A prospective study of maternal anti-HPA 1a antibody level as a potential predictor of alloimmune thrombocytopenia in the newborn

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ABSTRACT

Background
Neonatal alloimmune thrombocytopenia is most commonly due to transplacental passage of maternal anti-HPA 1a antibodies. A prospective study was carried out to evaluate the pattern and quantity of maternal anti-HPA 1a antibodies in order to predict the level of thrombocytopenia in the neonates.

Design and Methods
A monoclonal antibody immobilization of platelet antigen assay was used to detect antibodies in maternal samples from 1,990 HPA 1bb women. HLA DRB3*0101 typing was performed in all immunized women by sequencing the HLA *DRB3* gene when present.

Results
Primary immunization more often took place in connection with delivery than during the first pregnancy. There was a strong correlation between maternal antibody levels and the platelet counts in the newborn (R^2=0.49, p<0.001). A maternal antibody level above 3.0 IU/mL measured in gestational week 22 or 34 had a diagnostic sensitivity and specificity of 93% and 63%, respectively, for predicting the grade of neonatal thrombocytopenia. The women who were negative for HLA DRB3*0101 had significantly lower anti-HPA 1a antibody levels than those who were HLA DRB3*0101 positive (p<0.007). In contrast to primigravida, in whom anti-HPA 1a antibody levels increased during pregnancy, the antibody level decreased in 92 of 147 women who had been pregnant previously ($P_{92 \text{ or more of } 147}$ = 0.003). The anti-HPA 1a antibody level regularly increased after delivery.

Conclusions
Maternal anti-HPA 1a antibody levels in weeks 22 and 34 of pregnancy are good predictors of the degree of thrombocytopenia in the newborn both in the first and subsequent pregnancies. Most mothers became immunized at the time of delivery.

Key words: anti-HPA 1a, neonatal alloimmune thrombocytopenia.

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Introduction

Neonatal alloimmune thrombocytopenia (NAIT) in Caucasians is most commonly due to transplacental passage of maternal anti-HPA 1a antibodies (85%),[1] and is a condition that resembles hemolytic disease of the newborn. Complications of NAIT, such as intracranial hemorrhage, leading to neurological disability or death, have been reported to occur in 10-30% of affected babies.[2-4] At present, an accurate measurement of fetal platelet count can only be achieved through high-risk sampling of umbilical cord blood. Several prospective studies have shown a correlation between anti-HPA 1a antibody level and severity of thrombocytopenia in the newborn.[4-7] A correlation was also found in two retrospective studies,[8,9] whereas one other retrospective study did not find such a relationship.[10] In a recent trial, we documented that identification of women with anti-HPA 1a antibodies, close clinical follow-up during pregnancy, elective Cesarean section and transfusion of compatible platelets to neonates with severe thrombocytopenia, reduce NAIT-related morbidity and mortality.[11] The main purpose of this study was to describe the natural course of HPA 1a alloimmunity by recording maternal anti-HPA 1a antibody levels longitudinally throughout pregnancy and after delivery. We also studied the relationship between the platelet counts in the neonates and both the level of maternal anti-HPA 1a antibodies at delivery and the weighted mean of anti-HPA 1a antibody levels to which the neonates had been exposed during pregnancy. Finally, we tried to identify a maternal anti-HPA 1a antibody level that could be used as a predictive parameter for severe thrombocytopenia in the newborn.

Design and Methods

Samples

HPA 1a negative pregnant women (1,990 women) were tested for anti-HPA 1a antibodies approximately every fourth week during pregnancy.[11] Preliminary data from the first 232 HPA 1a negative women included in this study have been published previously.[5] Anti-HPA 1a antibodies were also analyzed and quantified around 6 weeks post-delivery in approximately 65% of the cases. Immediately after birth, the platelet count was determined in umbilical cord blood and/or from a capillary blood sample from the neonate. We defined severe thrombocytopenia as a platelet count $<50\times10^9$/L and moderate thrombocytopenia as a platelet count between 50×10^9/L and 149×10^9/L.

This study was approved by the Regional Committee for Medical Research Ethics, North Norway (approval number: P-REK V 13/1995).

HPA 1 typing

HPA 1 phenotyping was performed either by an HPA 1a typing assay (DiaMed AG, Switzerland) in accordance with the manufacturer's instructions, or by flow cytometry, as previously described,[12] using a fluoroescein isothiocyanate conjugated anti-CD61 antibody reacting specifically with the HPA 1a epitope[13] (clone SZ21, Immunotech, Marseilles, France). All HPA 1a-negative samples were genotyped by a 5´nuclease assay or melting curve analysis.[14,15]

Detection and quantitation of antibodies

Anti-HPA 1a antibodies were searched for using either a modification of the technique known as monoclonal antibody immobilization of platelet antigen assay (MAIPA)[16-18] or the indirect platelet suspension immunofluorescence test (PIFT) analyzed by flow cytometry.[19] Plasma samples from HPA 1a negative pregnant women from the northern part of Norway were screened for antibodies using MAIPA, whereas those from HPA 1a negative pregnant women from the southern part of Norway were screened for antibodies using the PIFT and, if antibodies were present, the samples were sent to the Department of Immunology and Transfusion Medicine, University Hospital of North Norway for identification of the antibodies by MAIPA. Anti-CD61, clone Y2/51 (Dako A/S, Glostrup, Denmark) was used to immobilize the glycoprotein GPIIb/IIIa complex for antibody detection.

For quantitation of anti-HPA 1a antibodies, a reference sample from one woman with a high level of anti-HPA 1a antibodies was used in two-fold dilutions from 1:2 to 1:64, to create a linear standard curve. The antibody concentration in this sample was equivalent to 15 IU/mL of the international anti-HPA 1a standard, (NIBSC product code 03/152, Hertfordshire, UK). Samples with high levels of antibodies were diluted so that the antibody concentration fell in the linear part of the standard curve. A negative sample was also run in parallel. The cut-off level for a positive test was an optical density of 0.1 at 490 nm. The weighted mean of anti-HPA 1a antibody levels during pregnancy was determined by calculating the area under the curve (AUC) describing the maternal anti-HPA 1a antibody level over time divided by the number of weeks of antibody measurements. The reproducibility of the MAIPA method was determined by calculating the critical difference from repeated intra-assay measurements of 14 samples with different amounts of anti-HPA 1a antibodies. The critical difference was found to be 30% and only changes in antibody levels exceeding this value were considered as clinically significant. An automatic microparticle enzyme linked immunoassay was used to detect Rubella antibodies (AxSYM RUBELLA IgG kit, Abbott Diagnostics Division, Abbott Park, IL, USA).

HLA DRB3 typing

HLA DRB3 typing was performed by sequencing the HLA DRB3 gene when present. The intron-located primers previously described by Kotsch *et al.*[20] were used for the polymerase chain reaction (PCR). The PCR product was sequenced in an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, USA). DNA from women whose sequencing results were difficult to interpret, and from HLA DRB3 negative women, were sent to the Tissue Typing Laboratory, Institute of Immunology, Rikshospitalet University Hospital, for HLA DRB3 typing by PCR-sequence-specific oligonucleotide probes

Table 1. Mean of maternal antibody levels at delivery and weighted mean of antibody levels measured in 161 non-compatible pregnancies.

	Number of neonates	*Mean antibody level at delivery, IU/mL (95% CI)*	*Weighted mean of antibody levels, IU/mL (95% CI)*
Platelet count [<50×10⁹/L	55	23.7 (15.0-32.3)*	22.0 (14.7-29.3)*
Platelet count [50-149×10⁹/L	33	4.2 (1.7-6.8)*	5.3 (2.7-7.9)*
Platelet count [>150×10⁹/L	75	1.4 (0.3-2.5)*	2.4 (1.1-3.7)*
Total	163		

*The 161 non-compatible pregnancies including two twin pregnancies. One pair of twins had platelet counts <50×10⁹/L, and the other pair had platelet counts >150x10⁹/L. *There was a statistically significant difference in antibody levels between the three groups; p<0.0001.*

and high resolution PCR-sequence-specific primers (GenoVision, Vienna, Austria).

Statistics

One way analysis of variance (ANOVA) was used to compare mean antibody levels in women who gave birth to neonates with normal platelet counts or moderate and severe NAIT. Twin births were regarded as one birth in the statistical calculations as the platelet counts of the two pairs of twins included in this study were in the same category (normal platelet counts in one pair and severe thrombocytopenia in the other). The paired sample t-test was used to compare anti-HPA 1a antibody levels before and after delivery. The Mann-Whitney U test was used to compare the anti-HPA 1a antibody levels in HLA DRB3*0101 positive and negative women.

To examine the association between maternal anti-HPA 1a antibody level and platelet count in the neonates, we used power fit regression analysis. The platelet count (Y) is given by the formula $Y=b_0 \times x^{b_1}$ where x is the anti-HPA 1a antibody level and b_0 and b_1 are constants.

We used a receiver operating characteristic (ROC) curve to evaluate maternal anti-HPA 1a level as a predictive parameter for severe NAIT.[21] An AUC of 1.0 indicates complete discrimination, whereas an AUC of 0.5 or less means absence of discrimination. The theoretically best cut-off value was calculated using Pythagoras' theorem as described by Peat and Barton.[22] The critical difference of the MAIPA indicated the minimal clinical relevant change in antibody level and was calculated according to Gluer *et al.*[23] The probability (p) of declining antibody levels during a pregnancy in a multigravida was calculated according to the binomial distributions (two-tailed test). Our calculations were based on the assumption that p=0.5.

Maternal antibody patterns and the number of children with severe NAIT were analyzed using the χ^2-test and calculation of relative risk (RR) with 95% confidence intervals (CI) when appropriate. The binomial sample distribution was calculated using an interactive calculator at the VassarStats website for statistical calculations.[24] Other statistical calculations were performed with SPSS for Windows computer software (Version 11.5, SPSS Inc., Chicago, IL, USA). p values <0.05 were considered statistically significant.

Results

Samples

The median gestational age with the 95% CI at which the first sample was taken was week 13+5 (week 12+6-week 14+4). The median (95% CI) gestational age at the time the last sample was taken was week 36+4 (week 36+0-week 36+6). The median (95% CI) gestational ages for the samples obtained around weeks 22 and 34 were 22+2 (week 21+6-week 22+5) and 34+0 (week 33+5-week 34+3), respectively.

Maternal anti-HPA 1a antibody level and platelet count in the newborn

Antibodies were detected in 170 pregnancies delivered by Cesarean section, as previously reported,[11] and in three pregnancies ending in vaginal deliveries. Twenty-four of these pregnancies were included in an earlier study.[5] In one of the 170 pregnancies a moderate amount of anti-HPA 1a antibodies was detected in the first sample (gestational week 34), but no anti-HPA 1a antibodies were found at the time of delivery. Due to lack of plasma for quantitative analysis in the first sample obtained, this pregnancy was excluded from our analysis. In 161 pregnancies, there was an HPA 1 incompatibility between the mother and the child.

Both maternal anti-HPA 1a antibody level at delivery and the weighted mean of anti-HPA 1a antibody levels during pregnancy were significantly higher in women who gave birth to severely thrombocytopenic children than in those who gave birth to children with moderate thrombocytopenia or a normal platelet count (Table 1). Regression analysis showed that there was a strong association between maternal anti-HPA 1a antibody level and the platelet count in the neonates (Figure 1).

Anti-HPA 1 antibody level as a predictor of severe NAIT

We then assessed the potential of maternal anti-HPA 1a antibody level for predicting severe NAIT (Figure 2). The theoretically best cut-off value for prediction of thrombocytopenia in the fetus was 3.0 IU/mL for antibody measurements close to delivery (sensitivity 81%, specificity 80%), and 5.0 IU/mL for the weighted mean of antibody levels (sensitivity 76%, specificity 81%). When using only one measurement close to delivery (week 34) and a cut-off level of 3.0 IU/mL, 10 of 55 pregnancies would have been lost from intervention in the Norwegian screening and intervention study.[11] However, when two antibody measurements were performed (at weeks 22 and 34) and a cut-off level of 3.0 IU/mL was used to identify cases at risk of NAIT, the sensitivity increased to 93% but the specificity decreased to 63% (Table 2). Using this approach, the number of false negative cases (maternal anti-HPA 1a level < 3.0 IU/mL, but severe NAIT) could be reduced from 10/55 neonates to 4/55.

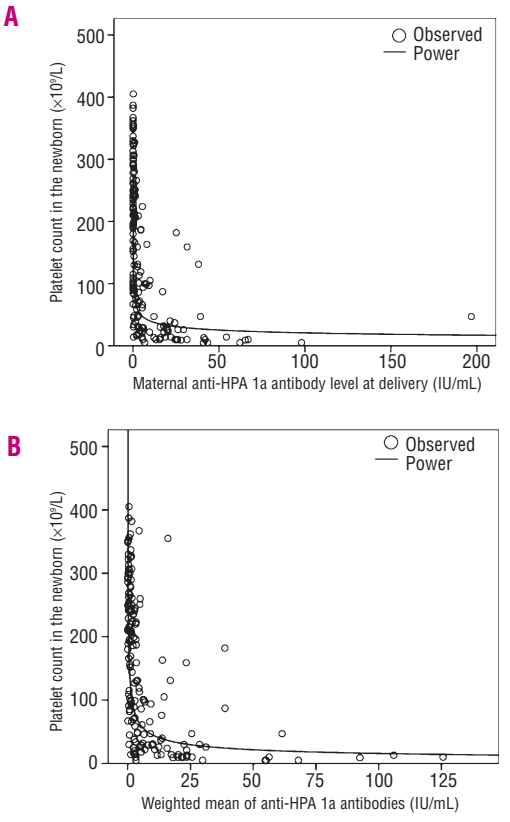

Figure 1. The association between maternal antibody level and platelet count in the newborn. (A) Maternal antibody level at the end of pregnancy and platelet count in the newborn. The equation of the regression curve is: $Y = 76 \times x^{-0.28}$. R^2 =0.49; p<0.001. (B) Weighted mean of anti-HPA 1a antibody levels during pregnancy and the platelet count in the newborn. The equation of the regression curve is: $Y = 127 \times x^{-0.45}$. R^2=0.45; p<0.001.

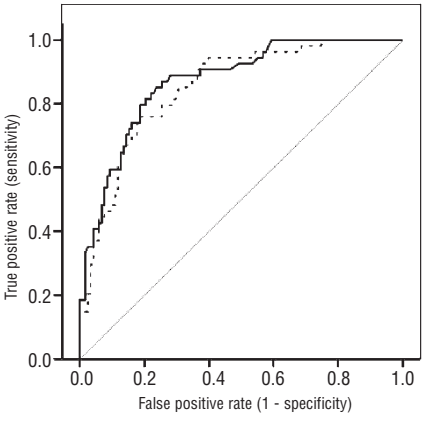

Figure 2. The receiver operating characteristic (ROC) curve showing the diagnostic performance of maternal anti-HPA 1a antibody level as a predictor of severe severe neonatal autoimmune thrombocythemia. The unbroken black line represents maternal antibody level close to delivery (week 34), and the hatched gray line the weighted mean. The unbroken gray reference line represents absence of discrimination. The calculated area under the curve was 0.87 (95% CI: 0.82-0.92) for maternal antibody levels close to delivery, and 0.85 (95% CI: 0.79-0.91) for the weighted mean of antibody levels, indicating equal diagnostic performance.

For 17 pregnancies (in 14 women) there was information of a previous child with severe NAIT. In seven of these 17 pregnancies, the baby had severe NAIT. An obstetric history of NAIT had a weaker positive predictive value than maternal anti-HPA 1a antibody level (Table 2).

Quantitation of anti-HPA 1a antibodies during pregnancy

Fourteen of 172 pregnancies in women with anti-HPA 1a antibodies were first pregnancies. One woman, alleged to be in her first pregnancy, was excluded because of elements of uncertainty. This woman had a high level of anti-HPA 1a antibodies in gestational week 7 but the antibodies were no longer present in week 20, suggesting immunization prior to the pregnancy. In 9 of the 13 remaining women, the antibodies were detectable in gestational week 22. The other four had detectable antibodies in gestational weeks 24, 26, 34 and 36. These 13 women had increasing anti-HPA 1a antibody levels towards term (Figure 3A). In the last sample before term the mean (±95% CI) anti-HPA 1a antibody level was 7.5 (±4.5) IU/mL whereas the weighted mean (±95% CI) of antibody level was 3.4 (±1.8) IU/mL. Six (46%) of these 13 first pregnancies culminanted with the birth of a child with severe thrombocytopenia.

In 92 of the 147 incompatible pregnancies in multigravida, there was a decline in antibody level during pregnancy (Figure 3B). The number of pregnancies with a decreasing antibody level was significantly higher than the number of pregnancies with no decline in antibody level ($P_{92 \text{ or more of } 147}$=0.003). The mean (±95% CI) antibody level in the 92 pregnancies in which antibody levels declined was 15.4 (±6.1) IU/mL in the first sample obtained and decreased to 5.6 (±2.4) IU/mL at the end of pregnancy, giving a weighted mean anti-HPA 1a antibody level of 9.6 (±4.2) IU/mL. Altogether 29 (32%) children in this group were severely thrombocytopenic.

For the pregnancies in which the antibody levels dropped to below 3.0 IU/mL by the end of pregnancy, the relative risk of severe thrombocytopenia in the neonates was 0.24 (95% CI: 0.13-0.45) relative to the group in which the antibody level in last sample was >3.0 IU/mL. In 33 pregnancies, no antibodies could be detected in the last sample before delivery. None of the babies born from these pregnancies were severely thrombocytopenic although seven had moderately reduced platelet counts (67×10^9/L-115×10^9/L).

In 25 multigravida, the antibody level increased from a mean of 11.6 (±6.1) IU/mL in the first trimester to 29.5 (±16.2) IU/mL in the last sample obtained before delivery, which was equivalent to a weighted mean of anti-HPA 1a antibody levels of 16.1 (±6.1) IU/mL (Figure 3C). Twelve (48%) children in this group had severe thrombocytopenia. In 24 multigravida, the antibody level remained low, fluctuating slightly throughout the pregnancy (Figure 3D). None of the children born from these women were thrombocytopenic.

Eleven of the pregnancies in multigravida were HPA Ibb compatible. The women who carried an HPA 1a negative fetus had persistently high levels of antibodies throughout pregnancy (Figure 3E). None of the compati-

ble children had thrombocytopenia. Figure 4 gives an overview of the different maternal anti-HPA 1a antibody patterns in the women and the outcome of their neonates.

In addition to these antibody patterns, another pattern was seen in six pregnancies in which an initially high level of anti-HPA 1a antibodies decreased by 20-30% during the course of the pregnancy. Since the decreases were less than the calculated critical difference, these six pregnancies were excluded from the large group of multigravida with decreasing antibody levels. Five of the children delivered from these six pregnancies had severe thrombocytopenia.

The relative risk of giving birth to a child with severe NAIT was zero if the anti-HPA 1a antibody level was >3.0 IU/mL in first trimester and subsequently became undetectable during gestation (multigravida, 0/15) compared to those pregnancies in which no anti-HPA 1a was detected in the first sample, but in which immunization took place during pregnancy and the anti-HPA 1a antibody level increased to >3.0 IU/mL (mainly first pregnancies, 6/10).

In all but six of the 147 pregnancies in women who had been pregnant previously, anti-HPA 1a antibodies were detected in the first sample obtained, usually in the first trimester. In the six pregnancies without detectable antibodies in the first samples, antibodies were detected in gestational weeks 14 (n=1), 20 (n=2), 22 (n=1), 24 (n=1) and 38 (n=1).

Post-partum levels of anti-HPA 1a

Anti-HPA 1a antibody levels were measured in samples taken approximately 6 weeks post-partum from 120 of the pregnancies with antibodies detectable during gestation. The post-partum antibody levels were significantly higher than those at the time of delivery in all non-compatible pregnancies (Figure 3A-D). The post-partum antibody levels in compatible pregnancies were not significantly higher than those at delivery (Figure 3E).

Post-partum samples from about 65% of the women who did not have detectable anti-HPA 1a antibodies during pregnancy were also analyzed for such antibodies after delivery. In 39 cases no anti-HPA 1a antibodies could be detected at delivery, but antibodies were present approximately 6 weeks later (Figure 3F). By adjusting for the whole study group we estimate that approximately 60 women became anti-HPA 1a positive after delivery.

Anti-HPA 1a antibody level and HLA DRB3*0101 status

Approximately 90% of the immunized women expressed HLA DRB3*0101.[11] The mean (±95%CI) maternal antibody level close to delivery was 10.3 (±3.6) IU/mL in HLA DRB3*0101 positive women, compared to 0.4 (±0.3) IU/mL in HLA DRB3*0101 negative women (p=0.007). Two HLA DRB3*0101 negative women gave birth to children with moderate thrombocytopenia (platelet count: 59×10^9/L and 94×10^9/L). There were no differences in the HLA DRB3*0101 status between women immunized early in pregnancy, those immunized in the last trimester of pregnancy and those with anti-HPA 1a antibodies detected 6 weeks after delivery.

Anti-rubella antibodies

Samples from ten women were examined antibodies against rubella. In all cases anti-rubella antibodies were present and their levels stable throughout gestation, contrasting with the anti-HPA 1a antibody levels that either decreased (n= 8) or increased (n=2) in these pregnancies (*data not shown*).

Discussion

A correlation between the level of antibodies to the HPA 1a antigen and severity of NAIT has been described by several investigators.[4-9] Results from the present work, including 161 non-compatible pregnancies, further substantiate this relationship. The theoretically best antibody cut-off level for predicting an outcome appraises sensitivity and specificity equally, but is not necessarily the optimal level for use in clinical practice since the consequences of false-negative and false-positive results may be very different. Although maternal antibody levels decreased in the

Table 2. The diagnostic sensitivity and specificity, as well as positive and negative predictive values of maternal antibody level and obstetric history as predictors of severe neonatal autoimmune thrombocythemia (neonatal platelet count <50×10^9/L).

Predictor	*Neonatal platelet count <50×10^9/L*	*Neonatal platelet count >50×10^9/L*	*Diagnostic sensitivity (95% CI)*	*Diagnostic specificity (95% CI)*	*Positive predictive value (95% CI)*	*Negative predictive value (95% CI)*
Anti-HPA 1a > 3.0 IU/mL in either week 22 or 34	50*	42	93% (81-89%)	63% (54-72%)	54% (43-63%)	95% (86-98%)
Anti-HPA 1a ≤ 3.0 IU/mL in both weeks 22 and 34	4	72*				
Previous history of NAIT	7	10	13% (6-26%)	92% (84-96%)	41% (19-66%)	70% (61-77%)
No previous history of NAIT	47*	109*				

*The calculation was based on the data from the 168 pregnancies for which antibody levels were assayed in both gestational weeks 22 and 34. *Including one twin pregnancy.*

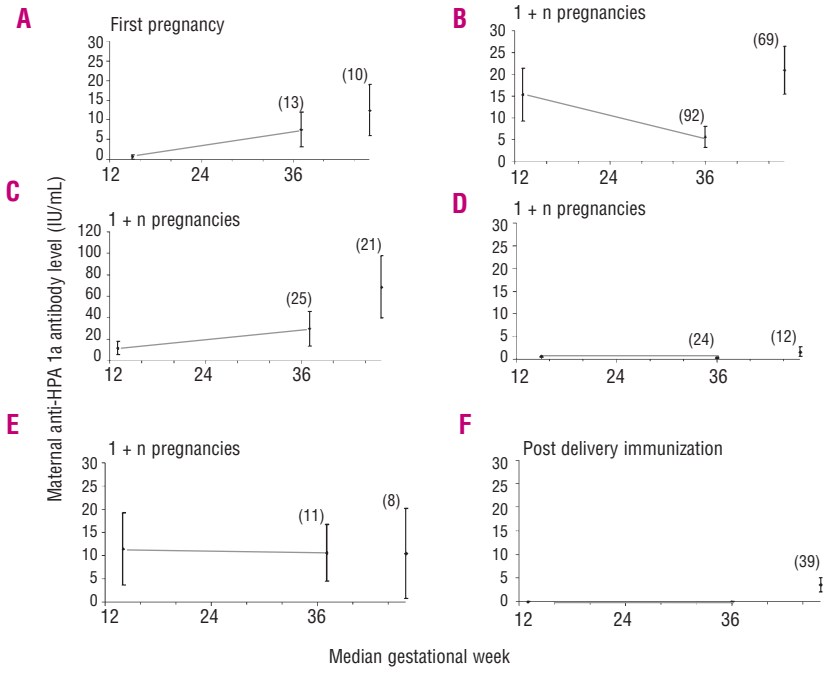

Figure 3. Different patterns of anti-HPA 1a level (mean ± 95% CI) during pregnancy (median gestational week of the first and last samples) and after delivery. (A) First pregnancies. (B) Pregnancies in women who had previously been pregnant and who had decreasing anti-HPA 1a antibody levels. (C) Pregnancies in women who had previously been pregnant and who had increasing anti-HPA 1a antibody levels. (D) Pregnancies in women who had previously been pregnant and who had persistently low levels of anti-HPA 1a antibody. (E) Pregnancies in women who had previously been pregnant and who gave birth to an HPA 1a negative child. (F) Pregnancies in which anti-HPA 1a antibodies were detected post-partum but not during pregnancy. The number of pregnancies is indicated in parentheses. Antibody levels were higher post-partum than at delivery in all non-compatible pregnancies [*p*=0.010 in group (A), *p*<0.0001 in group (B), *p*=0.004 in group (C) and *p*=0.026 in group (D)]. No increase in antibody level was found between delivery and 6 weeks post-partum in compatible pregnancies (*p*=0.127 group (E)).

majority of non-compatible subsequent pregnancies, such that several women had low levels of anti-HPA 1a antibodies at term, some of these women still gave birth to children with severe NAIT. However, by measuring antibody levels twice, at approximately gestational weeks 22 and 34, and using a cut-off level of 3.0 IU/mL, the diagnostic sensitivity for predicting severe thrombocytopenia of the newborn was 93%. An additional advantage of measuring antibody levels early and late in pregnancy, rather than only once closer to delivery, is that appropriate clinical follow-up and possible interventions can be planned if the anti-HPA 1a level in the first sample is very high.

The weighted mean of antibody levels as a predictive parameter cannot discriminate between those women with antibody levels rising from zero to >3.0 IU/mL, and those with antibody levels decreasing to below 3.0 IU/mL. We have shown that women in the former group have a high risk of giving birth to children with severe NAIT, whereas the risk in the latter group is low. This indicates that the antibody level close to delivery may be the best predictive parameter for women who have not previously been pregnant.

Recently, Ghevaert *et al.*[10] published the results of a retrospective study in which no correlation could be found between the maternal anti-HPA 1a antibody level and platelet count in the neonates. The explanation for the divergence between their results and ours may be differences in selection criteria. In our study, women were included prospectively after HPA 1 typing, whereas Ghevaert and his group mainly included women retrospectively on the basis of their having had a child with a bleeding tendency. They also included some women with a previous history of a child with NAIT.

It was shown in a European collaborative study on the antenatal management of feto-maternal alloimmune thrombocytopenia[25] that a previous child with NAIT was the best predictor of subsequent pregnancies at risk of NAIT. Although one could argue that a previous history of thrombocytopenia may have been missed in our study, and could have biased the analyses, we found that women may have a severely affected child even if their previous child or children did not have clinical signs of NAIT. Moreover, women who gave birth to children with severe NAIT in one pregnancy did not necessarily give birth to an affected child in the next one.[11] Our results indicate that the maternal anti-HPA 1a antibody level has equal predictive power as the obstetric history.

Antibody responses to the HPA 1a antigen have been reported to be restricted to women who are HLA DRB3*0101 positive.[26] In our study, however, approximately 10% of the immunized women did not have this particular HLA class II antigen.[11] Quantitation of the anti-HPA 1a levels revealed that these were significantly lower in HLA DRB3*0101 negative women, than in women who were HLA DRB3*0101 positive, probably reflecting that the antigen presented in the context of HLA DRB3*0101 is more immunogenic than peptides presented by other HLA class II molecules.

Altogether, anti-HPA 1a antibodies were detected in 172 pregnancies from a population of 1,990 HPA 1a negative women. Only 13 of these 172 pregnancies were first pregnancies. This is surprising, since severe NAIT has been reported to occur frequently in first born children.[2] On the other hand, it was calculated that 60 women seroconverted after delivery. This may indicate that the primary immune response was established in association with, or after, the delivery, most probably due to feto-maternal bleeding. Thirteen primigravida and 6 multigravida converted from an antibody negative to antibody positive status during pregnancy. The time of conversion could not be determined for the remaining multigravida, because anti-HPA 1a was detected in the first sample collected during pregnancy. Although we cannot exclude the possibility that our MAIPA technique is not sufficiently sensitive to

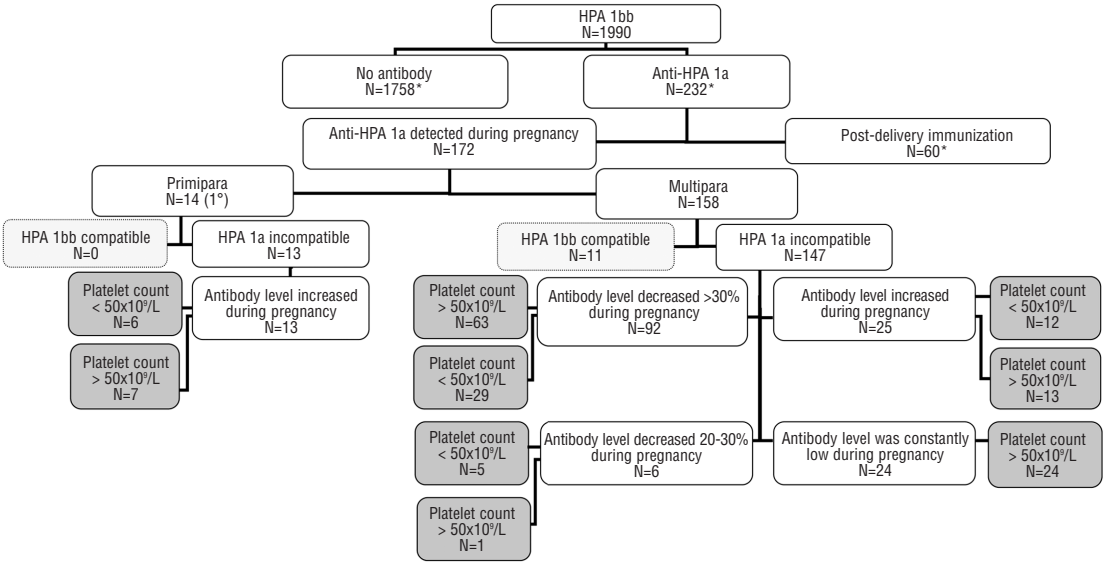

Figure 4. **A flow diagram showing the maternal anti-HPA 1a antibody patterns and the outcome of the neonates (severe neonatal autoimmune thrombocythemia or not). *As post-delivery samples were obtained from approximately 65% of the women, the numbers given have been adjusted to reflect the expected numbers of the whole group.° One woman was excluded because it was unclear whether she really was a primipara. She had a high level of anti-HPA 1a antibodies in week 7 of her pregnancy and the antibodies disappeared by gestational week 20, suggesting immunization prior to this pregnancy.**

detect low levels of maternal anti-HPA 1a antibodies present during a first pregnancy, our observations do suggest that immunization occurs after delivery in a relatively high proportion of HPA 1a negative women giving birth to HPA 1a positive children.

Our results suggest that the immunization pattern for NAIT in many women is similar to that occuring in hemolytic disease of the newborn. It is not, therefore, unlikely that immunization against HPA 1a could be prevented in a similar way as for the RhD antigen, i.e. by administration of anti-HPA 1a antibodies after any event associated with an increased risk of feto-maternal bleeding, such as an abortion, amniocentesis or delivery. Either purified polyclonal human anti-HPA 1a immunoglobulin G or recombinant human HPA 1a antibodies could be used for this purpose.[27]

We and others have previously reported that anti-HPA 1a antibody levels decrease throughout pregnancy in women who have had a previous pregnancy.[9,28-30] The fact that the levels of antibodies to the rubella antigen were stable during pregnancy disproves the hypothesis that the decrease in anti-HPA 1a antibody levels could be due to the normal hemodilution that occurs during pregnancy. Furthermore, women with anti-HPA 1a antibodies and an HPA 1a negative fetus did not experience decreasing anti-HPA 1a levels during pregnancy. The decrease in antibody levels in multiparous women may also have implications when evaluating possible treatment with high-dose intravenous immunoglobulin in women who have previously had a child with NAIT. The natural decline in anti-HPA 1a antibody levels in multiparous women to a low level with no clinical significance may explain why the incidence of children with NAIT is lower in these women. Indeed, when the maternal anti-HPA 1a antibody level decreased and became undetectable before delivery, there was a very low risk of thrombocytopenia in the newborn.

In conclusion, we have presented further evidence for the strong association between maternal anti-HPA 1a antibody level and platelet count in the newborn. The preterm antibody level has equal positive predictive value for NAIT as obstetric history and a much higher diagnostic sensitivity. Expression of HLA DRB3*0101 seems to be a prerequisite for producing clinically significant levels of anti-HPA 1a antibodies. In previously immunized women anti-HPA 1a antibody levels tend to decrease during pregnancy, sometimes to undetectable levels. Immunization seems to occur more often after delivery than during the first non-compatible pregnancy.

Authorship and Disclosures

BS and AH were accountable for conception of the study; MKK collected the data and performed the experiments; MKK and JKK performed statistical analyses; the paper was written by MKK and BS, with contributions from AH and JKK. All authors designed the research, analyzed the data, critically reviewed the paper and approved the version for publication.

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