Fetal fluid and protein dynamics
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Introduction

Fetal medicine is a relatively young and fast growing field of medicine. The first successful fetal treatment was the intrauterine blood transfusion for Rhesus hemolytic disease in 1963 (by Dr. A.W. Liley). Since then, both the number of indications as the number of fetal treatment options have expanded rapidly. With the introduction of ultrasound, prenatal diagnosis of a wide range of fetal diseases became possible. In addition, ultrasound guided techniques enabled the development of minimally invasive prenatal treatments. Therefore, ultrasound and fetal medicine are inextricably linked. Sonographic observations provided a growing insight in the physiology and the pathophysiology of human fetuses. However, as a young and developing field of medicine, many questions still have to be answered at a fundamental level.

Physiology is the basis for our understanding of both health and disease state. Research in this area in human adults has resulted in the development of many diagnostic tools, medicines and other treatment modalities. Furthermore, experimental animal studies have provided many answers on questions that were impossible to investigate in humans. However, distinct differences can make it difficult to translate findings from animal experimental models to the human situation. Especially the human placenta is a unique organ, not comparable to that of any other mammal. Further, lessons can be learned from studies in premature neonates of the same gestational age as a fetus. However, radical changes take place after birth, especially in the cardiovascular system, making direct comparisons often impossible.

As a tertiary fetal therapy center, the LUMC has an obligation, besides high quality patient care and performance of clinical trials, to develop scientific projects on basic research level. A unique possibility is provided to study human fetal (patho-) physiology, by the access to the fetal circulation and amniotic fluid during treatment of several fetal diseases. Both the etiology of prenatal diseases and the reaction of fetuses on prenatal treatment can be investigated. Potentially treatable fetal diseases include fetal anemia, twin related problems, primary cardiac failure, primary hydrothorax and other causes of non-immune hydrops fetalis. All of these diseases can lead to abnormal amniotic fluid volumes, i.e. oligo- or polyhydramnios, which can result in a premature delivery of a neonate in a critical condition. Moreover, all of these diseases inevitably lead to the development of hydrops fetalis, eventually leading to intrauterine fetal demise. To the surviving fetuses, impaired neurological outcome poses a serious threat.
The development of abnormal amniotic fluid volumes and hydrops fetalis form the final common pathway of all these fetal diseases and are in fact shifts in fluid and protein in the different fetal compartments. These shifts take place between the amniotic fluid and the intravascular and the interstitial compartment in both the fetus and the placenta. The mechanisms involved in these **fetal fluid and protein dynamics** will be investigated in the studies described in this thesis. With increasing insight in the pathophysiological processes, improvements can be made in the diagnosis of disease stages, in timing of fetal treatment, in new treatment modalities and even in preventive strategies for these high risk prenatal conditions.

From clinical practice, several questions arose. First of all, diagnosis of fetal anemia and the timing of intrauterine blood transfusion has been a subject of interest at our department since several decades. One of the oldest diagnostic tools, measuring bilirubin content in amniotic fluid, developed by Bevis in 1956 [1] and introduced into clinical practice by Liley in 1961 [2], has been a clinically useful tool for many years. Surprisingly, detailed studies on the background of this test are lacking. Secondly, treatment of fetuses with intrauterine blood transfusion still carries a substantial risk of complications or fetal loss. Treatment methods were developed empirically, and are practically unchanged since the late 1980s. More basic knowledge on fetal condition during developing anemia and on fetal reaction to blood transfusion could potentially lead to adaptation and refinement of management protocols, with increased safety of this procedure.

Finally, the studies described in this thesis are aimed to bring forth an increased understanding of fetal physiology in general. This can contribute to the development of fetal therapy for a wider range of obstetric complications as oligo- and polyhydramnios and intrauterine growth restriction.
Background

The studies in this thesis were performed in fetuses with hemolytic alloimmune anemia. Yearly, around 90 intrauterine blood transfusions are performed in the LUMC to treat this disease.

Fetal hemolytic alloimmune anemia

Hemolytic alloimmune anemia used to be the main cause of hydrops fetalis [3] and one of the most important causes of perinatal death before 1960 [4]. It was commonly referred to as erythroblastosis fetalis. In red cell alloimmunization a woman’s immune system is sensitized to foreign red blood cell surface antigens, stimulating the production of IgG antibodies. The most common routes of maternal sensitization are via blood transfusion or after feto-maternal hemorrhage. Feto-maternal hemorrhage can occur for example during spontaneous or induced abortion, ectopic pregnancy, trauma, invasive obstetric procedures, and delivery, espacially traumatic parturition, with consequences for a subsequent pregnancy. The antibodies can cross the placenta and, if the fetus is positive for the red blood cell surface antigens, lead to hemolysis of fetal red blood cells and fetal anemia. Of the more than 50 different antigens causing hemolytic disease in the fetus and newborn, the D antigen of the Rhesus blood group system (Rh D) causes the most cases of prenatal severe hemolytic disease in the fetus and newborn [5;6]. With the introduction of intrauterine blood transfusion, the possibility of prenatal detection of anemia, improved neonatal care and last but not least the preventive administration of anti-D immunoglobulins, an enormous decrease has taken place of alloimmune anemia and immune hydrops fetalis, in the last 40 years [7;8].

Diagnosis of fetal hemolytic anemia

The diagnosis of fetal hemolytic anemia can be established by fetal blood sampling. However, the invasive nature of this procedure introduces a risk to the pregnancy and to further boosting of alloimmunization. Signs of fetal anemia can be observed with ultrasound i.e. cardiomegaly, hepato- and splenomegaly and signs of hydrops. Furthermore, fetal anemia can be predicted by Doppler blood flow measurements or by amniotic fluid analysis. Both of these diagnostic tools are quite accurate in prediction of fetal anemia [9;10], however, the measurement of the peak systolic velocity in the middle cerebral artery can predict severe anemia with higher accuracy than bilirubin determinination in amniotic fluid [9]. Moreover, the great advantage of sonographic measurements is the fact that it is not harmfull for the pregnancy. However, below 18 weeks and above 36 weeks of gestation, the measurement of the peak systolic velocity in the middle cerebral artery appears less accurate or more
difficult to obtain. Also, maternal obesity, abnormal position of the fetus or concomitant pathology can make prediction of fetal anemia with ultrasound difficult. Then measurement of bilirubin content (usually delta OD450 measurement) by amniocentesis may help the clinician in timing of the more invasive cordocentesis and a first transfusion. The so-called Liley or Queenan charts show the cut-off values for bilirubin content in amniotic fluid that indicate the risk of fetal anemia. The diagnosis of fetal anemia is finally confirmed by the sampling of fetal blood.

**Intrauterine transfusion**

Intrauterine transfusion is an ultrasound guided procedure. Puncture of the umbilical vein is performed either at the cord insertion, through the anterior placenta, or in the intra-abdominal hepatic portion of the umbilical vein. Fetal blood is sampled for analysis and an intravascular blood transfusion can be performed. Another option is an intraperitoneal transfusion of donor blood. Red cells will then be absorbed from the peritoneal cavity, through lymphatic drainage, towards the intravascular compartment. Although this method was replaced by the intravascular method in the 1980s, renewed interest has brought it back to use recently, often in combination with intravascular transfusion to prolong the transfusion-interval. At the LUMC, intrauterine transfusions are performed as early as 16 weeks of gestation and repeat transfusions are given every 2-5 weeks up to 35 weeks [11]. After birth, aimed between 36 and 38 weeks of gestation, phototherapy, transfusions and/or exchange transfusions may be necessary to treat recurrent anemia and hyperbilirubinemia. The procedure related risk of fetal loss is 1.6% for every intrauterine transfusion [12]. Risk factors are low gestational age and severe hydrops. Improvement of the most commonly used fetal therapy is therefore still an important subject of investigation.

**Hydrops fetalis**

Hydrops fetalis is the condition where a fetus retains an abundant amount of fluid. It is defined as the presence of an abnormal fluid collection in two or more fetal compartments. It can be recognized on ultrasound as fetal ascites, pericardial effusion, hydrothorax, (generalized) subcutaneous tissue edema, placental edema or polyhydramnios. Hydrops fetalis can be classified as immune or non-immune hydrops, based on whether or not alloimmunization underlies the etiology. This classification was traditionally used, since non-immune hydrops usually was not treatable and implied a poor prognosis. Recently, classification as anemic or non-anemic hydrops has been proposed [13]. Nowadays, this is a useful classification, since many hydropic fetuses can benefit from an intrauterine blood transfusion. Besides alloimmune hemolytic anemia, this includes cases of Parvo B19 viral infection or feto-maternal
hemorrhage. Other groups of causes can be identified that can benefit from fetal therapy [14]. One of these groups are twin related problems, such as twin reversed arterial perfusion sequence or twin-to-twin transfusion syndrome, that can be treated with laser ablation of intertwin connecting blood vessels. Other causes include primary hydrothorax that can benefit from thoraco-amniotic shunt placement or fetal arrhythmia that can benefit from transplacental drug treatment. Chromosomal, genetic, or metabolic disorders or congenital infections as CMV should be excluded since these are generally non-curable causes of hydrops fetalis.

The similarity between both anemic and non-anemic hydrops fetalis is the occurrence of cardiovascular changes, either as a primary or a secondary effect. Understanding of the cardiovascular pathophysiology in fetal hemolytic anemia and immune hydrops can therefore be helpful in the understanding of many other fetal diseases and might improve different types of fetal therapy.
Outline of the thesis

The studies described in this thesis explore fetal pathophysiology in hemolytic anemia and immune hydrops fetalis. Measurements performed in fetal blood as well as in amniotic fluid, before or during intrauterine transfusion, were used for our research.

The studies in this thesis can be summarized as follows:

The mechanism behind the curve of the so-called Liley chart has never been fully understood. In chapter two, we investigated the relation between bilirubin concentration in fetal blood and that in amniotic fluid. We hypothesized on the most plausible pathway for bilirubin to enter and leave the amniotic fluid.

In chapter three, we tested the hypothesis that the concentration of bilirubin is determined by the binding to albumin. Thereby we tried to explain the relation between fetal anemia and the Liley chart. This led to the next question: how does albumin enter and leave the amniotic fluid?

In chapter four, we reviewed the available evidence on the origin of albumin in amniotic fluid and the transport mechanisms that determine amniotic fluid composition. We speculate on the function of albumin in amniotic fluid and propose directions for future research and development of new fetal therapy strategies.

In chapter five, we investigated whether low albumin concentration was a causative or secondary effect in the development of hydrops fetalis. Concentration of albumin in fetal blood was analyzed to assess the relation with severity of anemia and severity of hydrops.

Fetal cardiovascular physiology may be distinct from adults and even from neonates. In chapter six, the maintenance of blood volume was investigated. The effect of severity of anemia and the presence of hydrops on total fetoplacental blood volume were analyzed.

In chapter seven, we investigated the extravascular fluid shift that takes place from the fetal circulation during intrauterine transfusion. The effect of volume and speed of transfusion, and the severity of anemia and presence of hydrops were analyzed.
Purpose of this thesis was to gain insight in human fetal (patho-)physiology. In chapter eight, old, current en acquired knowledge of fetal fluid and protein dynamics is described. Furthermore, implications for current practice that followed from our studies are discussed and implications for future research are proposed.

Finally, chapter nine summarizes the results of the presented studies.

References
