Risk of HPA-1a–immunization in HPA-1a–negative women after giving birth to an HPA-1a–positive child

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BACKGROUND: Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is the platelet counterpart of hemolytic disease of the newborn. Most severe cases of FNAIT are caused by antibodies against human platelet antigen-1a (HPA-1a). HPA-1a–negative women giving birth to an HPA-1a–positive child are at risk of becoming HPA-1a–immunized, particularly women who are HLA-DRB3*01:01–positive. The aim of the study was to estimate the risk of HPA-1a–immunization in both HPA-1a–negative/HLA-DRB3*01:01–positive and HPA-1a–negative/HLA-DRB3*01:01–negative women after delivery of an HPA-1a–positive child.

STUDY DESIGN AND METHODS: A literature search was conducted, which identified 10 prospective FNAIT studies. The risk of becoming HPA-1a–immunized postpartum was calculated by Bayes' theorem. The results of HLA-DRB3/4/5 typing of 212,472 European Caucasians from the National Marrow Donor Program were used as estimate of the frequency of the HLA-DRB3*01:01 allele.

RESULTS: In HPA-1a–negative/HLA-DRB3*01:01–positive women, the risk of HPA-1a–immunization after delivery of an HPA-1a–positive child was estimated to 12.7% (95% confidence interval, 8.6%–16.8%) as compared to 0.5% (95% confidence interval, 0.1%–0.9%) in women who were HPA-1a–negative/HLA-DRB3*01:01–negative. Potential differences between nulliparous and multiparous and the role of one versus two doses of HLA-DRB3*01:01 could not be determined.

CONCLUSION: In HPA-1a–negative/HLA-DRB3*01:01–positive women, the risk of HPA-1a–immunization is 12.7% after delivery of an HPA-1a–positive child, which is 25 times higher than in HPA-1a–negative/HLA-DRB3*01:01–negative women. Thus, the risk of HPA-1a–immunization in high-risk pregnancies is in the same range as the risk of RhD immunization in RhD-negative women after delivery of a RhD-positive child without RhD prophylaxis.

FNAIT is a rare disease that affects around 1 in 1000 fetuses/newborns.[1] The clinical consequences of FNAIT span a continuum from no symptoms to petechiae, mucosal bleeding, hematomas, retinal bleeding, and intracranial hemorrhage (ICH), which may lead to intrauterine death or lifelong disability.[2] The incidence of FNAIT-associated intracranial hemorrhage has been estimated to be around 1 in 10,000 newborns.[3] Given an annual birth rate of around 10 million in Europe and North America, this incidence rate translates to approximately 1000 cases of FNAIT-associated ICH.

In Caucasians, 85% of the FNAIT cases are caused by an alloantibody to HPA-1a located on the β3 integrin (GPIIIa),[4] which is present on the platelet surface from gestational week 16.[5] The alloantibody (anti–HPA-1a IgG) traverses the placenta and causes fetal thrombocytopenia.[3] There are two allelic variants of the β3 integrin molecule, HPA-1a and HPA-1b,

ABBREVIATIONS: FNAIT = fetal/neonatal alloimmune thrombocytopenia; HPA-1a = human platelet antigen-1a; ICH = intracranial hemorrhage.

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which is the result of a substitution of proline for leucine at amino acid 33 of the β3 integrin molecule.[6]

For more than two decades it has been known that the vast majority of women who are HPA-1a–immunized carry the HLA-DRB3*01:01 allele.[7,8] It has been demonstrated that the HLA-DRB3 molecules on antigen-presenting cells, of which the β-chain is encoded by the HLA-DRB3*01:01 allele, present the peptide that harbors the HPA-1a polymorphism to antigen-specific T cells.[9–11] These T cells in turn provide help to HPA-1a–specific B cells that eventually develop into anti–HPA-1a–producing plasma cells.

The risk of HPA-1a–immunization after delivery of an HPA-1a–positive child is not known. The aim of the present study was to calculate the risk of HPA-1a–immunization in two groups of HPA-1a–negative women—women who are HLA-DRB3*01:01–positive versus women who are HLA-DRB3*01:01–negative.

METHODS

An electronic literature search was performed in PubMed to identify prospective studies that contained information about three core variables: 1) the frequency of HPA-1a–negative individuals, 2) the proportion of HPA-1a–negative women who become HPA-1a–immunized postpartum, or 3) the proportion of HPA-1a–immunized women who are HLA-DRB3*01:01–positive. The following keywords were used: "mass screening [MeSH Terms]" AND "thrombocytopenia, neonatal alloimmune [MeSH Terms]" OR "alloimmune thrombocytopenia [MeSH Terms]" OR "HPA-1a." The search had no start date. One of the authors (JKK) selected the studies, and the other author (KJO) validated the primary selection by checking that the selected studies had a prospective design, and that each study contained at least one of the three above-mentioned core variables. There were no queries to solve. As of May 31, 2018, the search resulted in 1207 publications. Of these, we identified 11 prospective studies that contained data on one or more of the above-mentioned variables.[12–23] One study[16] was excluded from the analysis because data from this paper were also included in a subsequent study.[17] In addition, data regarding the frequency of HPA-1a–negative individuals were obtained from the PREVFNAIT study, a recently completed large prospective FNAIT study in Poland,[23,24] and interim data from a prospective study from the Netherlands, "A Step Towards Routine HPA Screening in Pregnancy: The HIP Study."[25,26] Finally, the results of HLA-DRB3/4/5 typing of 212,472 European Caucasians from the National Marrow Donor Program were used to estimate the frequency of the HLA-DRB3*01:01 allele in the general population.[27]

As no single study contained all information necessary to calculate the HPA-1a–immunization risk of HPA-1a–negative/HLA-DRB3*0101–positive or HPA-1a–negative/HLA-DRB3*0101–negative women giving birth to an HPA-1a–positive child, data from the literature search were used to calculate the immunization risk by applying Bayes' theorem. This basic probability theory theorem states that the probability of Event A on condition of Event B is calculated as $P_{A|B} = P_{B|A} \times P_A/P_B$. The numerator on the right-hand side is the probability that both Event A and Event B happen, while the denominator is the probability of Event B; hence, the ratio gives the probability of Event A when Event B has already happened. The theorem can be extended when more than two different events are relevant as here with HPA-1a–immunization risk, HPA-1a–negative women, and HLA-DRB3*01:01–positive women. When each of the probabilities is estimated as a proportion from a binomial distribution, the sampling variance for a proportion follows directly from this assumption. The combined expression derived using Bayes' theorem is therefore a product and ratio of estimates coming from binomially distributed variables. The variance of this expression cannot be calculated exactly. An approximate formula may be derived using the principle of error propagation, which basically linearizes the expression and then calculates the approximate variance. To confirm the calculation of the sampling variance by error propagation a series of 100,000 Monte Carlo experiments based on binomial distributions was conducted.

The data did not allow separate analyses for nulliparous and multiparous women. Hence, the risk calculations pertained to all women at risk, both nulliparous and multiparous. The events for the formal derivation of the relevant formula are defined in Table 1.

For some of the identified studies, information was available for more than one of the above-mentioned events, and these studies could therefore in principle be included in estimating more than one of the events above. However, by including data regarding more than one event from a study, the calculation of the variance contribution would lead to nonvanishing covariance. The derivation of the covariance between two of the above-mentioned events from one publication requires that cross tabulations are available, which is

TABLE 1. Event definitions assuming the woman is HPA-1a–negative

Event	Description
A	Woman becomes HPA-1a–immunized postpartum
B	Woman is HLA-DRB3*01:01–*positive* – general population
C	Woman is HLA-DRB3*01:01–*negative* – general population
B | A	Woman is HLA-DRB3*01:01–*positive* given that she is HPA-1a–immunized
C | A	Woman is HLA-DRB3*01:01–*negative* given that she is HPA-1a–immunized
D	Child is HPA-1a–positive
A | B, D	Woman becomes HPA-1a–immunized postpartum given that she is HLA-DRB3*01:01–*positive* and the child is HPA-1a–positive
A | C, D	Woman becomes HPA-1a–immunized postpartum given that she is HLA-DRB3*01:01–*negative* and the child is HPA-1a–positive

TABLE 2. Risk of immunization postpartum without considering the women's HLA-DRB3*01:01 status or the HPA-1a type of the fetus*

	No. of women		
	Antibody positive	Denominator	Percentage
No. of women immunized before pregnancy	152	1,990	7.6
No. of women immunized during study	19	1,990–152	1.0
Estimated no. of women immunized postpartum†	39	(1,990–152–19) × 0.65	3.3

* The data were derived from the hitherto largest prospective FNAIT study.[17]
† Samples for antibody analysis were only received from 65% of the women. See text for details.

generally not the case. The applied approach is therefore based on a selection among all studies, so that each study contributes to just one of the events, and assuming then that all events are estimated independently.

Of the 10 identified prospective FNAIT studies, postpartum immunization was assessed in only one study. This study was carried out in Norway between 1996 and 2004 and is the largest prospective FNAIT study conducted to date.[17] Pregnancies at risk of anti–HPA-1a–associated FNAIT were identified by routine HPA-1a typing of all pregnant women in two health regions in Norway; more than 100,000 pregnant women were HPA-1a typed. HPA-1a–negative women were invited to a program entailing frequent assessments of anti–HPA-1a during pregnancy and close clinical follow-up of HPA-1a–immunized women, including frequent ultrasonographic examinations of the fetal brain. All HPA-1a–immunized women were also HLA-DRB3*01:01 typed, and they were offered delivery by cesarean section 2 to 4 weeks before term. All nonimmunized HPA-1a–negative women were encouraged to have a blood sample examined 6 weeks postpartum to see if they had become immunized after delivery.

RESULTS

Based on the results from the Norwegian screening and intervention study,[17,28] it was possible to estimate the risk of immunization after pregnancy. HPA-1a antibodies could be detected in 210 of 1,990 HPA-1a–negative women at least at one time point during the study. Of the 210 HPA-1a–immunized women, 19 were immunized *during pregnancy* and 39 were immunized *postpartum;* that is, antibodies were detected approximately 6 weeks after delivery by using the monoclonal antibody–specific immobilization of platelet antigens assay.[28] The remaining 152 women had antibodies in the first sample collected during pregnancy. As the median gestational age, at which the first sample was collected, was week 13 + 5, it was considered unlikely that immunization should have occurred in the current pregnancy, and hence it was assumed that these women had been HPA-1a–immunized during a previous pregnancy or delivery. There were 1,780 women in whom antibodies could not be detected during pregnancy. Although these women were encouraged to provide a blood sample for antibody analysis 6 weeks postpartum, only 65% of these nonimmunized women were examined for anti–HPA-1a *postpartum.*[28] Consequently, only 1,182 women who were not immunized *during pregnancy* were examined for HPA-1a antibodies *postpartum* (Table 2).

Four of the 10 prospective studies determined the HLA-DRB3*01:01 status of the HPA-1a–immunized women.[14,17,21,22] To ensure independence of the probabilities, the study by Kjeldsen-Kragh et al.[17] was not used to estimate the frequency of the HLA-DRB3*01:01 allele among HPA-1a–immunized women because results from this study were used to estimate the risk of postpartum HPA-1a–immunization of HPA-1a–negative women (risk irrespective of their HLA-DRB3*01:01 status and fetal HPA-1a type). Thus, the three prospective FNAIT studies by Williamson et al.,[22] Maslanka et al.,[18] and Turner et al.[21] were used to estimate the frequency of the HLA-DRB3*01:01 allele in HPA-1a–immunized women. In these three studies, 73 of 81 (90.1%) HPA-1a–immunized women were HLA-DRB3*01:01–positive. This estimate is not much different from the percentage of HPA-1a–immunized women who were HLA-DRB3*01:01–positive in the study by Kjeldsen-Kragh et al.[17] In this study, 180 of 198 (90.9%) HPA-1a–immnunized women carried the HLA-DRB3*01:01 allele. In contrast, the frequency of the HLA-DRB3*01:01 allele is much lower in the general population. Of the 10 previously published prospective FNAIT studies, two studies determined the HLA-DRB3*01:01 status in women with and without HPA-1a antibodies.[21,22] Although the studies by Williamson et al.[22] and Turner et al.[21] contain data regarding the prevalence of the HLA-DRB3*01:01 allele in the general population, these two studies were not used for estimating the prevalence of this HLA allele in the general population because they were also used to determine the prevalence of HLA-DRB3*01:01 in HPA-1a–immunized women. For this reason, the data from National Marrow Donor Program were used for estimating the frequency of the HLA-DRB3*01:01 allele in the general population.[27] Of 212,472 European Caucasians, only 58,551 (27.3%) carried one or two copies of the HLA-DRB3*01:01 allele.[27] This estimate is not very different from the observed prevalence of the HLA-DRB3*01:01 allele in the studies by Williamson et al. (31.9%) and Turner et al. (35.3%).[21,22]

TABLE 3. Frequency of HPA-1a–negative individuals

Data source	No. of HPA-1a–negative individuals	Denominator	Percentage
Mueller-Eckhardt et al.[19]	26	1,211	2.1
Reznikoff-Etievant et al.[20]	27	860	3.1
Blanchette et al.[12]	81	5,000	1.6
Doughty et al.[14]	74	3,473	2.1
Durand-Zaleski et al.[15]	52	2,066	2.5
Davoren et al.[13]	54	3,272	1.7
The PREVFNAIT study[24]	598	24,259	2.5
The HIP study[26]	601	25,203	2.4
Total	**1,513**	**65,344**	**2.3**

The probability that an HPA-1a–negative pregnant woman gives birth to an HPA-1a–positive child is dependent on the frequency of HPA-1a–negative individuals in the population. All 10 published prospective studies, the Polish PREVFNAIT study,[24] and the Dutch HIP study[26] had data on the frequency of HPA-1a–negative individuals. However, to ensure that the probabilities are independent, the data from Kjeldsen-Kragh et al.,[17] Williamson et al.,[22] Maslanka et al.,[18] and Turner et al.[21] were not used for estimating the frequency of HPA-1a–negative individuals because these four studies have been used to estimate the *postpartum* immunization risk[17] and the frequency of the HLA-DRB3*01:01 allele among HPA-1a–immunized women,[18,21,22] respectively. The frequencies of HPA-1a–negative individuals in the remaining six published prospective FNAIT studies, the Dutch HIP study and the Polish PREVFNAIT study are listed in Table 3.

The estimate of 2.3% HPA-1a–negative individuals in the general population is very close to the observed frequency of HPA-1a–negative individuals in the studies by Kjeldsen-Kragh et al. (2.1%), Williamson et al. (2.4%), Maslanka et al. (1.8%), and Turner et al. (1.7%)[17,18,21,22]—the four studies that were not used for estimating the frequency of HPA-1a–negative individuals. Table 4 gives an overview of the data sources for estimation of the probabilities for the different events.

By using the data from Tables 2 and 3 in addition to HLA-DRB3/4/5 data from the National Marrow Donor Program, it is possible to estimate the HPA-1a–immunization risk among HPA-1a–negative/HLA-DRB3*01:01–positive women who give birth to an HPA-1a–positive child by using Bayes' theorem. With the above definitions of terms A, B, and D, we can calculate

$$P_{A|B,D} = P_{B,D|A} \times P_A / P_{B,D}$$
$$= P_{B|A} \times P_{D|A} \times P_A / (P_B \times P_D).$$

As the genes encoding HLA-DRB3 and integrin β3, which hosts the HPA-1a/b polymorphism, are located on different chromosomes, it is reasonable to assume that events B and D are independent; hence, $P_{B,D} = P_B \times P_D$. Because the woman can be immunized only if the child is positive, we further have that $P_{D|A} = 1$, which leads to the final formula:

$$P_{A|B,D} = P_{B|A} \times P_A / (P_B \times P_D).$$

Similarly, the risk of an HPA-1a–negative/HLA-DRB3*01:01–negative woman becoming HPA-1a–immunized after delivery of an HPA-1a–positive child can be expressed as

$$P_{A|C,D} = P_{C|A} \times P_A / (P_C \times P_D).$$

P_A, $P_{B|A}$ and $P_{C|A}$ have been derived from publications related to three independent studies.[17,21,22,28] P_B and P_C have been derived from the results of HLA-DRB3/4/5 typing of 212,472 European Caucasians from the National Marrow Donor Program.[27] By applying the Hardy–Weinberg principle on the data from the six previously published prospective FNAIT studies[12–15,19,20] in addition to data from the Polish PREVFNAIT study[24] and the Dutch HIP study,[26] the probability of having an HPA-1a–positive child can be calculated as follows:

$$P_D = 1 - \sqrt{\text{Proportion of HPA-1a–negative individuals}}$$

The final estimation of the HPA-1a–immunization risk of HPA-1a–negative/HLA-DRB3*01:01–positive women after delivery of an HPA-1a–positive child is shown in Table 5.

The risk of becoming HPA-1a–immunized postpartum after delivery of an HPA-1a–positive child was calculated to be 12.7% (95% confidence interval [CI], 8.6%–16.8%) for women who are HPA-1a–negative/HLA-DRB3*01:01–positive, and to 0.5% (95% CI, 0.1%–0.9%) for women who are HPA-1a–negative/HLA-DRB3*01:01–negative (Table 5). The Monte Carlo experiments verified the calculated 95% CIs. Thus, the immunization risk is around 25 times higher for women who are HLA-DRB3*01:01–positive as opposed to women who are lacking this allele.

DISCUSSION

Although the association between HPA-1a–immunization and HLA-DRB3*01:01 has been known for more than two decades, the risk of becoming HPA-1a–immunized after delivery of an HPA-1a–positive child has not been known for women who have or women who lack this HLA allele. As no single study contains data for calculating the

TABLE 4. Data sources for estimating the probabilities of the different events

References	Information on different events				Event used for the formal calculations
	A	B and C	B | A and C | A	D	
Mueller-Eckhardt et al.[19]	No	No	No	Yes	D
Reznikoff-Etievant et al.[20]	No	No	No	Yes	D
Blanchette et al.[12]	No	No	No	Yes	D
Doughty et al.[14]	No	No	No	Yes	D
Durand-Zaleski et al.[15]	No	No	No	Yes	D
Williamson et al.[22]	No	Yes	Yes	Yes	B | A and C | A
Davoren et al.[13]	No	No	No	Yes	D
Maslanka et al.[18]	No	Yes	Yes	Yes	B | A and C | A
Turner et al.[21]	No	Yes	Yes	Yes	B | A and C | A
Kjeldsen-Kragh et al.[17]	Yes	No	Yes	Yes	A
The PREVFNAIT study[24]	No	No	No	Yes	D
The HIP study[26]	No	No	No	Yes	D
Gragert et al.[27]	No	Yes	No	No	B and C

TABLE 5. Calculation of the postpartum HPA-1a–immunization risk in women who are HPA-1a–negative/HLA-DRB3*01:01–positive and have given birth to an HPA-1a–positive child

Term	Observed* and estimated risks (%)	Sampling standard error†	95% confidence interval‡	Tables	Publications
P_A	39/1,182 = 3.3%	0.5%		Table 2	[17,28]
$P_{B\|A}$	73/81 = 90.1%	3.3%			[14,18,21,22]
$P_{C\|A}$	8/81 = 9.9%	3.3%			[14,18,21,22]
P_B	58,551/212,472 = 27.6%	0.1%			[27]
P_C	153,921/212,472 = 72.4%	0.1%			[27]
P_D	$1-\sqrt{\frac{1.513}{65.344}}=84.8\%$	0.2%		Table 3	[12,13,15,19,20,24,26]
$P_{A\|B,D}$	12.7%		8.6%, 16.8%		
$P_{A\|C,D}$	0.5%		0.1%, 0.9%		

* The observed frequencies are derived from the publications in the right column.

† The sampling variance for P_A, $P_{B|A}$, $P_{C|A}$ and P_C is found as $V = n(N\text{-}n)/N^3$, where N is the total sample and n is number of cases. The table presents the corresponding standard error, that is, the square root of the variance. For P_D the square root transform leads to $V = (N - n)/(2\ N)^2$.

‡ The confidence interval is calculated from an approximate total variance derived from the four sampling variances using the error propagation principle. For $P_{A|B,D}$ this leads to the formula:
$V_{tot} = (P_{B|A}/(P_B \times P_D))^2 \times V_A + (P_A/(P_B \times P_D))^2 \times V_{B|A} + (P_{B|A} \times P_A/(P_B{}^2 \times P_D))^2 \times V_B + (P_{B|A} \times P_A/(P_B \times P_D{}^2))^2 \times V_D$ and for $P_{A|C,D}$:
$V_{tot} = (P_{C|A}/(P_C \times P_D))^2 \times V_A + (P_A/(P_C \times P_D))^2 \times V_{C|A} + (P_{C|A} \times P_A/(P_C{}^2 \times P_D))^2 \times V_C + (P_{C|A} \times P_A/(P_C \times P_D{}^2))^2 \times V_D$

immunization risk, data from 12 prospective studies on FNAIT, as well as the results of HLA-DRB3/4/5 typing of 212,472 European Caucasians from the National Marrow Donor Program were used for calculation of the immunization risk by applying Bayes' theorem. Women who are HPA-1a–negative/HLA-DRB3*01:01–positive have a 12.7% risk of becoming HPA-1a–immunized postpartum, whereas the risk is only 0.5% for women who are HPA-1a–negative/HLA-DRB3*01:01–negative (Table 5).

For some of the studies, information was available for more than one type of event used for the calculation. However, the approach used for calculation of the CI of the immunization risk requires that the events are independent, as inclusion of different types of events will lead to nonvanishing covariance. Thus, one may speculate to what extent this elimination of data could have biased the calculation of the immunization risk. However, inclusion of events (A, B, C, B | A, C | A and D) from all 12 clinical trials in the models did not change the risk estimates presented above, indicating that elimination of data from some of the clinical studies has not led to bias of the results.

As the propensity to develop HPA-1a antibodies is closely related to HLA-DRB3*01:01, it would have been interesting to know if the risk of immunization is related to the dose of this allele; that is, is the immunization risk in women who are homozygous for HLA-DRB3*01:01 different from the immunization risk in women who are heterozygous or hemizygous for this allele? Titze et al.[29] have examined how the dose of HLA-DRB3*01:01 influences the maternal anti–HPA-1a level and neonatal platelet count. The data from this study were derived from the Norwegian screening and intervention study,[17] and for this reason, it was not possible to make separate risk analyses for women who are homozygous versus women who are either heterozygous or hemizygous for this allele, because only one core variable from each study could be included in the

calculations, as explained in the Methods section. Nevertheless, Titze et al.[29] showed that there is a significant relationship between the dose of HLA-DRB3*01:01 and antibody levels; those very few HLA-DRB3*01:01–negative women who become immunized typically produce the lowest levels of anti–HPA-1a, followed by women who have one copy of this allele and with the highest antibody levels in the group of women who are homozygous for HLA-DRB3*01:01. The opposite trend was seen with regard to neonatal platelet counts: HPA-1a–immunized women negative for HLA-DRB3*01:01 give birth to children with normal platelet counts or occasionally moderately reduced platelets counts usually without clinical consequences, whereas newborns of women carrying one copy of this allele have lower platelet counts and women homozygous for HLA-DRB3*01:01 give birth to children with the most severe thrombocytopenia.[29]

Wienzek-Lischka et al.[30] have also examined if there is a difference in neonatal platelet count in children born of women who have one or two doses of HLA-DRB3*01:01, and reached the conclusion that the neonatal platelet count is not different between these two groups. The population examined by Wienzek-Lischka et al.[30] originated from mother–child samples that were referred to their reference laboratory due to suspicion of FNAIT. Thus, it is highly likely that this selection of samples is biased toward more severe FNAIT cases. The fact that only 1.9% of the women in their population were HLA-DRB3*01:01–negative, as opposed to 7.9% in the study by Kjeldsen-Kragh et al.[17] and 28% in the study by Turner et al.[21] confirms that the population studied by Wienzek-Lischka et al.[30] is biased toward the more severe cases. Also, Wienzek-Lischka et al.[30] did not include nullizygous, but only hetero−/hemizygous and homozygous in their analysis, as opposed to the study by Titze et al.[29] Consequently, the selection bias may have prevented Wienzek-Lischka et al.[30] from detecting an effect of the HLA-DRB3*01:01 zygosity status on neonatal platelet count.

Unfortunately, due to lack of data, the current study can address neither the questions related to the dose effect of HLA-DRB3*01:01 nor the potential differences between nulliparous and multiparous women. Further studies are necessary for shedding light on these questions. Another weak point is that the frequency of HPA-1a–immunization postpartum was examined only in the Norwegian screening and intervention study,[17] whereas data regarding the other variables were available from more than one study (see Table 4). Thus, it cannot be excluded that the postpartum immunization risk may vary somewhat in other Caucasian populations. Moreover, as fetal-maternal bleeding is supposed to play a key role for HPA-1a–immunization, it could also be envisaged that different obstetric practices (cesarean section, assisted vaginal [operative] delivery, ordinary vaginal delivery, etc.) between countries may influence the volume of fetal-maternal bleeding and thus also the risk of becoming HPA-1a–immunized. It is not possible to eliminate this weakness because there are no data that support or disprove differences in immunization risk between different Caucasian populations. The authors believe such a potential bias will be minor, and therefore, the 95% CI of the estimated immunization risk is considered relevant and the most precise estimate that can be achieved at the present time.

For the past 10 years it has been discussed whether it is time to start screening for pregnancies at risk of FNAIT.[1,31,32] On national levels, the national health authorities in Norway, the United Kingdom, and the Netherlands have also been discussing if general HPA-1a typing of pregnant women should be included as part of the routine antenatal health care program, in the same way as RhD typing of pregnant women has been practiced for more than four decades for identifying pregnancies at risk of hemolytic disease of the fetus and newborn. However, no country has yet adopted HPA-1a typing for FNAIT screening, primarily because there are still unanswered questions regarding both how to treat pregnancies at risk and FNAIT epidemiology, particularly how to identify women at the highest risk of having the pregnancy complicated by FNAIT.

Until now, the risk of postpartum immunization has not been known for the approximately 0.5% of the women who are HPA-1a–negative/HLA-DRB3*01:01–positive. The current study has shown that the risk of postpartum immunization is 12.7% (CI, 8.6%–16.8%) for this small group of women. In comparison, women who are RhD negative who give birth to a RhD-positive child have an approximately 7% risk of becoming RhD immunized if not treated with prophylactic anti-D immunoglobulin after delivery of a RhD-positive child.[33]

The current study makes it possible to calculate the number of women who should be offered close clinical follow-up due to HPA-1a–immunization if general screening for FNAIT is implemented. Of a hypothetical cohort of 100,000 pregnant women without HPA-1a antibodies, 538 will be HPA-1a–negative/HLA-DRB3*01:01–positive, of whom 68 will become HPA-1a–immunized postpartum (Fig. 1). These 68 women will represent three-fourths of all immunization cases because one-fourth of the cases occur during pregnancy.[17] Hence, of a starting population of 100,000 nonimmunized women, it is reasonable to assume that 23 women will be immunized during pregnancy. Thus, 91 women will develop HPA-1a antibodies either during pregnancy (23) or postpartum (68). The 68 women who develop antibodies after delivery will be at risk of having a subsequent pregnancy complicated by FNAIT. As FNAIT-associated ICH has been shown to occur in 1 of 10,000 pregnancies[3] (from the number of ICH cases [6] and the total population [59,475], the binomial proportion and the corresponding Wald-based 95% CI can be calculated to be 0.2–1.8), we can expect around 10 cases of ICH among the newborns/fetuses of these 91 HPA-1a–immunized women.

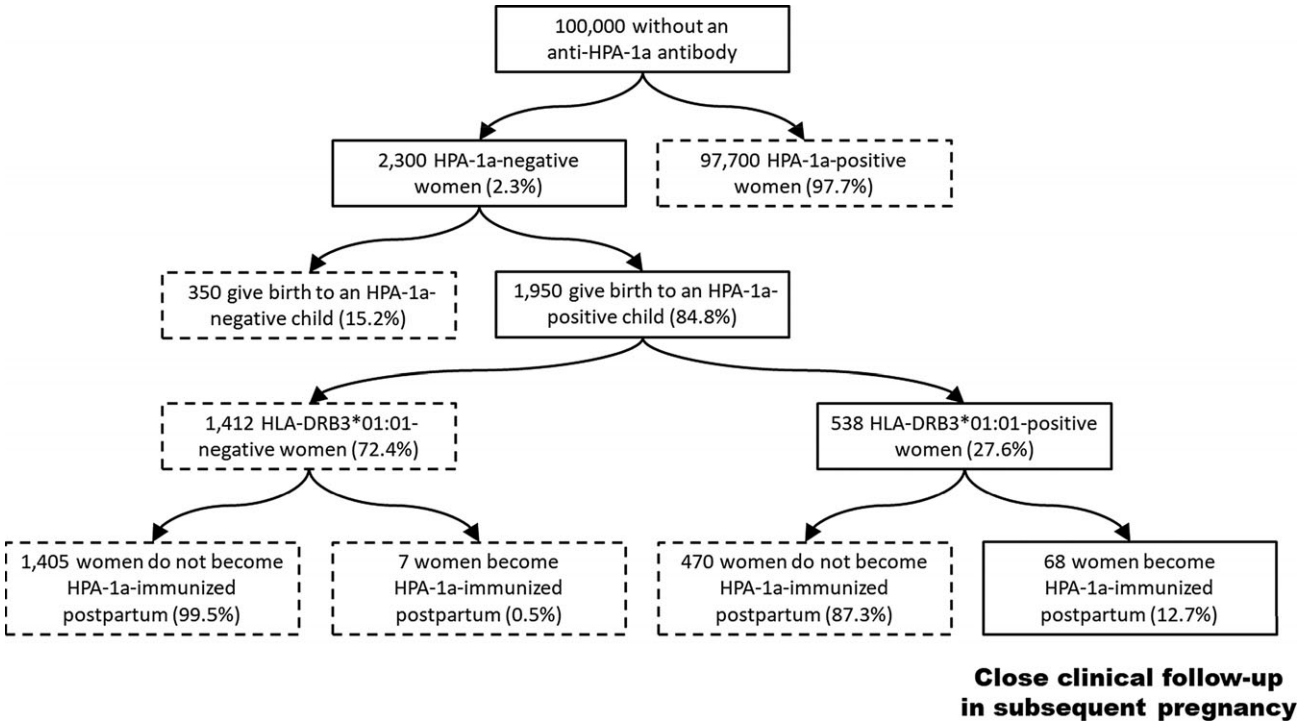

Fig. 1. The number of women who would become HPA-1a–immunized postpartum of a hypothetical cohort of 100,000 women. Women belonging to boxes with dashed lines are at very low risk of having a subsequent pregnancy complicated by FNAIT. It might be considered just to offer the eight HPA-1a and HLA-DRB3*01:01–negative women who become HPA-1a–immunized ordinary clinical follow-up during a subsequent pregnancy because the risk of having a fetus/child with severe FNAIT is very low (see text for explanation).

The fact that only 91 of 100,000 women are at high risk of having their current or subsequent pregnancy complicated by FNAIT reduces the number of women who potentially should be treated with high-dose intravenous immune globulin to prevent ICH. Intravenous immune globulin treatment is very costly, but it seems justified to treat 91 women to prevent 10 cases of ICH when taking into consideration the immense health care and societal costs that are associated with the care of a child with ICH.

Of the 100,000 pregnant women there will be 1,412 who are both HPA-1a and HLA-DRB3*01:01–negative, and 7 of these will also develop HPA-1a antibodies (Fig. 1). However, it could be questioned if special clinical follow-up during pregnancy would be needed for these women, as the vast majority will develop only very low levels of anti–HPA-1a,[29] and most of them will give birth to children with normal platelet count or occasionally moderate thrombocytopenia with no or few clinical consequences. Thus, in four large prospective FNAIT studies,[17,18,21,22] which altogether involved more than 150,000 pregnant women, there were 220 HPA-1a–immunized women who were HLA-DRB3*01:01 typed, 19 of whom were HLA-DRB3*01:01–negative. None of these 19 women gave birth to a child with severe thrombocytopenia (platelet count $<50 \times 10^9$/L). The view that close clinical follow-up may be not necessary for HLA-DRB3*01:01–negative women has also recently been suggested by the platelet immunology group in Gießen, Germany.[30]

Implementing FNAIT screening could also be accomplished by HPA-1a typing followed by screening for HPA-1a antibodies in HPA-1a–negative women every trimester through pregnancy. This approach is used by some centers when a woman is serendipitously identified to be HPA-1a-negative and becomes pregnant with an HPA-1a–positive fetus. The advantage of this testing strategy is that there is need for neither HLA-DRB3*01:01 typing nor fetal HPA-1a typing. However, the disadvantage is that more than two-thirds of the women will be at very low risk of having their pregnancy complicated by FNAIT because they are HLA-DRB3*01:01–negative. As these women are not identified but are examined for HPA-1a antibodies every trimester, there is a risk that some may perceive stress during pregnancy because they fear they may give birth to a thrombocytopenic child.

The current study has provided an important piece to the jigsaw puzzle that constitutes FNAIT epidemiology, information that could be useful for health authorities and decision makers when the question of introducing screening for FNAIT is on the table.

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CONFLICT OF INTEREST

JKK is one of the founders and owners of Prophylix AS, a Norwegian biotech company that leads the EU-founded PROFNAIT Consortium, which is developing a hyperimmune anti–HPA-1a IgG for the prevention of fetal and neonatal alloimmune thrombocytopenia.

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