Chapter 7

Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies

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Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies. Submitted for publication.
ABSTRACT

Objective To evaluate the clinical significance of fetal thrombocytopenia in Rhesus D alloimmunized pregnancies.

Study design Fetal platelet counts were measured before 914 intrauterine blood transfusions in 318 Rhesus D alloimmunized pregnancies, and correlated with the presence and severity of hydrops, hemorrhagic complications and perinatal outcome.

Results Severe thrombocytopenia (platelet count < 50 x 10⁹/L) was found in 25/914 (3%) of all fetal blood samplings (22 fetuses), and in 7/30 (23%) severely hydropic fetuses. Prolonged bleeding (> 300 sec) occurred in 14/215 (7%) cases of transamniotic blood sampling, all with platelets > 50 x 10⁹/L. In 5/23 (22%) fetuses with hemorrhagic complications platelet count was < 50 x 10⁹/L. Perinatal mortality in fetuses with severe thrombocytopenia was 8/22 (36%).

Conclusion Thrombocytopenia is common in hydropic anemic fetuses. Severe thrombocytopenia is associated with a poor prognosis, irrespective of the presence of hydrops. The option of having platelets available at blood transfusion in severely hydropic anemic fetuses needs further study.
INTRODUCTION

Rhesus D alloimmunization in pregnancy may cause destruction of fetal red cells with progressive fetal anemia, which, untreated, may lead to fetal hydrops and perinatal death. As Rhesus D antigens are exclusively expressed on the surface of the red cells, all sequellae of Rhesus D antibodies are direct consequences of the resulting fetal anemia and hemolysis. In our 20-year experience of treating Rh-alloimmunized pregnancies with intrauterine intravascular blood transfusion for Rhesus D induced fetal anemia, we encountered a number of anemic fetuses in which we additionally, and mostly unexpectedly, found severe thrombocytopenia. A few studies suggested an association between low platelet counts and fetal hydrops1-3.

Fetal thrombocytopenia may have grave consequences, such as intracranial hemorrhage (ICH) and prolonged, possibly life-threatening bleeding from the puncture site in the umbilical cord. Although thrombocytopenia is generally defined as a platelet count below 150 x 10^9/L, for severe thrombocytopenia with a risk for bleeding problems, a cut-off level of 50 x 10^9/L is commonly used1,2,4,5. Some authors recommended that intrauterine blood transfusions for fetal anemia should be combined with a platelet transfusion when fetal platelets were below 50 x 10^9/L.

The aim of our study was to evaluate the incidence and clinical consequences of severe fetal thrombocytopenia in pregnancies complicated by Rhesus D alloimmunization.

MATERIALS AND METHODS

Since 1965, the Leiden University Medical Center is the national referral center for the intrauterine treatment of fetal anemia in The Netherlands. We retrospectively evaluated prospectively collected data from all patients with red cell alloantibodies treated with one or more intrauterine transfusions from January 1988 till December 2005. From this cohort, we selected pregnancies with anti-Rhesus D (including a combination of anti-D and anti-C) alloantibodies in which fetal blood sampling was performed for suspected anemia, with known platelet counts. In a previous study, we found that in our series of Kell alloimmunized pregnancies, severe fetal thrombocytopenia did not occur6.

All fetal blood samplings were done by inserting a 22 or 20 G needle under continuous ultrasound guidance into the umbilical vein. A sample of 2-3 mL of
pure fetal blood was taken, followed by injection of saline to confirm correct placement of the needle tip. From the sample, 0.2 mL was immediately aspirated into a Sysmex F800 micro cell counter (C.Goffin, IJsselstein, The Netherlands) present in the procedure room, for assessment of hemoglobin concentration, hematocrit, MCV, and platelet count. Another 0.5 mL was collected into an ethylenediaminetetra-acetic acid solution and immediately sent to the hospital's central hematology laboratory for the same measurements and reticulocyte and erythroblast counts. In case of an automated platelet count < 100 x 10^9/L, a manual count was done. For this study, we used the data obtained from the central laboratory.

Within one minute following the fetal blood sampling, the fetal hemoglobin and hematocrit levels were available from the cell counter, and packed donor red cells were transfused until the desired post transfusion hematocrit of around 45% was reached. Details on our treatment method have been described previously. Transfusion number, gestational age and presence of hydrops at the time of the fetal blood sampling were recorded. Hydrops was further subdivided into mild or severe hydrops, according to previously described criteria. In summary, the presence of a distinct rim of ascites with or without pericardial effusion, hydrops was classified as early or mild hydrops. Hydrops was classified as severe when ascites was abundant (free floating intra-abdominal organs) with or without pericardial effusion, skin edema, and pleural effusion.

Possible associations between severe thrombocytopenia and hydrops, hemorrhagic complications and perinatal outcome were assessed. Hemorrhagic complications were defined as fetal distress during fetal blood sampling with evidence of prolonged bleeding or formation of a hematoma, or evidence of fetal or neonatal ICH. In most cases, successful transfusion causes hydrops to resolve, therefore we assessed the occurrence of thrombocytopenia according to the severity of hydrops using only data from the first fetal blood sampling.

In transfusions performed via transamniotic needling of the cord, the time of bleeding visible on ultrasound from the puncture site was measured after removing of the needle. We investigated a possible association between the bleeding time and the degree of thrombocytopenia.

STATISTICAL ANALYSIS

The association between fetal hemoglobin concentration and fetal platelet count was analyzed using correlation and regression analysis. The platelet counts be-
between the nonhydropic group, the mild hydrops group and the severe hydrops group were analyzed using analysis of variance followed by pair wise comparisons using the Mann-Whitney test, with $p$-value of less than 0.01 was considered statistically significant, to allow for multiple comparisons. Perinatal mortality rates between the severe thrombocytopenia groups and the other pregnancies were compared using the Fisher exact test.

RESULTS

A total of 982 fetal blood samples were performed in 318 pregnancies with Rhesus D alloimmunization. Platelet count data were unavailable from 68 procedures, leaving 914 blood sampling procedures for analysis. None of the women or fetuses suffered from other diseases known to be associated with fetal thrombocytopenia, such as infection, immune thrombocytopenic purpura, alloimmune thrombocytopenia or chromosomal abnormalities. Fetal hydrops was present in 88/318 (28%) of the Rhesus D alloimmunized pregnancies at the time of the first fetal blood sampling. In 30/318 (9%) the hydrops was classified as severe. Three fetuses were still hydropic at the second transfusion, with severe hydrops in two of them. A median of three transfusions were performed per pregnancy, with a range of 1 to 8. Overall perinatal survival was 296/318 (93%). Demographic characteristics and outcome of pregnancies of the cohort, divided into three groups according to the severity of hydrops, are summarized in Table 1.

Table 1: Demographic characteristics of 318 pregnancies complicated by RhD alloimmunization

<table>
<thead>
<tr>
<th></th>
<th>Nonhydropic</th>
<th>Mildly hydropic</th>
<th>Severely hydropic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at first IUT (weeks)</td>
<td>28 (16-35)</td>
<td>25 (17-34)</td>
<td>26 (17-34)</td>
<td>0.006</td>
</tr>
<tr>
<td>No of IUT</td>
<td>3 (1-8)</td>
<td>3 (1-6)</td>
<td>3 (1-6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>36 (23-38)</td>
<td>35 (26-37)</td>
<td>34 (18-38)</td>
<td>0.001</td>
</tr>
<tr>
<td>Vaginal birth, n (%)</td>
<td>143 (62)</td>
<td>37 (64)</td>
<td>14 (47)</td>
<td>0.233</td>
</tr>
<tr>
<td>Birth weight (gram)</td>
<td>2690 (650-3930)</td>
<td>2665 (1238-3695)</td>
<td>2312 (190-3600)</td>
<td>0.066</td>
</tr>
<tr>
<td>Antenatal death, n (%)</td>
<td>6 (3)</td>
<td>3 (5)</td>
<td>6 (20)</td>
<td>0.001</td>
</tr>
<tr>
<td>Overall survival, n (%)</td>
<td>221 (96)</td>
<td>55 (95)</td>
<td>20 (67)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values given as median (range) or actual numbers (percentage)
IUT: intrauterine transfusion
Fetal thrombocytopenia (platelet count < 150 x 10^9/L) was found in 241/914 (26%) fetal blood samples. Moderate thrombocytopenia (< 100 x 10^9/L) was present in 85/914 (9%) fetal blood samples, while in 25/914 (3%) platelet counts were below 50 x 10^9/L. These 25 fetal blood samples were taken from 22 fetuses. In 12 cases, severe thrombocytopenia was found at the first fetal blood sampling, in 2 cases at the second, in 6 cases at the third, in 1 case at the fourth and in 1 case at the fifth fetal blood sampling. In Table 2, the fetal platelet counts at the first fetal blood samplings are given and compared according to the severity of hydrops. The median platelet count was significantly lower in the severe hydrops group, compared to the other two groups (p < 0.01).

Table 2: Fetal platelet count and hemoglobin concentration at the first fetal blood sampling in 318 RhD alloimmunized pregnancies according to the severity of hydrops

<table>
<thead>
<tr>
<th></th>
<th>Nonhydropic</th>
<th>Mildly hydropic</th>
<th>Severely hydropic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (x 10^9/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>230</td>
<td>58</td>
<td>30</td>
<td>0.001</td>
</tr>
<tr>
<td>33 (14)</td>
<td>156 (47-334)</td>
<td>23 (77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (1)</td>
<td>2 (1)</td>
<td>7 (3)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin conc. (g/dL)</td>
<td>6.2 (1.9-13.2)</td>
<td>3.5 (1.5-8.7)</td>
<td>3.0 (1.5-4.8)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values given as median (range) or actual numbers (percentage)

Figure 1 shows the correlation between the fetal platelet count and the hemoglobin concentration at first fetal blood sampling for the complete cohort (R^2 = 0.224, p < 0.001).

In Figure 2, the duration of bleeding from the puncture site as seen on ultrasound is given in relation to the platelet count before intrauterine transfusion in cases where transamniotic cord needling was done and platelet counts were available (n = 215). The correlation is weak but statistically significant (R^2 = 0.042, p = 0.003). The mean time of visible bleeding was 135 seconds (range 1-600). In 14 fetuses, prolonged bleeding occurred, defined as a duration of > 300 sec; all had platelet counts above 50 x 10^9/L. The 7 fetuses with platelet counts below 50 x 10^9/L had a median bleeding time of 180 sec, range 60-240, which was not different from the group of fetuses with platelet counts > 50 x 10^9/L (p = 0.62).
**Figure 1:** Correlation between fetal platelet count and fetal hemoglobin concentration at the time of the first fetal blood sampling in 318 RhD alloimmunized pregnancies

![Figure 1](image1.png)

R² Linear = 0.224  
\( p < 0.001 \)

Hb fetal hemoglobin concentration  
IUT intrauterine transfusion

**Figure 2:** Correlation between fetal platelet count before intrauterine blood transfusion and the duration of bleeding from the puncture site as seen on ultrasound, in 215 cases of transamniotic fetal blood sampling in RhD alloimmunized pregnancies

![Figure 2](image2.png)

R² = 0.042  
\( p = 0.003 \)
In a previous study, we analyzed complications of intrauterine transfusions, dividing them in procedure-related or disease-related complications. Using the same definitions, we found 23 procedure-related complications in this cohort of 914 transfusions (3%). In five of these 23 cases (22%), fetal platelet count was found to be below 50 x 10^9/L. In 891 transfusions without procedure-related complications, severe fetal thrombocytopenia was found in 20 blood samples (2%) (p < 0.001). Details of the 5 complications associated with low platelet counts were analyzed further, revealing that 3/5 showed excessive hemorrhage or hematoma formation had occurred. One of these survived after an emergency caesarean section at 34 weeks. This neonate had a small grade I bleeding and adverse neurological outcome, possibly related to perinatal asphyxia which caused infantile encephalopathy.

In the group of survivors with fetal thrombocytopenia during at least one fetal blood sampling, 2/14 (14%) had an ICH at cranial ultrasound in the first week of life, versus 4/282 (1%) in the group of fetuses without a severe thrombocytopenia (p < 0.05). These six ICH’s consisted of four small grade I bleedings, possibly related to prematurity, one large combined subarachnoid and tentorial bleeding (in the severe thrombocytopenic group) and one older hematoma located in the left frontal lobe in a neonate with a congenital cerebellum defect (in the non-severe thrombocytopenic group).

Perinatal mortality in the severely thrombocytopenic group at any fetal blood sampling was 8/22 (36%), significantly higher than the mortality of 14/296 (5%) in fetuses with platelet counts that never dropped below 50 x 10^9/L (p < 0.001).

In Table 3, the separate and combined effects of fetal thrombocytopenia and hydrops on perinatal mortality are given.

**Table 3:** Perinatal mortality and severity of hydrops according to fetal platelet count (plt) in pregnancies with RhD alloimmunization

<table>
<thead>
<tr>
<th>Plt &gt; 50 x 10^9/L</th>
<th>Plt &lt; 50 x 10^9/L</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perinatal mortality</td>
<td>Perinatal mortality</td>
<td>P-value</td>
</tr>
<tr>
<td>No hydrops</td>
<td>8/220 (4%)</td>
<td>1/10 (10%)</td>
</tr>
<tr>
<td>Mild hydrops</td>
<td>2/55 (4%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>Severe hydrops</td>
<td>4/21 (19%)</td>
<td>6/9 (67%)</td>
</tr>
<tr>
<td>All fetuses</td>
<td>14/296 (5%)</td>
<td>8/22 (36%)</td>
</tr>
</tbody>
</table>
COMMENT

Our evaluation of a large cohort of severe Rhesus D alloimmunized pregnancies demonstrates that, in addition to low hemoglobin levels, these fetuses may have decreased platelet counts. Fetal thrombocytopenia occurred more often in hydropic anemic fetuses and was inversely correlated with the severity of hydrops. Perinatal mortality was also highly associated with severe thrombocytopenia, as more than a third of the fetuses in this group died, while more than 95% of non-thrombocytopenic fetuses survived.

Our results confirm findings from two earlier smaller studies. Van den Hof and Nicolaides found platelet deficits > 2SD below the mean for gestational age in one third of fetuses with immune hydrops, and in only 2% of nonhydropic anemic fetuses\(^1\). Saade \textit{et al}. found severe thrombocytopenia in 10% of the hydropic anemic fetuses versus 2% in the non-hydropic group\(^3\).

In our non-hydropic and mildly hydropic groups however, thrombocytopenia was also associated with poor outcome.

In our study, none of the fetuses with prolonged bleeding had severe thrombocytopenia, and in the severely thrombocytopenic group, bleeding time was within the normal limits. A limitation of this analysis was that this measurement was only possible in transamniotic cordocentesis, while we performed most of our transfusions directly in the cord root or in the intrahepatic portion of the umbilical vein, in which case bleeding either does seem to not occur or is less visible on ultrasound. One previous study reported on post puncture bleeding, and showed an increased mean bleeding time in eight procedures with a fetal platelet count below \(50 \times 10^9/\text{L}\). Their mean bleeding time after transamniotic umbilical venous cord puncture with a 22 G needle was 144 seconds, remarkably similar to our findings. They also performed \textit{in vitro} experiments, and estimated an expected blood loss from a venous puncture site to be between 5 and 13 mL/min and probably less in vivo. The impact of such blood loss depends on the fetoplacental volume and thus on gestational age. In extreme cases, prolonged bleeding from the puncture site may cause fetal compromise but other factors than thrombocytopenia are apparently involved.

The main clinically relevant question is whether we can prevent deaths or adverse outcome related to fetal thrombocytopenia using intrauterine platelet transfusions, as was suggested by Saade \textit{et al}.\(^3\).

One option would be to have platelets available at any intrauterine blood transfusion in a severely hydropic fetus, including the ability to rapidly perform
a platelet count during the procedure. In our series, this would have meant the preparation and costs for 30 platelet transfusions at a total of 914 (3%) red cell transfusions, actually giving the platelets in nine cases to potentially save three of the six fetuses that died, as the other three deaths were not related to hemorrhagic complications.

A second option would be to include also the mildly hydropic cases. This would mean preparing platelets for 88/914 (10%) transfusions, transfusing platelets in three additional cases to possibly save one additional child.

At least the first option seems a reasonable one. However, platelet transfusion itself may be associated with additional complications due to the extra volume given. This may particularly affect severely hydropic, thus already compromised fetuses. Secondly, there is no guarantee that platelet transfusion prevents hemorrhagic complications in fetuses with thrombocytopenia. Adverse outcome in fetuses suffering from alloimmune thrombocytopenia due to bleeding after cordocentesis despite rapid platelet transfusion has been reported\textsuperscript{10,11}. Despite the relatively large series of patients, our study cannot provide strong evidence in favor or against prophylactic platelet transfusion in red cell alloimmunization.

Our analysis was not designed to solve the problem of the etiology of thrombocytopenia in anemic fetuses. Both an increased consumption, increased destruction, a decreased production, or a combination may play a role. In most cases severe thrombocytopenia existed already at the first fetal blood sampling. This rules out the important suppressive role of intra uterine platelet transfusion as a cause for fetal thrombocytopenia.

Given the rarity of this disease, the best way to increase our understanding of the various aspects of thrombocytopenia in red cell alloimmunized pregnancies is a prospective international multicenter collaboration.

In conclusion, our study shows that fetuses with severe anemia due to Rhesus D alloimmunization, particularly those with severe hydrops, may show a decreased platelet count. This group of fetuses constitutes the highest risk group for adverse outcome. Clinicians managing these pregnancies may consider having platelets available when transfusing these fetuses, although at present there is no evidence that this policy does more good than harm.
REFERENCES


FETAL THROMBOCYTOPENIA: PREVENTIVE STRATEGIES