CME/SAM

Fetal and neonatal alloimmune thrombocytopenia: predictive factors of intracranial hemorrhage

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BACKGROUND: In Caucasians, fetal/neonatal alloimmune thrombocytopenia (FNAIT) is most frequently caused by maternal alloimmunization against the human platelet antigen HPA-1a. The most serious complication of severe FNAIT is intracranial hemorrhage (ICH). ICH mainly occurs in utero; therefore, there is a need to identify noninvasive predictive factors of ICH to facilitate early identification of this condition and to determine response to maternal therapy.

STUDY DESIGN AND METHODS: We studied gynecologic and immunogenetic variables of severe cases of anti-HPA-1a FNAIT within three groups: Group I, FNAIT without ICH; Group II, FNAIT with ICH; and Group III, suspected FNAIT cases without detectable maternal anti-HPA-1a alloantibodies.

RESULTS: ICH was associated with a poor outcome because it led to death in 59% of cases. Multigravida (two or more pregnancies) was overrepresented in Group II, consistent with the high concentrations of maternal HPA-1a alloantibody and the frequent detection of a strong newborn-specific HLA class I antibody response at delivery. The proportion of HLA-DRB4*01:01P (*01:01 or *01:03) women was similar in Groups I and II, but this allele was overrepresented in Group III, in which FNAIT was less severe than in the other two groups. Finally, antenatal intravenous immunoglobulin therapy tended to be more effective in HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(+) women than for HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(–) women.

CONCLUSION: The number of gestations is a predictive factor of ICH in anti-HPA-1a–alloimmunized women. Maternal immunogenetic variables should be investigated in the context of maternal immunization and may predict response to maternal therapy in subsequent pregnancies.

Fetal/neonatal alloimmune thrombocytopenia (FNAIT) results from the destruction of fetal platelets (PLTs) by maternal alloantibodies that cross the placenta and target fetal PLT antigens inherited from the father. FNAIT is one of the most common causes of early onset severe isolated thrombocytopenia ($<50 \times 10^9$/L) in newborns and occurs in approximately one in 1000 births.[1] In Caucasians, the most frequently involved antigen in severe FNAIT is the human PLT antigen (HPA)-1a partly because antibody against this antigen impairs angiogenesis.[2] The HPA-1 antigenic system is defined as leucine-to-proline amino acid change at Residue 33 of the mature glycoprotein IIIa (β3 integrin). The presentation of HPA-1a peptides and subsequent production of anti-HPA-1a alloantibodies is associated with a particular human leukocyte antigen (HLA), HLA-DRB3*01:01.[3] In 90% of cases of HPA-1a fetomaternal alloimmunizations, the mother carries this HLA Class II molecule.[4] Recently, Loewenthal and colleagues[5] suggested

ABBREVIATIONS: FNAIT = fetal/neonatal alloimmune thrombocytopenia; ICH = intracranial hemorrhage; IQR(s) = interquartile range(s); MAIPA = monoclonal antibody-specific immobilization of platelet antigens; NSA(s) = newborn-specific HLA antibody(-ies); wg = weeks of gestation.

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that HLA-DRB4*01:01 also plays a role in HPA-1a peptide presentation. They observed that the combination of both DRB3*01:01 and DRB4*01:01 antigens was associated with more severe cases of FNAIT and affects response to antenatal treatment with IVIG.

No antenatal screening program exists; therefore, FNAIT is mostly diagnosed when a fetus or newborn is affected.[6] The most serious outcome of severe FNAIT is intracranial hemorrhage (ICH), leading to neurologic sequelae in 20% of reported cases or death in 10%.[7] Approximately 80% of ICH cases occur in utero.[8] Strategies of antenatal management have been developed given the high recurrence rate of severe thrombocytopenia in subsequent pregnancies with incompatible fetuses.[9-11] Such pregnancies are managed by the maternal administration of IVIG,[9] with an estimated rate of effectiveness near to 75%[6] when combined with corticosteroids, leading to the delivery of newborns with a "safe" PLT count of higher than 50×10^9/L. Only a small series of patients with ICH associated with FNAIT have been reported so far and no noninvasive predictive factors have been linked to its occurrence except the presence of ICH in a previous child.[12]

Here, we analyzed gynecologic and immunogenetic variables in patients with FNAIT with HPA-1a fetomaternal incompatibility to identify risk factors of ICH and factors predicting response to maternal therapy. In particular, we focused on obstetric history (number of gestations at the time of FNAIT diagnosis), maternal anti-HLA Class I alloimmunization, and maternal anti-HPA-1a alloantibody concentration and maternal HLA Class II typing.

MATERIALS AND METHODS

Study design

Cases were identified between 1987 and 2012 and referred to the Institut National de la Transfusion Sanguine for immunologic examination of PLTs after fetal or neonatal ICH or severe neonatal thrombocytopenia ($<50 \times 10^9$ PLTs/L). Cases were selected according to PLT genotype of the mother (HPA-1bb) and the infant (HPA-1ab). Informed consent for genetic investigations was obtained according to the Declaration of Helsinki. Three cohorts were defined according to the severity of the cases and the results of serologic tests.

The first two groups included FNAIT patients for whom maternal anti-HPA-1a alloantibodies were detectable by the gold standard "monoclonal antibody-specific immobilization of PLT antigens" method (MAIPA[13]). The first group included newborns without ICH ("Group I," n = 52 women), and the second included newborns with ICH ("Group II," n = 27 women) diagnosed either during pregnancy (17 cases) or at delivery (10 cases). The third group included HPA-1bb women with HPA-1a fetomaternal incompatibility but without detectable anti-HPA-1a alloantibodies. In this suspected FNAIT group, all infants were born with severe neonatal thrombocytopenia and no ICH was recorded ("Group III," n = 19 women).

Immunologic examination of PLTs

PLT genotype was determined by a high-throughput method based on beadchip technology (BioArray, Immucor, Warren, NJ). PLT antibodies were detected and anti HPA-1a alloantibodies were quantified by MAIPA, as previously described.[13,14]

HLA typing

HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 typing was performed by the polymerase chain reaction (PCR)-sequence specific oligonucleotide typing method, following the manufacturer's instructions (One Lambda, Canoga Park, CA).[15] Fluorescence was measured with a flow analyzer (Luminex 100, Luminex, Austin, TX). HLA-DRB3, -DRB4, and -DRB5 were genotyped by sequence-specific PCR primers (Olerup SSP, Stockholm, Sweden).[16]

HLA-DRB4*01:01 differs from HLA-DRB4*01:03 by one amino acid (Gly135Ser) that is located outside the HLA binding site; consequently, these alleles are considered phenotypically identical (HLA-DRB4*01:01P). The same situation was observed for HLA-DQB1*02:01 and HLA-DQB1*02:02 alleles (Gly135Asp), corresponding to the HLA-DQB1*02:01P phenotype.

The HLA Class II genotype frequencies of these three groups were compared with that of a "reference population" group of 100 healthy Caucasian hematopoietic stem cell donors. The HLA frequencies in this control group were similar to those recorded for Caucasians in an allele frequency website, which validates our control (www.allelefrequencies.net).

Detection of HLA antibodies

Maternal serum collected at delivery was screened for anti-HLA Class I antibodies (HLA-A and -B) by single antigen beads coated with purified recombinant HLA Class I antigens[17] (LabScreen single antigen Class I, One Lambda). Fluorescence was measured with a flow analyzer (Luminex 100, LABScan 100, Luminex). Sera were classified as positive for HLA antibodies if the normalized mean fluorescence intensity (MFI) was more than 1500. Newborn-specific HLA antibodies (NSAs) were defined by the presence in maternal serum of an HLA antibody against the newborn PLT HLA Class I alleles. MFI values of more than 3000 were considered for the distinction between HLA alloimmunized women with low and high levels of NSAs.

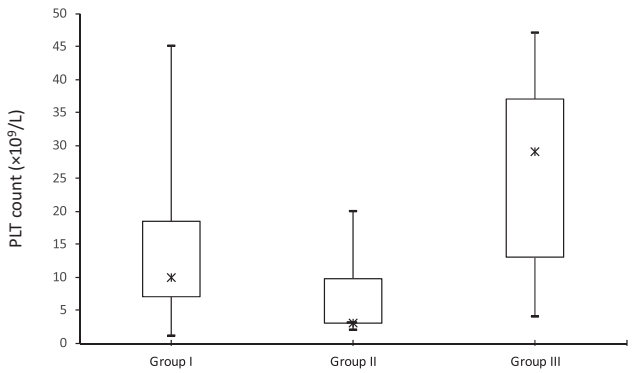

Fig. 1. Comparison of newborn PLT count at delivery. *Median value.

Gynecologic variables

The number of gestations per woman was reported. The predicted weight of infants was estimated according to the term of delivery using the table of Shinozuka and colleagues,[18] to identify "small for gestational age" newborns.

Therapy during subsequent pregnancies

Women treated with IVIG received 1 g of IVIG per kg/week starting from 20 to 28 weeks of gestation (wg) until delivery, usually associated with corticosteroids (1 mg/kg/day) starting at approximately 30 to 35 wg. Therapy was considered a success if the infant was born with a "safe" PLT count of more than 50×10^9/L.

Statistical analysis

Comparisons between percentages were performed with the Fisher's exact test, using the computer software (MedCalc, Mariakerke, Belgium). Comparisons of medians were done with the Mann-Whitney U test. The odds ratio (OR) with 95% confidence interval (CI) were used to evaluate the potential risk factor of FNAIT with ICH or suspected FNAIT. Newborn PLT counts were described by the median and the interquartile ranges (IQRs). Tests were considered significant if p values were less than 0.05.

RESULTS

Clinical description of the index cases

Gynecologic history

Multigravida was more common in Group II (81%) than in Group I (48%; p = 0.007; OR, 4.66; 95% CI, 1.43-18.22) or Group III (42%; p = 0.02; OR, 5.82; 95% CI, 1.09-35.9).

Clinical severity, ICH, and outcome

The median newborn PLT count was significantly lower in infants born to anti-HPA-1a–alloimmunized women than in those born to nonalloimmunized women (10×10^9/L; IQR, 5×10^9-14.5×10^9/L in Groups I and II vs. 29×10^9/L; IQR, 13×10^9-37×10^9/L in Group III; $p < 0.001$) and was lowest in the ICH group (3×10^9/L; IQR, 3×10^9-9.75×10^9/L in Group II vs. 10×10^9/L; IQR, 7×10^9-18.5×10^9/L in Group I; p = 0.004; Fig. 1).

ICH occurred in utero in 17 of the 27 cases, once during the second trimester of pregnancy (n = 1; 22 wg) and mostly during the third trimester (n = 16; 29-41 wg). In three of these 17 cases, ICH occurred during the first pregnancy. In the 10 newborns with ICH only identified at delivery, no fetal imaging had been performed during pregnancy. Pregnancies were carried to delivery in 100% of cases in Group I and in 89% in Group III, whereas half of all pregnancies in Group II were aborted (52%).

In Group II, ICH led to fetal or neonatal death in 59% of cases (four in utero deaths, 10 therapeutic abortions and two neonatal deaths). None of the infants died in Group I (Group II vs. Group I; $p < 0.001$) and the mortality rate was 11% in Group III (Group II vs. Group III; p = 0.002). Gestational term and birth weight at delivery were similar in the three groups.

Immunogenetics and anti-HPA-1a alloimmunization in the index cases

Maternal antibody concentrations were measured in the immediate postnatal period for 30 women from Group I and 17 women from Group II. The median concentration of maternal alloantibodies was significantly higher in Group II than in Group I (p = 0.002; Table 1).

Maternal HLA Class II typing for DRB1, DQB1, and the second molecule of DRB (DRB3-4-5) was performed if DNA was available (n = 29 in Group I, n = 16 in Group II, and n = 19 in Group III; Table 2). The frequency of HLA-DRB3*01:01 was significantly lower in Group III (21.05%) and in the control group (33%) than in Groups I and II (84.44%; $p < 0.001$).

HLA-DQB1*02:01P was also more frequent in Groups I and II than in Group III (p = 0.03). By contrast, HLA-DRB4*01:01P tended to be highly represented in women who did not develop antibodies against HPA-1a (63.16% in Group III vs. 40% in Groups I and II; p = 0.11; OR, 2.53; 95% CI, 0.75-9.2) and was significantly more represented in Group III than in Group II (p = 0.04; OR, 4.89; 95% CI, 0.98-29.66). This antigen was also more frequently present in Group III than in the control group (63.1% vs. 36%, respectively; p = 0.04*). Focusing on the two anti-HPA-1a–alloimmunized groups the proportion of HLA-DRB4*01:01P-positive women is lower in the ICH group (25% in Group II vs. 48.28% in Group I) as well as the HLA-DRB3/DRB4 haplotype expression. Indeed, in the last group only three of 14 HLA-DRB3*01:01-positive women (18.75%) carried the HLA-DRB4*01:01P allele whereas 11 of 24 women (37.93%) in Group I showed this

TABLE 1. Clinical and biologic data

	Detectable anti HPA-1a alloantibodies		No anti HPA-1a antibody: Group III, n = 19	p values
	Group I (no ICH), n = 52	Group II (ICH), n = 27		
Gestation	G1: 27 (52%) G ≥ 2: 25 (48%)	G1: 5 (19%) G ≥ 2: 22 (81%)	G1: 7/12 (58%) G ≥ 2: 5/12 (42%)	Groups I/II: p = 0.007 Groups II/III: p = 0.02*
Therapeutic abortion	0 (0%)	10 (37%)	2	
In utero death	0 (0%)	4 (15%)	0 (0%)	
% of delivery	52 (100%)	13 (48%) But 2 babies died	17 (89%)	Groups I/II: p < 0.001 Groups II/III: p = 0.01
Mortality rate*	0/52 (0%)	16/27 (59%)	2/19 (11%)	Groups I/II: p < 0.001 Groups II/III: p = 0.002
Term at delivery	38.3 ± 1.6 n = 35	38.1 ± 2.1 n = 12	37.7 ± 2.8 n = 13	Group I/II: p = 0.814
% "Small for gestational age"	14/33 (42%)	5/12 (42%)	3/12 (25%)	Groups I + II/III: p = 0.539
Newborn PLT count ($\times 10^9$/L) (median)	10 (IQR, 7-18.5) n = 35	3 (IQR, 3-9.75) n = 10	29 (IQR, 13-37) n = 17	Groups I/II: p = 0.004 Groups I + II/III: p < 0.001
Maternal immunization				
Anti-HLA antibodies	Negative: 3 Positive: 11	Negative: 1 Positive: 4	Negative: 6 Positive: 7	Groups I/II: p = 1
Anti-HLA NSAs	Negative: 12 Positive: 2	Negative: 1 Positive: 4	ND†	Groups II/I: p = 0.017
Mean MFI value of NSAs	14735	16163	ND†	
Anti HPA-1a alloantibody concentration at delivery (IU/mL) (median)	22 ± 44 n = 30	99 ± 56 n = 17	No anti-HPA-1a detected	Groups I/II: p = 0.002

* Mortality resulting from both therapeutic abortions and ICH itself.
† Not determined; newborn HLA typing not assigned.

TABLE 2. Frequency of HLA-DQB1 and DRB alleles (%) in mothers of Groups I, II, and III

Alleles	Control group, n = 100	Group I, n = 29	Group II, n = 16	Group III, n = 19	p values
DRB3*01:01	33.00	82.76	87.50	21.05	Groups I + II/III: p < 0.001 Groups I + II/control: p < 0.001
DRB4*01:01P	36.00	48.28	25.00	63.16	Groups I + II/III: p = 0.11 Groups I/II: p = 0.20 Groups II/III: p = 0.04 Groups III/control: p = 0.04
DQB1*02:01P	43.00	65.52	62.50	31.58	Groups I + II/III: p = 0.03
DRB3*01:01(+) DRB4*01:01P(+)	7.00	37.93	18.75	5.26	Groups I/II: p = 0.17
DRB3*01:01(+) DRB4*01:01P(–)	26.00	44.83	68.75	15.79	
DRB3*01:01(–) DRB4*01:01P(+)	29.00	10.34	6.25	57.90	Groups III/control: p = 0.04
DRB3*01:01(–) DRB4*01:01P(–)	38.00	6.90	6.25	21.05	

combination. However, the differences does not achieve significance (respectively, p = 0.20 and p = 0.17).

HLA fetomaternal alloimmunization and fetal/neonatal thrombocytopenia

We investigated whether anti-HLA Class I antibodies specifically directed against NSAs aggravate fetal/neonatal PLT destruction leading to ICH. HLA antibodies identification was only performed in 19 cases because of sample shortage (14 cases in Group I and five cases in Group II; Table 1). The percentage of HLA-alloimmunized mothers was identical in Group I and II. Interestingly, in Group II, four of five women were positive for strong NSA (mean MFI value of 16,163; Table S1, available as supporting information in the online version of this paper), whereas in Group I, NSA with high MFI value (>3000) were only detected in two of 14 women (Group II vs. Group I; p = 0.02).

Subsequent IVIG-treated pregnancies: outcome according to maternal HLA typing

Only two women from Group II became pregnant again, probably because of the stress suffered during their previous pregnancy. Consequently, the outcome of subsequent pregnancies in this group could not be studied.

In Group I, 25 women had subsequent IVIG-managed pregnancies (31 pregnancies; 32 newborns) and no ICH was recorded. Twelve infants were born to

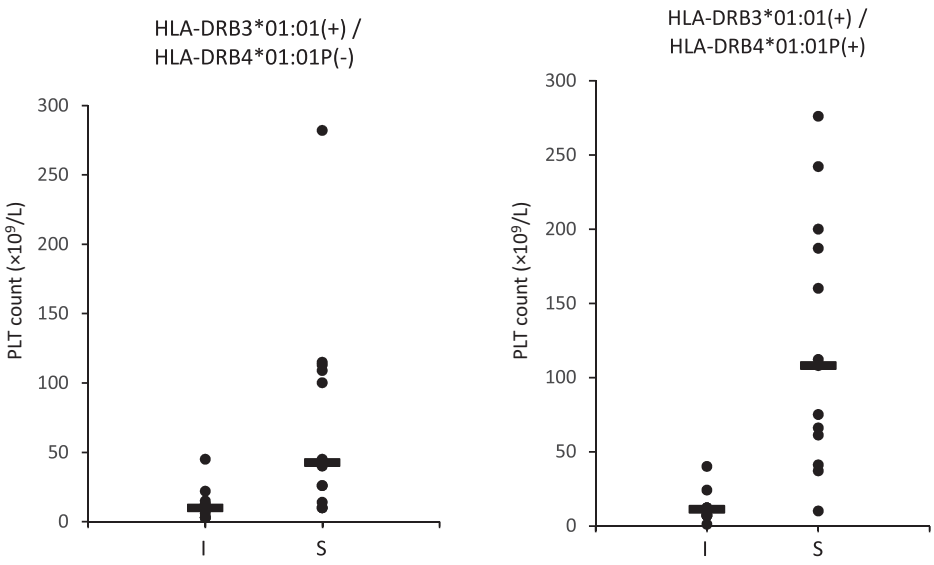

Fig. 2. Evolution of newborn PLT count at delivery according to maternal HLA-DRB3/DRB4 haplotype in index cases and in subsequent IVIG-treated pregnancies. (—) Median value; (|) newborn PLT count in index cases; (S) newborn PLT count in subsequent IVIG-treated pregnancies.

HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(–) women and 13 to HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(+) women. Respectively, 58 and 23% of them were severely thrombocytopenic at birth ($p = 0.11$; Fig. 2). The median newborn PLT count in the index cases was similar for these two groups (10; IQR: 5.25-13.5 vs 11; IQR: 7-12) but higher from those born to HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(+) IVIG-treated women (42.5; IQR, 23-110 vs 108; IQR, 61-187; $p = 0.08$; Fig. 2).

DISCUSSION

The aim of the study was to determine noninvasive predictive factors of ICH in women with anti-HPA-1a FNAIT and of response to maternal therapy in subsequent pregnancies. All cases were treated in France and referred to our laboratory for immunologic testing; consequently, they reflect a homogeneous cohort in terms of pregnancy management and laboratory testing.

We divided severe cases of FNAIT (neonatal PLT counts $< 50 \times 10^9$ PLTs/L) into three groups according to the occurrence of ICH and the presence of maternal anti-HPA-1a alloantibodies. The first two groups included women with FNAIT producing detectable maternal anti-HPA-1a alloantibodies either without ICH (Group I) or with ICH (Group II). Although small, the cohort of ICH is one of the largest reported to date from a single center.[19,20] The third group contained women with fetomaternal incompatibility bearing infants with severe neonatal thrombocytopenia, despite the absence of detectable maternal anti-HPA-1a alloantibodies (Group III).

We compared gynecologic and immunogenetic variables between these three groups. This report confirms that ICH is associated with a poor outcome, because ICH led to death in 59% of cases.

PLT counts in newborns were lower and maternal HPA-1a alloantibody concentrations at delivery were higher in the ICH group than in all other groups. The high proportion of multigravida in women of this group leading to a heightened immune response in successive pregnancies may partly explain this result. These observations led us to investigate maternal immunogenetic background, to understand better the immunization process underlying the production of maternal alloantibodies and to predict the occurrence of ICH as early as possible.

Antibody production depends on T helper cell activation resulting from the interaction between T-cell receptor and HLA Class II-peptide complex.[21] The reactivity of maternal T lymphocytes in response to HPA-1a peptides may predict the severity of thrombocytopenia in the fetus.[22] The immune response to HPA-1a peptide containing the core residues (Trp25-Leu33) is restricted to HLA-DRB3*01:01.[22] The binding cavity of this HLA Class II molecule is composed of five residues (Tyr30, Phe37, Leu38, Val57, and Trp61), which enable a very close interaction with HPA-1a Leu33 of the β_3 integrin.[23] The frequency of HLA-DRB3*01:01 was significantly lower in Group III than in Groups I or II but was similar to that of the control group, which is consistent with previous studies.[3,24] Conversely, we found no difference in the frequency of HLA-DRB3*01:01 between Group I and Group II. Furthermore, HLA-DQB1*02:01 was overrepresented in anti-HPA-1a–alloimmunized women probably because of

linkage disequilibrium with HLA-DRB3*01:01.[25] Recently, Loewenthal and coworkers[5] showed that HLA-DRB4*01:01P also binds the HPA-1a epitope leading to an immune response. Nevertheless, the model of HLA-DRB4*01:01P crystal structure published by the authors shows that the binding cavity of this variant is less hydrophobic than that of HLA-DRB3*01:01. In addition, the Leu33 of the β_3 integrin cannot make close contact with the small amino acid Ala38 of the HLA-DRB4*01:01P antigen binding cleft. These differences suggest that HLA-DRB3*01:01 has a better avidity for HPA-1a epitope than HLA-DRB4*01:01P. The HLA-DRB frequencies among mothers in our cohort support this assumption. Indeed, the HLA-DRB4*01:01P allele was more frequent in women who did not develop antibodies against HPA-1a than in those who did (63.16% in Group III vs. 40% in Groups I and II). These results are consistent with those of L'Abbé and colleagues[4] who found this allele in 15 of 36 (41.7%) HPA-1a–negative alloimmunized women and in seven of 10 HPA-1a–negative nonalloimmunized women despite them having had an HPA-1a–positive children. Interestingly, this antigen was also more frequent in Group III than in the control group. Women in Group III have a high risk of anti-HPA-1a FNAIT (severe thrombocytopenia and fetomaternal incompatibility); however, infants born to these women had a higher median newborn PLT counts than those born to anti-HPA-1a–alloimmunized women. It is possible that such antibodies were not detected by the MAIPA assay because of their low avidity.[26] Further studies should be carried out to examine the association between low-avidity anti-HPA-1a antibodies and expression of HLA-DRB4*01:01P and also to investigate the use of antenatal treatment in further pregnancies for women with previous history of less severe suspected FNAIT. Nevertheless, we failed to identify a protective role of HLA-DRB4*01:01P in HLA-DRB3*01:01(+) alloimmunized women. Indeed, although the proportion of women carrying this antigen tended to be lower in Group II than in Group I, the difference was not significant ($p = 0.17$). Furthermore, women carrying the HLA-DRB4*01:01P allele and those not carrying the allele had a similar mean anti-HPA-1a concentration at delivery (43.8 IU/mL vs. 60.1 IU/mL, respectively; $p = 0.72$; data not shown) and bore infants with a similar median newborn PLT counts (10×10^9; IQR: 7×10^9-12×10^9 vs. 10×10^9; IQR, 5×10^9-20×10^9/L, respectively; data not shown).

Although PLTs express the HLA-A and -B antigens on their membrane[27] the role of anti-HLA Class I in FNAIT is not fully understood.[28,29] Some studies report that maternal anti-HLA Class I antibody can cause FNAIT.[30-32] Consequently, we also searched for maternal anti-HLA Class I antibodies against newborn PLT HLA antigen (NSAs). We more frequently detected NSAs with a high MFI value in the ICH group than in the Group I ($p = 0.02$*). This difference cannot be exclusively the result of the high proportion of multigravida in Group II, because the proportion of HLA-alloimmunized women in Group II and Group I was similar and NSAs should be present in all HLA-alloimmunized women. As previously suggested, there is probably a relationship between the strength of antibodies assessed by MFI and their clinical relevance.[33] Interestingly, in the only NSA-negative case of Group II, we detected anti HLA-C*02:02 alloantibody in maternal serum (MFI 7432) that were specifically directed against newborn HLA antigen (data not shown). HLA-C antigens are generally weakly expressed on PLTs but their expression varies among individuals[34,35] and HLA-C antibodies have already been reported in cases of FNAIT;[31] therefore, this antibody may be clinically relevant. Our data must be interpreted with caution because of the limited number of samples. However, these findings suggest that strong NSAs may aggravate FNAIT in anti-HPA-1a–alloimmunized women. Moreover, Marin and colleagues[36] prosed that anti-HLA and PLT antibodies have a synergistic effect in the pathogenesis of FNAIT.

Our study shows that ICH is mainly detected during the third trimester between 26 and 34 wg, consistent with the results of Tiller and colleagues.[19] Thus, antenatal therapy should begin relatively early during pregnancy to ensure that the fetus is protected from bleeding. Fetal response to therapy is very important to consider. The desired outcome of therapy is 1) absence of ICH and 2) a fetal PLT count of more than 50×10^9/L allowing vaginal delivery. Due to the risk of invasive procedures such as fetal blood samplings,[6,37] a fetal PLT count is nowadays rarely obtained. Therefore, noninvasive maternal variables have to be considered. We previously reported that high maternal alloantibody concentrations before delivery and multigravida were predictive of poor response to antenatal therapy.[38] However, in the current patient series, no ICH was documented in women treated by IVIG, despite the delivery of severely thrombocytopenic infants. Therefore, we hypothesize that IVIG protects the cerebral endothelium, as reported recently in a mouse model.[39] Interestingly, among women treated by IVIG, infants with PLT counts at delivery of fewer than 50×10^9/L tended to be born more frequently to HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(–) women than to HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(+) women (58% vs. 23%, respectively; $p = 0.11$), suggesting that the treatment was more effective in HLA-DRB3*01:01(+)/HLA-DRB4*01:01P(+) women. Our results are inconsistent with those published by Loewenthal and colleagues,[5] but they used a PLT count threshold of 100×10^9/L to define therapy failure. The choice of this threshold is questionable because a full-term newborn with alloimmune thrombocytopenia and a PLT count of between 50×10^9 and 100×10^9/L does not need any treatment.

In conclusion, we found that the number of gestations is predictive of ICH in severe cases of anti-HPA-1a FNAIT. Repeated immune stimulation results in strong

maternal immunization against both HLA Class I and HPA-1a fetal PLT antigens. Our results also suggest that HLA-DRB4*01:01P plays an important role in anti-HPA-1a FNAIT and this antigen may be associated with the efficacy of IVIG treatment in HLA-DRB3*01:01 women. Nevertheless, we cannot determine from our data whether the absence of this antigen is a risk factor of ICH in anti-HPA-1a–alloimmunized women. A larger cohort is necessary to validate our observations. Maternal immunogenetic factors should be evaluated in all suspected cases of FNAIT because these factors may predict the development of maternal immunization and the response to antenatal therapy in subsequent pregnancies. This is also important for the design of new therapies to modulate the maternal response[40-42] and to prevent its deleterious effects on the fetus.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:
Table S1. Newborn-specific HLA antibodies (NSA) in maternal serum after delivery of the index cases.