Management of Alloimmune Thrombocytopenia: Antenatal Diagnosis and in utero Transfusion of Maternal Platelets

By C. Kaplan, F. Daffos, F. Forestier, W.L. Cox, D. Lyon-Caen, M.C. Dupuy-Montbrun, and Ch. Salmon

Neonatal alloimmune thrombocytopenia (NAIT) can cause severe bleeding in the central nervous system (CNS) and death or severe neurologic sequelae. The expression of the PL$^{A1}$ antigen is detectable as early as 19 weeks of gestation. Alloimmunization can therefore lead to fetal thrombocytopenia very early in pregnancy. Until recently, we have had no means of detecting and assessing the severity of fetal thrombocytopenia during pregnancy. The level of the maternal antibody is not of a predictable value since 20% of the mothers had no circulating antibodies in our series. An alternative approach is to carry out investigations on fetal blood samplings. This management leads to an exact knowledge of the fetal status and antenatal diagnosis is feasible as early as the 21st week of gestation. Early diagnosis facilitates appropriate management and makes possible such therapeutic options as in utero maternal platelet transfusions. We report our experience in the antenatal diagnosis and management of nine cases with in utero transfusion in the six cases with severe thrombocytopenia. All neonates did well, with no signs of bleeding at birth. No side effects of therapy were noted after a period ranging from 6 months to 3 years.

Neonatal alloimmune thrombocytopenia (NAIT) due to maternal immunization against fetal platelet antigens affects ~1 in 3,000 live births. The PL$^{A1}$ antigen is the antigen most frequently implicated. This condition must be investigated further because of the associated high perinatal morbidity and mortality, most being secondary to intracranial hemorrhage (ICH). Generally, ICH has been assumed to occur during delivery, but increasing evidence shows that ICH may have already occurred in utero. The risk of subsequent pregnancies being affected is very high (88% to 97%).$^{2,4}$ and management must be aimed at preventing severe complications, especially birth trauma.

The expression of the PL$^{A1}$ antigen is detectable as early as 19 weeks of gestation. Alloimmunization can therefore lead to fetal thrombocytopenia very early in pregnancy. Of PL$^{A1}$-negative women who become sensitized to the PL$^{A1}$ antigen, the highest risk group is comprised of women who are HLA-B8, DR3.$^{1}$ Detection of thrombocytopenia early in pregnancy is important if a woman has a previous history of NAIT or if she is at high risk because her first child may be affected.$^{4}$ Early diagnosis facilitates appropriate management and makes possible such therapeutic options as in utero maternal platelet transfusions. Preliminary data have been presented,$^{2}$ and we report our experience in the antenatal diagnosis and management of nine cases.

MATERIALS AND METHODS

Patients. Nine women were considered at risk for NAIT. In seven cases, a previous child had NAIT, sometimes with severe neurologic sequelae. The pertinent clinical features of each patient are summarized in Table I. In five cases, the women were referred to our center for the second pregnancy, in case 3 for the third pregnancy, and in case 6, for the fourth pregnancy. In the two other cases, there was no previous history of NAIT, but the women were considered at risk because they were PL$^{A1}$ negative, HLA-B8 DR3 and PL$^{A1}$ incompatible with the putative father. One patient had a sister whose child died in the neonatal period with clinical consequences of severe alloimmune thrombocytopenia.

Fetal blood samplings and in utero transfusion. Fetal blood sampling (FBS) was done by direct puncture of the umbilical vein near the cord insertion under ultrasound guidance as previously described.$^{9}$ Three to four milliliters blood was taken and divided into aliquots for assessment of purity,$^{10}$ typing, and serologic studies. Platelet count and volume were performed on a Coulter S + Il. Low platelet count was checked with a manual technique using a Unopette (Becton Dickinson, Grenoble, France). The in utero transfusions were performed using the same procedure as for FBS. Selective immobilization of the fetus was performed before transfusion by intravenous (IV) injection of vecuronium (0.1 mg/kg). Fetal movements were thus avoided during transfusions. The transfusion volume was calculated according to the formula: V = VSF (C3 - C1)/C2, where VSF = estimated fetal blood volume (according to fetal weight estimation), C1 = platelet concentration before transfusion, C2 = platelet concentration in the product to be transfused (maternal platelet concentrate), and C3 = platelet concentration desired at the end of transfusion. This simple formula can be used for platelet transfusion, since the change in fetoplacental total blood volume induced by the transfusion is not significant.

Maternal platelets were obtained after centrifugation of whole blood or by an automated plasmapheresis a few hours before fetal transfusion. After separation of the platelets, the mother was infused with the RBCs. The maternal platelets were carefully washed, resuspended in normal AB plasma to remove any remaining anti-PL$^{A1}$ antibody, and irradiated before transfusion.

When there was a Rhesus-positive fetus with a Rhesus-negative mother, anti-D gammaglobulin prophylaxis was given. The quantity given depended on whether there had been transplacental access to the fetal circulation.

Serologic studies. Determination of the PL$^{A1}$ typing and quantification of platelet-associated immunoglobulin (PA-IgG) and circulating platelet antibodies were carried out according to the methods of Soulier et al.$^{11}$ and Muller et al.$^{12}$

Prenatal management. The initial FBS is performed at ~21 weeks of gestation, allowing enumeration of the fetal platelets, PL$^{A1}$ typing, and quantification of the PA-IgG. When the diagnosis of thrombocytopenia secondary to alloimmunization was made, our approach was to advise careful rest until 37 weeks of gestation. Ultrasound examination was done monthly throughout pregnancy,
Table 1. Maternal and Sibling History

<table>
<thead>
<tr>
<th>Case</th>
<th>Mother Ts parity</th>
<th>Sibling history</th>
<th>Platelet count (10^9/L)</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Maternal anti-PL A antibody at birth of sibling</th>
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<tr>
<td>1</td>
<td>G2P2</td>
<td>NAIT</td>
<td>10</td>
<td>Exchange-TS</td>
<td>No seqs</td>
<td>Neg. 1/16 after ab 2 1/16</td>
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<tr>
<td>2</td>
<td>G2P2</td>
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<td>25</td>
<td>TS random</td>
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<td>Pos., no titer.</td>
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<td>3</td>
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<td>4</td>
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<td>16</td>
<td>TS PL A neg.</td>
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<tr>
<td>5</td>
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<td>NAIT, purpura</td>
<td>10</td>
<td>Maternal TS</td>
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<tr>
<td>6</td>
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<td>NAIT, purpura</td>
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<td>No Tt spontaneous</td>
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<tr>
<td>7</td>
<td>G2P2</td>
<td>NAIT</td>
<td>17</td>
<td>IS PIA I,</td>
<td>No seqs</td>
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Table 2. Clinical and Laboratory Data

<table>
<thead>
<tr>
<th>Case</th>
<th>Week of Gestation</th>
<th>Fetal Platelet Count (10^9/L)</th>
<th>PA-IgG on Maternal Platelet</th>
<th>Anti-PL A antibody</th>
<th>In Utero Transfusion</th>
<th>Fetal Platelets After TS (10^9/L)</th>
<th>Fetal Platelets at Birth (10^9/L)</th>
<th>Way of Delivery</th>
<th>Birth</th>
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<td>51</td>
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<td>47</td>
<td>C M</td>
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<td>C F</td>
<td>2,720</td>
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<td>10</td>
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<td>Y(120.10^9)</td>
<td>180</td>
<td>190</td>
<td>C F</td>
<td>1,920</td>
<td>1-10</td>
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<tr>
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<td>C F</td>
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<td>5</td>
<td>Y(9.6.10^9)</td>
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<td>110</td>
<td>C F</td>
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<td>10-10</td>
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<td>8</td>
<td>37</td>
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<td>V F</td>
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<tr>
<td>9</td>
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<td>1/8</td>
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<td>190</td>
<td>180</td>
<td>V F</td>
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<tr>
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<td>15</td>
<td>15.000</td>
<td>Y(200.10^9)</td>
<td>180</td>
<td>190</td>
<td>V F</td>
<td>2,600</td>
<td>9-10</td>
</tr>
</tbody>
</table>

C: cesarean section; V: vaginal delivery; s, sec; w, weight.
At the end of pregnancy, in six cases (cases 2 through 7) FBS done in the third trimester revealed a severe thrombocytopenia with a fetal platelet count of less than 20 x 10^9/L. In one case (case 1), the platelet count was at 50 x 10^9/L. All fetuses, except case 1, received in utero transfusions and had a prompt increase in platelet count. Delivery was by cesarean section in the first four cases and vaginal in the last three (Table 2). In case 4, vaginal delivery was proposed, but was not feasible because of obstetric conditions (breech presentation and a small pelvis).

All infants had a clinical examination and hematologic study performed at birth and repeated during the neonatal period. Both a cerebral ultrasound and brain scan were performed as well. All neonates did well and had no signs of bleeding at birth. In case 2, which was our first case to be treated by in utero transfusion, we also transfused maternal platelets just after birth, but this appeared not to be necessary in the subsequent cases.

After birth, the platelet counts remained within the normal range in all but one case. This neonate, case 5, at age 10 days, received another maternal platelet transfusion because of decreasing platelet levels (16 x 10^9/L) and the appearance of clinical purpura. After this second transfusion, the platelet count remained stable and the outcome was good. No adverse side effects of therapy were noted after a period ranging from 6 months to 3 years. There has been no evidence of relapse.

Maternal anti-PLA1 antibody levels were measured during pregnancy. The results of serial estimations were variable (Table 2). In cases 1 and 2, anti-PLA1 antibody was negative, cases 3 and 4 showed no modification, cases 5 and 6 had a noticeable decrease from 1 in 16 to 1 in 1 (nondiluted) and from 1 in 16 to 1 in 4, respectively and case 7 increased from negative to 1 in 8.

At birth, PLA1 typing was done on cord blood and was repeated in three cases, 2 and 7 days after birth. We observed that PLA1 positive platelets were detectable at birth; on day 2, ~50% of the platelets were PLA1 positive, but 100% were positive by 7 days of age, demonstrating the rapid circulation of the infant's platelets.

Two women were considered at risk for NAIT with no previous history. In one case, the fetus was sampled at 24 weeks of gestation; the platelet count was normal and the platelet typing was PLA1 negative. The delivery was at full term without problem. In the second case, although the fetus was PLA1 positive, the platelet count remained within the normal range from 25 weeks of gestation (340 x 10^9/L) until 38 weeks (350 x 10^9/L) and vaginal delivery was therefore allowed.

**DISCUSSION**

Considering the severity of NAIT and the high risk of recurrence in subsequent pregnancies, the management of pregnancies at risk is therefore exacting. Because transplacental passage of platelet antibodies can occur as early as the fourteenth week of gestation, the possibility of maternal sensitization is an important issue in the antenatal prediction of fetal damage. It is not reliable to depend on monitoring of serial maternal anti-PLA1 antibodies, as we have shown that the maternal antibody level was not useful in predicting fetal thrombocytopenia, 20% of the women having no detectable circulating antibodies. When present, there was no correlation between the antibody level and the severity of the fetal thrombocytopenia. This study confirms that there is no adequate means for detecting and assessing the severity of fetal thrombocytopenia without performing FBS.

FBS is a much less invasive procedure than fetoscopy. The duration is usually <10 minutes and 3 to 4 mL pure fetal blood can be obtained. The risk of fetal bleeding and possible exsanguination from the puncture site on the cord is an important consideration. There was no excessive or unusual cord bleeding despite severe thrombocytopenia. The only serious bleeding observed in the center occurred in a case of Glanzmann's thrombasthenia.

Fetal platelet counts are very reliable, provided strict control is maintained in assessing the purity of the fetal blood sample. Activation of coagulation by amniotic fluid leads to platelet aggregation and will cause false results. The gross contamination by amniotic fluid is detected by the decrease of hematologic parameters and smaller quantities by the change in coagulant factor activities. Detection of maternal blood contamination relies on visualization of the hematologic parameters, RBC antigen typing, and estimation of βHCG. PLA1 typing on fetal platelets is feasible as early as 20 weeks of gestation; in NAIT, however, severe thrombocytopenia and the presence of maternal antibodies coating the fetal platelets can lead to difficulties in interpreting the platelet groups. The timing of the first FBS is open to debate since no prevention is possible during pregnancy. We believe that FBS should be performed as early as possible (ie, 18 to 21 weeks of gestation) for four major reasons: (a) detection of PLA1-negative fetuses not at risk of thrombocytopenia; (b) prenatal counselling (rest, frequent ultrasound examinations) in case of early fetal thrombocytopenia; (c) better knowledge of the natural history of the disease; and (d) possible prevention by maternal and/or fetal therapy in the near future. The appropriate management of NAIT will have a major effect on the perinatal outcome.

There is no effective prevention for fetal bleeding during pregnancy; the estimated incidence of fetal bleeding is 10%. We have demonstrated the short efficacy of platelet transfusions, and similar results have been obtained by Waters et al. Maternal treatment with IV globulin, used in only one case, had no apparent effect on the fetal platelet count, and it is not feasible to transfuse the fetus repeatedly until birth. Maternal plasma exchange to attempt to decrease antibody levels has been suggested, but the effect is not certain and must be reevaluated as there is no demonstrable correlation between fetal platelet levels and maternal antibody titer.

To prevent severe complications, especially birth trauma, delivery by cesarean section, or fetal scalp vein platelet count to determine the most appropriate mode of delivery, has been proposed. Fetal scalp vein samplings are not easy to perform, however, and false results can be obtained if there is contamination with amniotic fluid causing platelet aggregation with false thrombocytopenia. Fetal bleeding, especially ICH, can occur at the onset of labor, and intrapartum cesarean section is not without risk for the infant. Transfusion of the infant
with platelets negative for the antigen to which the mother has developed an antibody is the preferable treatment. In NAIT resulting from anti-PLA\textsuperscript{1}, the mother is always PLA\textsuperscript{1} negative and is the most convenient donor. In our experience, antenatal maternal platelet transfusions promptly increased the in utero platelet count, therefore protecting the infant during delivery and immediately afterward, which is the time of greatest risk for ICH. Only one neonate required a repeat platelet transfusion at 10 days of age. No adverse effects were noted.

In the higher risk group, FBS should be performed during the first pregnancy for immunologic criteria or for family history (for example, if the patient has a sister with a history of NAIT), as this problem affects the first pregnancy in most cases.\textsuperscript{4,4} In any case, the risk of antiplatelet immunization due to the procedure itself should be considered, although the dose of platelets necessary for sensitization is unknown. In the absence of traumatism the high frequency of NAIT at first gestation suggests that the mechanism of immunization is very complex and differs from immunization against D antigen.

In conclusion, only FBS provides precise information on the fetal platelet status. Management using FBS is safe and feasible. Severe thrombocytopenia at the end of pregnancy can be treated by in utero transfusion of maternal platelets. The mother is usually the most convenient donor. This series illustrates the potential value of umbilical cord transfusions in allowing safe delivery, with the possibility of vaginal delivery when the fetal platelet count after transfusion is >150 \times 10^9/L. This will lead to the birth of healthy infants without bleeding in the neonatal period, thereby reducing the neurologic sequelae.

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