 18 weeks onwards.

It is still unknown how bilirubin enters the amniotic fluid. We studied *(chapter 4)* the relationship between bilirubin concentration in amniotic fluid and fetal blood in rhesus D alloimmunized anemic fetuses in order to speculate on the possible pathway of bilirubin from fetal blood to amniotic fluid.

Part 2: Ultrasonographic approach

We have been performing intrauterine transfusions with a large volume load since many years. We investigated *(chapter 5)* the effects of acute large increases of hematocrit on fetal blood flow velocities. This therapeutic experiment may help to explain the relationship between hematocrit and blood flow velocities in the fetus.

Theoretically, fetal anemia may lead to a reactive increase in cardiac contractility and, consequently, to an increased cardiac wall thickness and an increased cardiac size. In everyday practice, sonographers often mention their impression that the heart is increased in size in a fetus with anemia. Therefore, to test this hypothesis and to explore potential applications, we assessed *(chapter 6)* the diagnostic value of cardiac size measurements in the prediction of severe alloimmune anemia.

Another impression of sonographers during intra-uterine transfusions is that the contractility of the fetal heart is decreased after intrauterine transfusion. Therefore, we assessed the effect of fetal anemia and intrauterine transfusion on ventricular shortening fraction *(chapter 7)*.

It is hoped that the studies in this thesis will add to a better understanding of the physiologic changes and to a more accurate diagnosis and hence to the timely therapeutic intervention in fetal anemia.

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