Prediction of the fetal status in noninvasive management of alloimmune thrombocytopenia

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Fetal/neonatal alloimmune thrombocytopenia is the most common cause of severe thrombocytopenia in the fetus and in an otherwise healthy newborn. To counter the consequences of severe fetal thrombocytopenia, antenatal therapies have been implemented. Predictive parameters for fetal severe thrombocytopenia are important for the development of noninvasive strategy and tailored intervention. We report here data concerning 239 pregnancies in 75 HPA-1bb women. Analysis of the index cases (diagnosis of fetal/neonatal alloimmune thrombocytopenia) did not show any significant correlation between the severity of the disease and the maternal genetic background (ABO blood group and HLA-DRB3 allele). Subsequent pregnancies were managed, and therapy effectiveness was evaluated. The highest mean newborn platelet count was observed for a combination of intravenous immunoglobulin and steroids (135 × 10^9/L; 54 newborns) compared with intravenous immunoglobulin alone (89 × 10^9/L; 27 newborns).

Methods

Patient cohort

Samples from 75 women (239 pregnancies), their partners, and infants were referred to our laboratory by clinicians in a context of F/NAIT. Informed consent for genetic investigations was obtained in accordance with the Declaration of Helsinki.

Data on individual cases from different centers in France and Switzerland were collected by our unit for diagnosis and therapy counseling. Diagnosis of alloimmunization was done either during pregnancy (FAIT) or at delivery (NAIT; index cases). All women were HPA-1bb, and the fathers were HPA-1aa, except in 3 cases, where fetal genotyping confirmed fetomaternal incompatibility for managed pregnancies. Severe fetal or neonatal thrombocytopenia was defined as a platelet count less than 50 × 10^9/L.

Subsequent managed pregnancies were classified according to a therapeutic option (chosen by each obstetric team), and homogeneity of the subgroups was carefully controlled (cases collected between 1981 and 2009):

- Steroids only (prednisone 0.5 mg/kg per day). These pregnancies occurred in the 1980s, before the implementation of the IVIG treatment. Two pregnancies were not included in this group because of short therapy.
- IVIG infusions only (1 g/kg per week, except for 1 case: 1 g/kg per 2 weeks).
- Steroids plus IVIG (1 g/kg per week, except for 1 case: 1 g/kg per 2 weeks).
- IVIG infusions only (1 g/kg per week, except for 1 case: 1 g/kg per 2 weeks).

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IVIG infusions (1 g/kg per week) plus steroids (prednisone 0.5 mg/kg per day): Steroids were administered only during the last trimester for most of the cases.

The number of IVIG infusions was recorded when possible. In 1 case, the mother received 2 g/kg per 3 weeks. Two managed pregnancies were not included in this group because of short therapy (3 IVIG injections).

The delivery was vaginal in the index cases and by cesarean section for all subsequent pregnancies. The term of delivery was not considered as a biologic criterion, as cesarean section is always planned.

For a limited number of cases, fetal blood sampling was performed to determine the fetal platelet count.

Maternal genetic parameters

HLA-DRB3 allele was determined by PCR-SSP (Dynal Allset SSP kit; Invitrogen) and ABO blood group was indicated by the clinicians.

Anti–HPA-1a quantification

The maternal anti–HPA-1a alloantibodies were quantified as previously described. Concentrations were given according to the international standard containing 100 IU/mL (NIBSC), corresponding to 813 arbitrary units per milliliter with our in-house reference serum. The maternal alloantibody concentration was determined at delivery in 57 cases.

To follow through the managed pregnancies, the anti–HPA-1a alloantibody concentration samples for 34 pregnant women were obtained from 17 plus or minus 5 weeks of gestation to delivery (6 ± 2 sera quantified per pregnancy).

Fetal and neonatal status and response to antenatal treatment

Therapy response was defined by the following criteria: (1) absence of ICH and (2) safe platelet count of 50 × 10^9/L or more.

Fetal and neonatal status and treatment effectiveness were analyzed taking into account the following data: (1) gynecologic history: gestation (abortions) and parity; (2) antenatal therapy; (3) maternal genetic parameters; (4) maternal anti–HPA-1a concentration during the managed pregnancy and at delivery; and (5) newborn platelet count at delivery and bleeding manifestations.

Statistical analysis

Qualitative variables are described using numbers and percentages, and quantitative variables by mean plus or minus SD. Univariate analysis was performed to assess the existence of a link between IVIG therapy efficiency and explanatory variables. Because of the small size of the groups, a multivariable analysis could not be made. We used the Mann-Whitney U test for mean comparisons and the Fisher exact test for percentage comparisons, using SAS software release 9.1 (SAS Institute). All tests were considered to be statistically significant at P < .05. The follow-up of the antibody concentrations was weighted by the weeks of gestation between the first and the last quantification, using MedCalc Version 8.0 software. Predictive values were determined by the receiver operating characteristic curves.

Results

**F/NAIT is a severe disease, and the first offspring is at high risk**

The diagnosis of maternal platelet alloimmunization was established either during the pregnancy (9 women) or at delivery (66 women). In this cohort, the pregnant women were primigravida in 51% of the cases.

In these index cases, severe thrombocytopenia was observed in 86% of the newborns from first pregnancies and 79% from multigravida (no statistically significant difference; Table 1). The deleterious consequences of this alloimmunization were evidenced by the severity of the fetal and neonatal conditions: 9 cases of ICH were documented (7 of 9 multigravida), and 3 cases were diagnosed in the postnatal period, leading to death for 2 neonates.

In the past history of multigravida (data available for 39 of 58 prediagnosis pregnancies), we observed 16 spontaneous abortions, voluntary abortion in 13 cases, medical abortion in 1 case (Down syndrome), and a fetal death because of a cardiac malformation.

Eight babies were born before the diagnosis of NAIT: 6 infants had no medical problem despite the HPA-1 fetomaternal incompatibility (no platelet count performed at the time), and 2 were thrombocytopenic but the NAIT diagnosis was not done at the time.

**Neonatal thrombocytopenia is not correlated with postdelivery maternal alloantibody concentration**

To search for factors associated with a severe condition in the index cases, the maternal antibody concentration was measured at delivery and maternal genetic factors (ABO and HLA-DRB3*01:01) were considered. The results show no significant relationship between the alloantibody concentration at delivery and the neonatal platelet counts, even if the antibody concentration at delivery for primigravida is significantly lower than for multigravida (P = .0032 Table 1).

In regard to the maternal blood group A or O (ABO distribution similar to the distribution in the French population), no significant difference emerged between the concentrations of the untreated mothers of blood groups A and O.

We did not observe any significant correlation between HLA-DRB3 allele and the maternal alloantibody concentration at delivery or the neonatal platelet counts.

**The most efficacious treatment for fetal alloimmune thrombocytopenia is maternal therapy with IVIG and steroids**

The control population “A” (no treatment administered during the pregnancy) included 62 index cases of NAIT discovery and 4 subsequent unmanaged pregnancies. These 4 cases do not induce a change in the statistical analysis.

Ninety-two pregnancies were managed using one of the following options: steroids (treatment category B, 11 pregnancies), IVIG
No ICH was recorded among the 92 managed pregnancies, and this was considered as the first criterion of treatment effectiveness (Table 2).

Steroids only were given during at least 7 weeks for 11 pregnancies (treatment category B). After therapy, the mean newborn platelet count was twice the mean platelet count for category A but still less than the “safe” threshold of 50 × 10^9/L for 8 of 11 newborns (73%). Seven newborns (64%) received a postdelivery treatment (platelet transfusion and/or IVIG).

Twenty-seven pregnancies were managed with IVIG only (treatment category C). The mean newborn platelet count was significantly increased (89 × 10^9/L) compared with non-managed pregnancies (category A). However, a severe thrombocytopenia less than 50 × 10^9/L was observed in 12 cases (44%).

Fifty-one pregnancies (3 twins) received both IVIG and steroids (treatment category D). The mean newborn platelet count (135 × 10^9/L) is higher than category C. Most important, only 13 newborns (27%) were severely thrombocytopenic.

To summarize, the mean newborn platelet count dramatically increased with IVIG plus steroids, and this is not because of variable IVIG courses (13.9 ± 4.3 for category C and 14.4 ± 4.2 for category D). Therefore, a significant lower proportion of newborns required postnatal treatment compared with the other groups, from 79% for category A to 26% for category D.

The maternal alloantibody concentration during pregnancy is predictive of fetal thrombocytopenia and is a prognostic factor for therapy response

In this study, we confirm that a high maternal alloantibody concentration measured before 28 weeks of gestation and before any treatment is correlated with a severe fetal thrombocytopenia.12 We refined the antibody concentration threshold, now corresponding to 28 IU/mL. This threshold allows improvement in the sensitivity (81.2%) and the specificity (83.3%) of the testing, with high positive and negative predictive values (86.7% and 76.9%, respectively; Table 3). Below the threshold of 28 IU/mL, the antibody concentration cannot be considered as a predictive parameter of the fetal status. This maternal parameter allows recognition of high-risk fetuses and the need for therapy.

The antibody concentration was observed during 34 managed pregnancies treated with IVIG with or without steroids. When women had had successive treated pregnancies, the analysis was reviewed only with the first treated pregnancy of each mother, but this did not change the statistics.

### Table 2. Response to therapy according to the antenatal management

<table>
<thead>
<tr>
<th>Parameter/treatment category</th>
<th>Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of women/pregnancies/newborns</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 66/66/66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 11/11/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 23/27/27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 46/51/54</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of intracranial hemorrhage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of newborns with severe thrombocytopenia (&lt; 50 × 10^9/L platelets)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 54 (82%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 8 (73%)</td>
<td>A/B: .4398</td>
<td></td>
</tr>
<tr>
<td>C 12 (44%)</td>
<td>A/C: .0003*</td>
<td></td>
</tr>
<tr>
<td>D 13 (27%)</td>
<td>A/D: &lt; .0001*; B/D: .0044*</td>
<td></td>
</tr>
<tr>
<td><strong>Mean newborn platelet count, × 10^9/L (95% CI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 23 (18-28) (n = 66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 46 (21-71) (n = 11)</td>
<td>A/B: .0074*</td>
<td></td>
</tr>
<tr>
<td>C 89 (55-123) (n = 27)</td>
<td>A/C: &lt; .0001*</td>
<td></td>
</tr>
<tr>
<td>D 135 (108-158) (n = 54)</td>
<td>A/D: &lt; .0001*</td>
<td></td>
</tr>
<tr>
<td><strong>No. of newborns who received a postnatal treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 42 (79%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 7 (64%)</td>
<td>A/B: .2682</td>
<td></td>
</tr>
<tr>
<td>C 16 (59%)</td>
<td>A/C: .0691</td>
<td></td>
</tr>
<tr>
<td>D 14 (26%)</td>
<td>A/D: &lt; .0001*; B/D: .0295*; C/D: .0066*</td>
<td></td>
</tr>
</tbody>
</table>

Treatment category A includes the index cases (n = 62) and 4 untreated subsequent pregnancies; category B, steroids alone; category C, IVIG; and category D, IVIG plus steroids.

CI indicates confidence interval.

*P < .05.

### Table 3. Maternal anti–HPA-1a alloantibody concentrations before 28 weeks of gestation and fetal platelet counts: statistical analysis

<table>
<thead>
<tr>
<th>Maternal antibody concentration, IU/mL</th>
<th>Platelet count, × 10^9/L</th>
<th>&lt; 50</th>
<th>≥ 50</th>
<th>P</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 28, n 3</td>
<td>.0016*</td>
<td>81.2</td>
<td>83.3</td>
<td>86.7</td>
<td>76.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 28, n 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

PPV indicates positive predictive value; and NPV, negative predictive value.

*P < .05.
The pattern of the antibody concentration follow-up was variable: steadily low antibody concentration (<28 IU/mL), decreasing toward delivery or increasing during the second or third trimester of pregnancy or just after delivery.

To analyze these curve tendencies, we measured the AUC of each antibody follow-up and weighted the AUC with the number of weeks’ gestation between the first and the last quantification. The weighted AUC was compared with the newborn platelet count at delivery, which was significantly higher for women who delivered severely thrombocytopenic newborns than with newborns with platelet count more than \( 50 \times 10^9/L \) \( (P = .0153 \text{ with the Mann-Whitney test}) \). The analysis of the receiver operating characteristic curve allowed the definition of a weighted AUC threshold of 24 IU/mL per week of gestation (Table 4).

### Multigravida and siblings with intracranial hemorrhage are risk factors for therapy failure

We analyzed the gynecologic history of the IVIG-treated women who delivered a severely thrombocytopenic neonate. Multigravida delivered 18 of 24 severely thrombocytopenic newborns and spontaneous abortion before the IVIG-treated pregnancy was delivered 18 of 24 severely thrombocytopenic newborns than newborns with platelet count more than \( 50 \times 10^9/L \) \( (P = .0153 \text{ with the Mann-Whitney test}) \). The analysis of the receiver operating characteristic curve allowed the definition of a weighted AUC threshold of 24 IU/mL per week of gestation (Table 4).

<table>
<thead>
<tr>
<th>Newborn platelet count, ( \times 10^9/L ) (95% CI)</th>
<th>&lt; 50</th>
<th>≥ 50</th>
<th>( P )</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>45 (28-62)</td>
<td>19 (10-28)</td>
<td>.0107*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( &lt; 24, n )</td>
<td>5</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ≥ 24, n )</td>
<td>9</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of newborns</td>
<td>14</td>
<td>23</td>
<td>.0153*</td>
<td>64.3</td>
<td>78.3</td>
<td>64.3</td>
<td>78.3</td>
</tr>
</tbody>
</table>

Weighted AUC and newborn platelet counts at delivery of 34 IVIG-treated pregnancies (37 newborns). CI indicates confidence interval; PPV, positive predictive value; and NPV, negative predictive value.

*\( P < .05 \) (CI for a-risk of 5%).

### Discussion

The present study focused on selected F/NAIT cases referred to our laboratory for investigation in a context of suspected alloimmune thrombocytopenia (at-risk population).

In this large series of cases, we searched for maternal parameters predictive of the severity of the disease, analyzed the efficacy of different therapeutic approaches in subsequent pregnancy, and looked for biologic markers predictive of response to therapy.

Case reports may overestimate the clinical severity we observe in the index cases, as profound thrombocytopenia was present in a large majority of the affected newborns. The retrospective nature of our series may have introduced a bias toward the most severe cases. However, it is noticeable that we found a high percentage of primigravida (51%) in the index cases confirming previous retrospective studies. In prospective studies, the incidence of alloimmunization in primigravida seems to be quite different. The discrepancies may be partly explained by the population enrolled in the study: at-risk versus nonselected population, the size of the screened population, and the ethnic diversity.

The severe thrombocytopenia in our index cases was not correlated either with the maternal alloantibody concentration at delivery or the maternal genetic background (ABO or HLA). However, studies for a relationship between maternal alloantibody concentration during the neonatal period and the newborn platelet counts have failed to reach a consensus, and the influence of maternal genetic factors on alloantibody production has been drawn from a prospective study. The retrospective nature of our series, as well as the differences in the gestation range, timing of maternal samplings, and the methodology used for the antibody titration have to be considered. The significantly higher postdelivery maternal anti–HPA-1a antibody concentration for the index cases in multigravida compared with primigravida may be explained by the stimulation of the maternal immune system during the previous pregnancy(ies).

Once diagnosed, as the disease is usually more severe in subsequent pregnancy with an incompatible fetus, antenatal therapy should be suggested. Antenatal management of F/NAIT has made considerable progress during the last 2 decades, from invasive strategies with fetal blood samplings and in utero platelet transfusion to noninvasive strategies. Nowadays maternal therapy is considered to be the first-line therapy.

In our study, subsequent pregnancies were treated according to one of the 3 options (steroids, IVIG, or both) and reduction of fetal blood samplings within the time period. During the first decade, one fetal blood sampling was performed for 77% of the pregnancies, and during the last 10 years, fetal blood sampling was done for only 27% of the pregnant women.

After antenatal therapy, no ICH was recorded whatever the option. Our study highlights that the most effective antenatal treatment is IVIG plus steroids, with a significant decrease in the proportion of newborns requiring postnatal treatment.

Because of the increasing trend toward proposing noninvasive strategies, development of biologic markers predictive of the severity of the fetal disease and of therapy effectiveness is important to be considered; however, this is highly dependent on the past history of F/NAIT.

In the present study, we show that maternal alloantibody concentration during pregnancy is predictive of the severity of the disease and of the response to therapy. In early gestation and before any therapy, we are able to confirm our previous results showing that maternal alloantibody concentration is predictive of severe fetal thrombocytopenia. This parameter is important to guide decisions on noninvasive treatment.
During managed pregnancy, the maternal antibody concentration follow-up may reflect a modulation of the maternal anti-HPA-1a IgG production and transportation across the placental barrier, and could also result from IVIG therapy modulation. The striking result we obtained was the observation, for the first time, that maternal alloantibody concentration measured during managed pregnancy could be a prognostic factor. The areas under the curves of the maternal alloantibody concentration weighted by the weeks of gestation were predictive of IVIG (with or without steroids) therapy failure. This parameter may be therefore used for salvage therapy and management at delivery to prevent severe hemorrhagic disorders of the neonate.

In conclusion, FNAIT is a severe disease, in a large number of cases occurring during first gestation, thereby preventing prophylactic approach in this context. Among the specific antenatal strategies, the combined therapy IVIG plus steroids was the most effective therapy. We have shown that maternal alloantibody concentration measurement and follow-up during pregnancy are essential predictive parameters in the assessment of fetal status and response to therapy, leading to the improvement of noninvasive strategies.

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Authorship
Contribution: G.B. performed the research, analyzed the data, and wrote the manuscript; M.D. performed the statistical analysis; C.M. performed the research; and C.K. designed the research, analyzed the data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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