Human platelet antigen antibody induction in uncomplicated pregnancy is associated with HLA sensitization

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BACKGROUND: Alloimmunization against human platelet antigens (HPAs) during pregnancy is rare but can lead to severe bleeding disorders, such as fetal and neonatal alloimmune thrombocytopenia.

STUDY DESIGN AND METHODS: In a cohort of 241 uncomplicated pregnancies, we investigated the immunogenicity of HPA mismatches and correlated HLA sensitization with HPA antibody formation. HPA antibodies were measured with a Luminex-based multiplex assay.

RESULTS: HPA mismatches were observed in 109 of 241 pregnancies (45%), but child-specific HPA antibodies were only found in two of 109 cases (2%), indicating a low immunogenicity. Only nine of 241 women (4%) had detectable HPA antibodies. HLA sensitization was identified as a strong and independent predictor for HPA antibody formation (hazard ratio, 10.2; 95% confidence interval, 1.8-193; $p = 0.006$), whereas the number of pregnancies was not.

CONCLUSION: Our observational data indicated a low immunogenicity of HPA and suggest that a broader immune response—inferred by HLA sensitization—is probably associated with HPA antibody induction.

To date, 35 different human platelet antigens (HPAs) are known (current list available at: http://www.ebi.ac.uk/ipd/hpa/table1.html). They are located on six different glycoproteins (GPs), such as GPIIb, GPIIIa, GPIbα, GPIbβ, GPIa, and CD109. HPAs were named in the order of their discovery. Some of them are biallelic (HPA-1 through HPA-5; HPA-15), and the more frequent allele became the subspecification "a," whereas the less frequent allele became "b."[1]

Alloantibodies against HPAs occur after immunization, in particular during pregnancy and after transfusion or transplantation.[2] These antibodies can be responsible for clinical syndromes, such as platelet transfusion

ABBREVIATIONS: FNAIT = fetal and neonatal alloimmune thrombocytopenia; GP(s) = glycoprotein(s); HPA(s) = human platelet antigen(s); MAIPA = monoclonal antibody immobilization of platelet antigens.

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refractoriness,[3] fetal and neonatal alloimmune thrombocytopenia (FNAIT),[4] and posttransfusion purpura.[5] FNAIT has a prevalence from one in 600 to one in 5000, with a mean prevalence of one in 1446; however, clinically detected cases are less frequent due to the heterogeneity of the disease.[6-8] The reason for this low prevalence is not fully known. Theoretically, it can be related to a low number of HPA mismatches occurring during pregnancy, a generally low immunogenicity of the HPA molecules, or a low clinical relevance of the HPA-antibodies in vivo. The most extensively explored HPAs are HPA-1a and HPA-5b.[9] Their mismatch rate in Caucasians is from 1 to 2.5% (HPA-1a) and 10% (HPA-5b), respectively. The assumed immunogenicity calculated by the frequency of HPA antibody formation in a mismatched mother-child constellation is reportedly approximately 10 to 12% (HPA-1a) and 20% (HPA-5b), respectively.[10-12] Severe complications, such as intracranial hemorrhage or intrauterine death, related to HPA-1a antibodies might occur in up to 20%, whereas they seem to be rare in individuals with HPA-5b antibodies.[10,12] These data—mainly based on observational screening studies—show that only a minority of mothers carrying an HPA-mismatched child develop HPA-antibodies. Furthermore, only some of these children eventually developed the clinical picture of FNAIT.

The low immunogenicity of HPA suggests that the initiation and the extent of the immune response against HPA might be shaped by specific factors of the mother's immune system. To date, some specific HLA molecules of the mother have been associated with HPA-antibody formation to a variable extent (i.e., HLA-DRB3*01:01 and HPA-1a antibodies, HLA-DR6, and HPA-5b antibodies).[10-12] We hypothesized that HLA sensitization in general indicates a more prominent immune response against the fetus and thus might also trigger or enhance an immune response against HPA molecules. If so, then one would expect that HLA sensitization confers a risk of developing HPA antibodies.

The aims of this study were: 1) to investigate the immunogenicity of individual HPA molecules, and 2) to determine whether HLA sensitization is associated with HPA sensitization. For this purpose, we used an unselected pregnancy cohort that was fully explored regarding HLA Class I sensitization and analyzed maternal sera for the presence of HPA antibodies with a relatively new Luminex multiplex assay that detects HPA-1a/b, HPA-2a/b, HPA-3a/b, HPA-4a/b, and HPA-5a/b antibodies. Mothers and children were also genotyped for HLA and HPA.

MATERIALS AND METHODS

Population and sample collection

This study was approved by the local ethics committee. After obtaining written informed consent, 301 consecutive women giving birth with a full term pregnancy at the University Hospital Basel between September 2009 and April 2011 were enrolled in the study.[13] All women had either their first full-term pregnancy or had previous children only from the same partner as the current live birth. A blood sample was drawn from the mother between days 1 and 4 after delivery for genetic and antibody analyses. Cord blood of the child was obtained immediately after delivery for genetic analyses. Samples were stored at −80°C until use. For the current study, we had sufficient samples from 241 of 301 mother and child pairs (80%) to perform all HPA analyses.

HPA antibody analysis

Serum samples from the mothers were thawed, centrifuged, and incubated during an hour with reconstituted PakLx Beads (LIFECODES Pak Lx Assay, Lot 3002075 and 3002191, Immucor GTI Diagnostics, Inc.).[14,15] After washing steps to remove unbound antibodies, anti-human immunoglobulin G antibody conjugated to phycoerythrin was added. After an incubation of 30 minutes, unbound conjugate was removed by another washing step. Testing for anti-HPA was performed on the Luminex 200 instrument. All procedures were performed according to the manufacturer's recommendations, and the manufacturer's software was used to define a positive result (i.e., the provider's ratio cutoff). Raw mean fluorescence intensity (MFI) data from all 241 samples were imported into a statistical program for further analysis and visualization.

HPA genotyping

For HPA genotyping, matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry-based single-nucleotide polymorphism genotyping was used. To extract DNA, both techniques—manual preparation using Necleon BACC3 reagents (Gen-Probe Life Sciences Ltd.) and automatic preparation by Chemagen magnetic bead technology (Perkin Elmer) —were used. Of 0.2-mL and 6-mL ethylenediaminetetraacetate-anticoagulated blood, 8 μg and 400 μg total genomic DNA was extracted, respectively. After amplification of DNA by Veriti 384-well plate cyclers (Life Technologies Europe B.V.), mass spectrometry was performed with hardware and software provided by Agena GmbH. Details are described elsewhere.[16,17]

HLA typing and HLA antibody analysis

High-resolution HLA-A/B/C/DRB1 typing of the mothers and their children was performed by either SSO DNA-typing (LABType HD; One Lambda) or sequencing-based typing (Histogenetics LLC). HLA-DRB3*01:01 was inferred from high-resolution HLA-DRB1 typing, which is in strong linkage disequilibrium. Assignment of HLA-DR6 included HLA-DRB1*13 and HLA-DRB1*14. HLA antibody analyses of the mothers' samples were performed by using single HLA antigen beads for Class I (iBeads Lot 1; One Lambda),

TABLE 1. Population characteristics

Characteristic	No. (%)
Age: Median [IQR], years	31 [29-34]
Ethnicity	
Caucasian	217 (90)
Non-Caucasian	22 (9)
Unknown	2 (1)
Gestation week at delivery, median [IQR]	40 [39-41]
No. of live births	
First	148 (61)
Second	75 (31)
≥Third	18 (8)
Mode of delivery	
Spontaneous	148 (61)
Cesarean section	38 (16)
Instrumental delivery	51 (21)
Not reported	4 (2)
No. of prior miscarriages	
None	190 (79)
One	33 (14)
More than one	18 (7)
Prior blood transfusions	2 (1)
Mother is Class I HLA sensitized	99 (41)
Mother has Class I child-specific HLA-antibodies	66 (27)
Mother's HLA background	
Associated DRB3*01:01 positive	47 (20)
DR6 positive	62 (26)

IQR = interquartile range.

which consist of a panel of 96 individual Class I antigens. A baseline, normalized MFI greater than 500 was regarded as a positive result. If one or more of the 96 individual beads revealed a positive result, then the mother was classified as "Class I HLA-sensitized." The number of positive beads was counted to estimate the broadness of reactivity against HLA Class I antigens. Assignment of child-specific HLA antibodies was performed by comparison of the positive bead specificities with the HLA typing of the child.[13]

Statistical analysis

We used the JMP 12.0.1 software package for statistical analysis. Categorical data are given as number (percentage) and were compared by chi-square test or Fisher exact test. Continuous data are presented as median values (with interquartile ranges) and were compared by Wilcoxon rank-sum test. Multivariable logistic regression was used to identify independent correlates for HPA antibody positivity of the mothers. Two-sided p values less than 0.05 were considered statistically significant.

RESULTS

Population characteristics

The population characteristics are detailed in Table 1. Ninety percent of the women were of Caucasian origin and had a median age at delivery of 31 years. All births were full-term pregnancies, with delivery at a median gestation week of 40. Sixty-one percent had their first full-term live birth, 39% had their second or later live birth. Twenty-one percent had prior miscarriages. Only two of 241 women (1%) had blood transfusions as additional potential sensitizing events. Ninety-nine of 241 mothers (41%) were "Class I HLA-sensitized," and 66 of 241 mothers (27%) had circulating Class I child-specific HLA antibodies. Twenty percent of the mothers carried HLA-DRB3*01:01, and 26% had a DRB1 allele belonging to the DR6 antigen group.

HPA genotyping and frequency of HPA mismatches

HPA genotyping results and the frequency of individual HPA mismatches of the 241 mother and child pairs are detailed in Table 2. Some HPA genes demonstrated 98% or greater presence (1a, 2a, 4a, 5a) or absence (4b). Overall, 109 of 241 (45%) pairs had at least one HPA mismatch (133 mismatches in total). Five HPA mismatches occurred with a frequency of 7 to 14% (i.e., 1b, 2b, 3a, 3b, 5b), while all other HPA mismatches had frequencies less than or equal to 1% (i.e., 1a, 2a, 4a, 4b, 5a).

Evaluation of the HPA antibody-detection assay

The raw MFI data from the 241 maternal serum samples analyzed for HPA antibodies are summarized in Fig. 1. The test used consists of 21 individual beads. The positive ("Pos") bead and the three negative control beads ("Con 1" to "Con 3") demonstrated very clear and consistent results.

The "HLA Class I" bead in the test used carries a mixture of HLA Class I molecules on the surface and is intended to detect concomitant sensitization against HLA. To evaluate the sensitivity and specificity of this HLA Class I bead, we compared the results with existing data obtained by a previous analysis with single HLA antigen beads, which is currently considered the most sensitive test to detect HLA antibodies.[13] Applying the same MFI cutoff of 500 to both assays, 49 of 241 women (20%) were positive for the HLA Class I bead from the HPA antibody test, and 99 of 241 women (41%) were positive for single HLA antigen beads. Thus, the sensitivity of the HLA Class I bead was lower compared with that of the single HLA antigen bead assay, although a direct comparison of the two tests is not allowed. Figure 2 shows that the MFI of the HLA Class I bead from the HPA antibody assay correlated well with the number of positive beads in the single HLA antigen bead assay ($r^2 = 0.78$). None of the women who were negative on the single HLA antigen bead assay were positive for the HLA Class I bead, illustrating the high specificity of the HLA Class I bead in the HPA antibody test.

TABLE 2. HPA genotyping and frequency of HPA mismatches*

	Mothers, n = 241		Children, n = 241		
HPA gene	Present	Absent	Present	Absent	Mismatch
HPA 1a	237 (98)	4 (2)	235 (98)	6 (2)	3 (1)
HPA 1b	54 (22)	184 (78)	60 (25)	181 (75)	27 (11)
HPA 2a	239 (99)	2 (1)	239 (99)	2 (1)	2 (1)
HPA 2b	31 (13)	210 (87)	42 (17)	199 (83)	26 (11)
HPA 3a	207 (86)	34 (14)	213 (88)	28 (12)	24 (10)
HPA 3b	152 (63)	89 (37)	159 (66)	82 (34)	33 (14)
HPA 4a	241 (100)		241 (100)		0 (0)
HPA 4b		241 (100)		241 (100)	0 (0)
HPA 5a	239 (99)	2 (1)	238 (99)	3 (1)	2 (1)
HPA 5b	51 (21)	190 (79)	45 (19)	196 (81)	16 (7)

* Data are reported as no. (%).

Frequency and correlates of HPA antibody positivity

Applying the HPA test provider's cutoff, only nine of 241 women (4%) had HPA antibodies. Three of those nine women had more than one detectable HPA antibody. The most frequent HPA antibody was against HPA-5b (n = 5), followed by HPA-1/3 (n = 2), and HPA-2 (n = 1) (Fig. 1).

Next, we investigated correlates for HPA antibody positivity defined by the provider's cutoff in a multivariable logistic regression model including parameters that reportedly were associated with HPA antibody induction (Table 3). The only significant correlate was the presence of Class I HLA sensitization (odds ratio, 10.2; 95% confidence interval, 1.8-193; $p = 0.006$). Second or later live birth and mother's HLA-DRB3*01:01 positivity demonstrated increased odds ratios but were not significant ($p = 0.23$ and $p = 0.44$, respectively) (Table 3). Interestingly, if the HLA Class I from the HPA antibody assay was used to define Class I HLA sensitization instead of the results from the single HLA antigen bead assay, then the multivariate analysis revealed no independent predictors (all $p \geq 0.09$).

Frequency of child-specific HPA antibodies and their correlation with clinics

As mentioned above, 109 of 241 (45%) mother and child pairs had at least one HPA mismatch. Using the provider's cutoff for HPA antibody positivity, only two of 109 HPA mismatches led to a measurable immune response. Therefore, the inferred overall immunogenicity of an HPA mismatch is 2% (two of 109 women), which is much lower than the immunogenicity of an HLA Class I molecule mismatch (i.e., on average, approximately 25%-30%[13]). Notably, both mothers who had child-specific HPA antibodies had also developed child-specific HLA antibodies. The immunogenicity of individual HPA mismatches is summarized in Table 4 and ranged from 0 to 13%.

Table 5 summarizes the two cases in which child-specific HPA antibodies were detected. Cases 1 and 2 were positive and demonstrated anti HPA-5b antibodies with rather high MFI values of 7963 and 3793, respectively. Both were Class I HLA-sensitized and had developed Class I child-specific HLA antibodies. No bleeding of the two newborns was noted, and both the mothers and their children were discharged from the hospital as intended by the clinical routine.

Interestingly, only two mothers had red blood cell alloantibodies. One mother had an anti-Lewis a alloantibody, one had an anti-M alloantibody, and neither had an HPA antibody.

DISCUSSION

The two key observations in our study were that: 1) HPA mismatches have low immunogenicity (i.e., 2%), and 2) HLA sensitization is a strong and independent predictor of HPA antibody formation. This suggests that a broader immune stimulation is required for HPA antibody induction, which can partially explain the rather low immunogenicity of HPA mismatches in uncomplicated pregnancy.

This is an intriguing observation, and we can only speculate on the potential mechanisms. It might be possible that, during an immune response against HLA molecules, peptides derived from mismatched HPA can more easily and efficiently induce an immune response. This phenomenon is well known in the context of microbial-induced autoimmunity and is usually referred to as bystander activation.[18] Alternatively, the combined sensitization might point toward a larger transfer of fetal cells into the mother's bloodstream.[19,20] Clearly, we do not advocate measuring HLA sensitization in pregnancies to assess the risk of HPA antibody formation, because measuring HLA sensitization by single HLA antigen beads is expensive and is not justified given the low incidence of harmful HPA antibodies.

Previously reported risk factors for HPA antibody formation, such as mother's DRB3*01:01, mother's DR6, and higher number of pregnancies, were not predictive in our univariate and multivariate analyses. Nevertheless, both

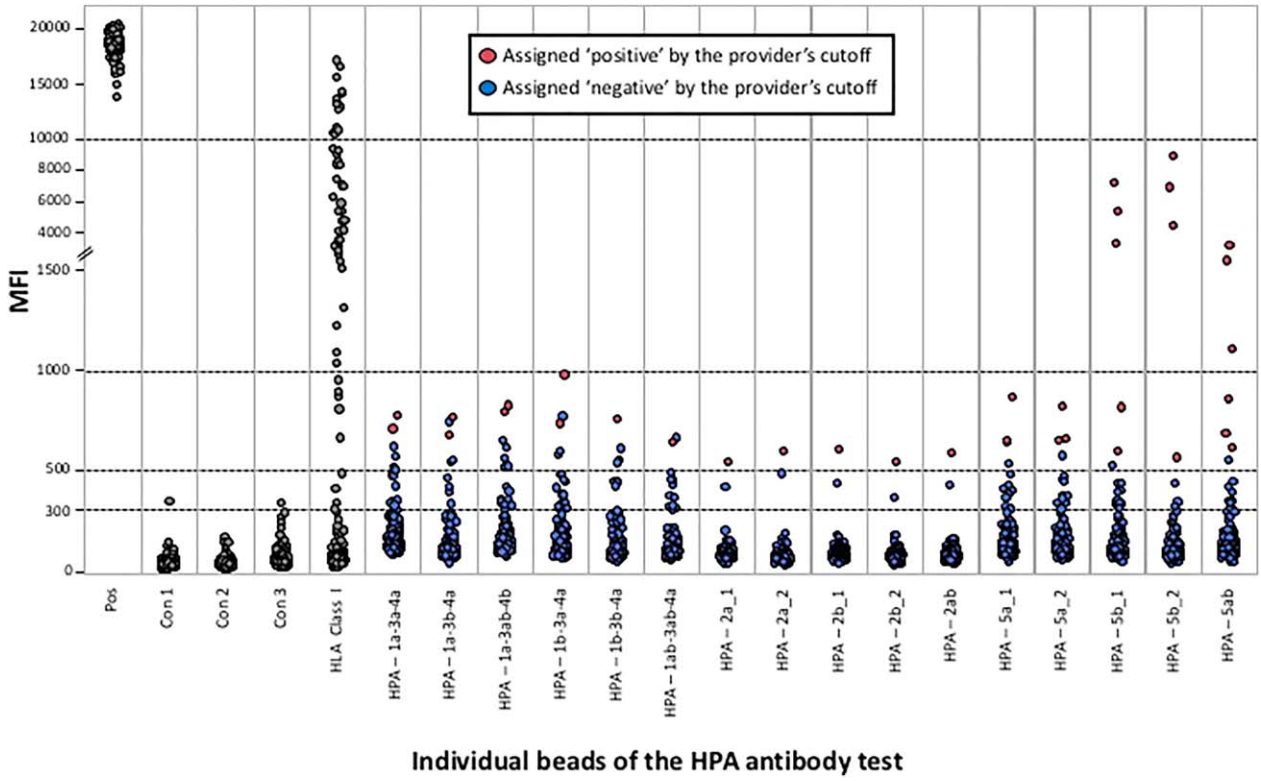

Fig. 1. HPA antibody test overview (n = 241 samples). The used HPA antibody test consists of 21 individual beads (four control beads, one bead to detect HLA Class I antibodies called "HLA Class I," and 16 beads covered with a single or multiple HPA antigen(s)). The raw mean fluorescence intensity (MFI) data from all 241 samples is shown. Red circles indicate positive results based on the provider's ratio cutoff.

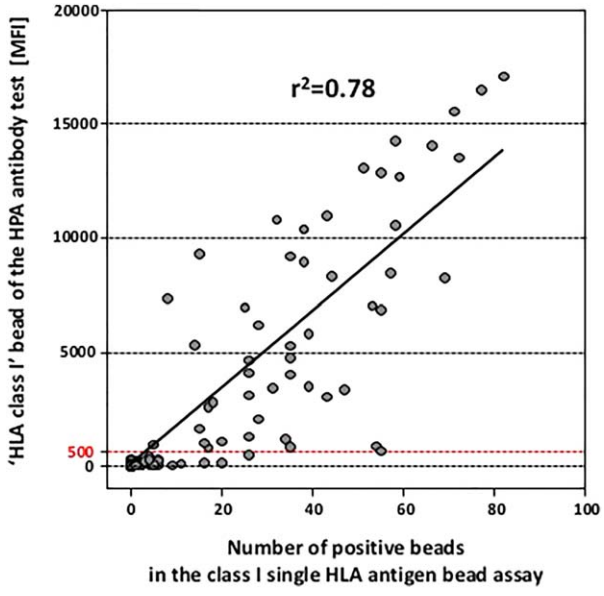

Fig. 2. Correlation of the "HLA Class I" bead of the HPA antibody test with the number of positive beads in the single HLA antigen bead assay (n = 241 samples). The arbitrary cutoff of MFI > 500 is marked in red.

mother's DRB3*01:01 and a second or later live birth had numerically higher frequency among mothers who had HPA antibodies, but the results did not reach significance. We assume that this is related to the low number of investigated pregnancies, which limited statistical power.

The numbers and specificity of HPA allele mismatches are comparable to those in other reports.[10] As in previous studies, no HPA-4b alleles were detected in our cohort.[21,22] Despite a high percentage of mother and child HPA mismatches (109 of 241 pairs; 45%), only two of 109 women (2%) developed child-specific alloantibodies. This finding is similar to previous studies, which described an alloimmunization rate of about 3%.[10] Overall, the rate of HPA sensitization is much lower compared with that of HLA sensitization during pregnancy, which is approximately 25 to 30%.[13]

The most frequently identified HPA antibody was against HPA-5b, which is similar to previous findings.[10] In line with previous publications, the newborns of the two mothers who had HPA-5b antibodies remained asymptomatic.[23] This might be explained by the lower frequency of HPA-5 antigen expression on platelets.[1] Because a routine blood count in neonates is not performed at our institution, it remains unclear whether mild thrombocytopenia without

TABLE 3. Correlates of HPA antibody positivity after delivery*

	HPA-Ab present, n = 9†	HPA-antibody absent, n = 232†	Univariate p value	Multivariate analysis OR [95% CI]	Multivariate analysis p value
Class I HLA-sensitized	8 (89)	91 (39)	0.004	10.2 [1.8-193]	0.006
≥Second live birth	6 (66)	87 (38)	0.09	2.4 [0.6-11.9]	0.23
Mother DRB3*01:01 positive	3 (33)	44 (19)	0.38	1.8 [0.4-7.6]	0.44
Mother DR6 positive	2 (22)	60 (26)	1.00	0.8 [0.1-3.8]	0.82

* Positive HPA antibody status was defined by the provider's ratio cutoff. The whole model had a correlation of determination (r^2) = 0.15 (p = 0.02).
† Data are reported as no. (%).
OR = odds ratio; CI = confidence interval.

TABLE 4. Immunogenicity of individual HPA mismatches*

HPA gene	Mismatch between mother (−) and child (+), no. of 241 (%)	Antibody against mismatched HPA, m/no. (%)
HPA 1a/1b/3a/3b	82 (34)	0/82 (0)
HPA 2a	2 (1)	0/2 (0)
HPA 2b	26 (11)	0/26 (0)
HPA 4a	0 (0)	—
HPA 4b	0 (0)	—
HPA 5a	2 (1)	0/2 (0)
HPA 5b	16 (7)	2/16 (13)

* Positive HPA antibody status was defined by the provider's ratio cutoff.

TABLE 5. Details of the two cases with child-specific HPA antibodies

Parameter	Case 1	Case 2
HPA mismatches	3b, 5b	2b, 5b
Child-specific HPA antibody (mean MFI of all beads)	5b (MFI 7963)	5b (MFI 3793)
Class I HLA sensitized	Yes	Yes
Class I child-specific HLA antibodies	Yes	Yes
Mother HLA		
A	01:01/32:01	11:01/24:02
B	08:01/51:01	07:02/39:06
C	07:01/14:02	07:02/07:02
DRB1	03:01/13:01	01:01/01:01
DRB6 positive	Yes	No
Associated DR3*01:01 positive	Yes	No
Age of mother, years	32	32
No. of live births	3	1
Gestation week at delivery	37	41
Blood loss at delivery	<500 mL	<500 mL
Discharge from hospital on	Day 4	Day 5
Bleeding of the child	None	None
Child's platelets measured	No	No

clinical symptoms was present. On the other hand, all newborns are examined by an experienced neonatologist. Therefore, we can exclude apparent clinical signs and symptoms of bleeding.

In contrast to previous studies—in particular studies designed for screening programs[11,12,24,25]—we consecutively included all pregnancies without limiting our investigations to HPA-1a–negative women. In addition, we performed HPA antibody testing in all pregnancies and not only in women who had an HPA mismatch with their children, as performed by Panzer and colleagues.[10] Irrespective of the above-mentioned screening studies, most work on the role of HPA antibodies in FNAIT have been performed retrospectively, including only cases with clinically overt FNAIT, thus introducing some selection bias.[26-28]

To the best of our knowledge, this is the first systematic detection of HPA antibodies in women after pregnancy performed by PakLx. Previous data clearly showed that PakLx is a sensitive antibody screening method almost equal to the gold standard of monoclonal antibody immobilization of platelet antigens (MAIPA) assay with 90.5% sensitivity and 98.4% specificity.[14] Compared with the MAIPA assay, Luminex technology is faster and easier to perform.

Because of the small number of patients and the lack of any case of FNAIT, we cannot provide information about the significance of an MFI value in our test. Other studies with different assays observed a correlation between antibody titers and the severity of thrombocytopenia.[11] In addition, other researchers reported that the detection of weak HPA antibodies, broadly reactive antibodies, and anti HPA-3 antibodies is more sensitive with the MAIPA test.[14,15] Because of the small amount of sera and our previous careful validation of the Luminex method, a parallel MAIPA analysis was not performed.

The major limitation of our study is the small number of pregnancies and thus the low number of women with HPA antibodies. Also, our results were limited to a Caucasian population. Another limitation is that all the samples were collected immediately after birth, which explains the lack of a longer and longitudinal follow-up, particularly for women with HPA immunization. Hence, alloimmunization that might have occurred in response to an exposure in the peri-delivery period would have been missed.

In addition, a longer follow-up would be very helpful to better understand the dynamics of alloimmunization during different pregnancies and the persistence of such antibodies.[11,29] Furthermore, one may speculate whether the immunogenicity of HLA and HPA can be directly compared, because their cellular expression and blood concentrations are different.

In conclusion, HPA mismatches have a 10-times lower immunogenicity than HLA mismatches in uncomplicated, full-term pregnancies. HLA sensitization is a strong and independent predictor for HPA antibody formation and suggests that a broader immune stimulation might be followed by HPA antibody induction. Further studies are required to uncover the exact mechanism of this observation.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

REFERENCES

1. Curtis BR, McFarland JG. Human platelet antigens—2013. Vox Sang 2014;106:93-102.
2. Veldhuisen B, Porcelijn L, Ellen van der Schoot C, de Haas M. Molecular typing of human platelet and neutrophil antigens (HPA and HNA). Transfus Apher Sci 2014;50:189-99.
3. Hod E, Schwartz J. Platelet transfusion refractoriness. Br J Haematol 2008;142:348-60.
4. McQuilten ZK, Wood EM, Savoia H, Cole S. A review of pathophysiology and current treatment for neonatal alloimmune thrombocytopenia (NAIT) and introducing the Australian NAIT registry. Aust N Z J Obstet Gynaecol 2011;51:191-8.
5. Roubinian NH, Leavitt AD. Shedding a little light on post-transfusion purpura. Transfusion 2015;55:232-4.
6. Murphy MF, Williamson LM, Urbaniak SJ. Antenatal screening for fetomaternal alloimmune thrombocytopenia: should we be doing it? Vox Sang 2002;83 Suppl 1:409-16.
7. Tiller H, Killie MK, Skogen B, Øian P, Husebekk A. Neonatal alloimmune thrombocytopenia in Norway: poor detection rate with nonscreening versus a general screening programme. BJOG 2009;116:594-8.
8. Davoren A, McParland P, Barnes CA, Murphy WG. Neonatal alloimmune thrombocytopenia in the Irish population: a discrepancy between observed and expected cases. J Clin Pathol 2002;55:289-92.
9. Peterson JA, McFarland JG, Curtis BR, Aster RH. Neonatal alloimmune thrombocytopenia: pathogenesis, diagnosis and management. Br J Haematol 2013;161:3-14.
10. Panzer S, Auerbach L, Cechova E, et al. Maternal alloimmunization against fetal platelet antigens: a prospective study. Br J Haematol 1995;90:655-60.
11. Williamson LM, Hackett G, Rennie J, et al. The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening. Blood 1998;92:2280-7.
12. Kjeldsen-Kragh J, Killie MK, Tomter G, et al. A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. Blood 2007;110:833-9.
13. Hönger G, Fornaro I, Granado C, Tiercy JM, Hösli I, Schaub S. Frequency and determinants of pregnancy-induced child-specific sensitization. Am J Transplant 2013;13:746-53.
14. Cooper N, Bein G, Heidinger K, Santoso S, Sachs UJ. A bead-based assay in the work-up of suspected platelet alloimmunization. Transfusion 2016;56:115-8.
15. Porcelijn L, Huiskes E, Comijs-van Osselen I, et al. A new bead-based human platelet antigen antibodies detection assay versus the monoclonal antibody immobilization of platelet antigens assay. Transfusion 2014; 54:1486-92.
16. Meyer S, Trost N, Frey BM, Gassner C. Parallel donor genotyping for 46 selected blood group and 4 human platelet antigens using high-throughput MALDI-TOF mass spectrometry. Methods Mol Biol 2015;1310:51-70.
17. Gassner C, Meyer S, Frey BM, Volmert C. Matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry-based blood group genotyping—the alternative approach. Transfus Med Rev 2013;27:2-9.
18. Getts DR, Chastain EM, Terry RL, Miller SD. Virus infection, antiviral immunity, and autoimmunity. Immunol Rev 2013; 255:197-209.
19. Kim YA, Makar RS. Detection of fetomaternal hemorrhage. Am J Hematol 2012;87:417-23.
20. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. Nat Rev Immunol 2011;11:264-74.
21. Ghevaert C, Rankin A, Huiskes E, et al. Alloantibodies against low-frequency human platelet antigens do not account for a significant proportion of cases of fetomaternal alloimmune thrombocytopenia: evidence from 1054 cases. Transfusion 2009;49:2084-9.
22. Randen I, Søorensen K, Killie MK, Kjeldsen-Kragh J. Rapid and reliable genotyping of human platelet antigen (HPA)−1, −2, −3, −4, and −5 a/b and Gov a/b by melting curve analysis. Transfusion 2003;43:445-50.
23. Rousseau J, Goldman M, David M. HPA-5b (Bra) neonatal alloimmune thrombocytopenia in Quebec: incidence and clinical outcome in 31 cases. Transfusion 2004;44:844-8.

24. Turner ML, Bessos H, Fagge T, et al. Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. Transfusion 2005;45:1945-56.
25. Bertrand G, Drame M, Martageix C, et al. Prediction of the fetal status in noninvasive management of alloimmune thrombocytopenia. Blood 2011;117:3209-13.
26. Ghevaert C, Campbell K, Walton J, et al. Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. Transfusion 2007;47:901-10.
27. Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion 2004;44:1220-5.
28. Mandelbaum M, Koren D, Eichelberger B, Auerbach L, Panzer S. Frequencies of maternal platelet alloantibodies and autoantibodies in suspected fetal/neonatal alloimmune thrombocytopenia, with emphasis on human platelet antigen-15 alloimmunization. Vox Sang 2005;89:39-43.
29. Schnaidt M, Wernet D. Platelet-specific antibodies in female blood donors after pregnancy. Transfus Med 2000;10:77-80.