Fetal and neonatal alloimmune thrombocytopenia: the proof of the pudding is in the eating

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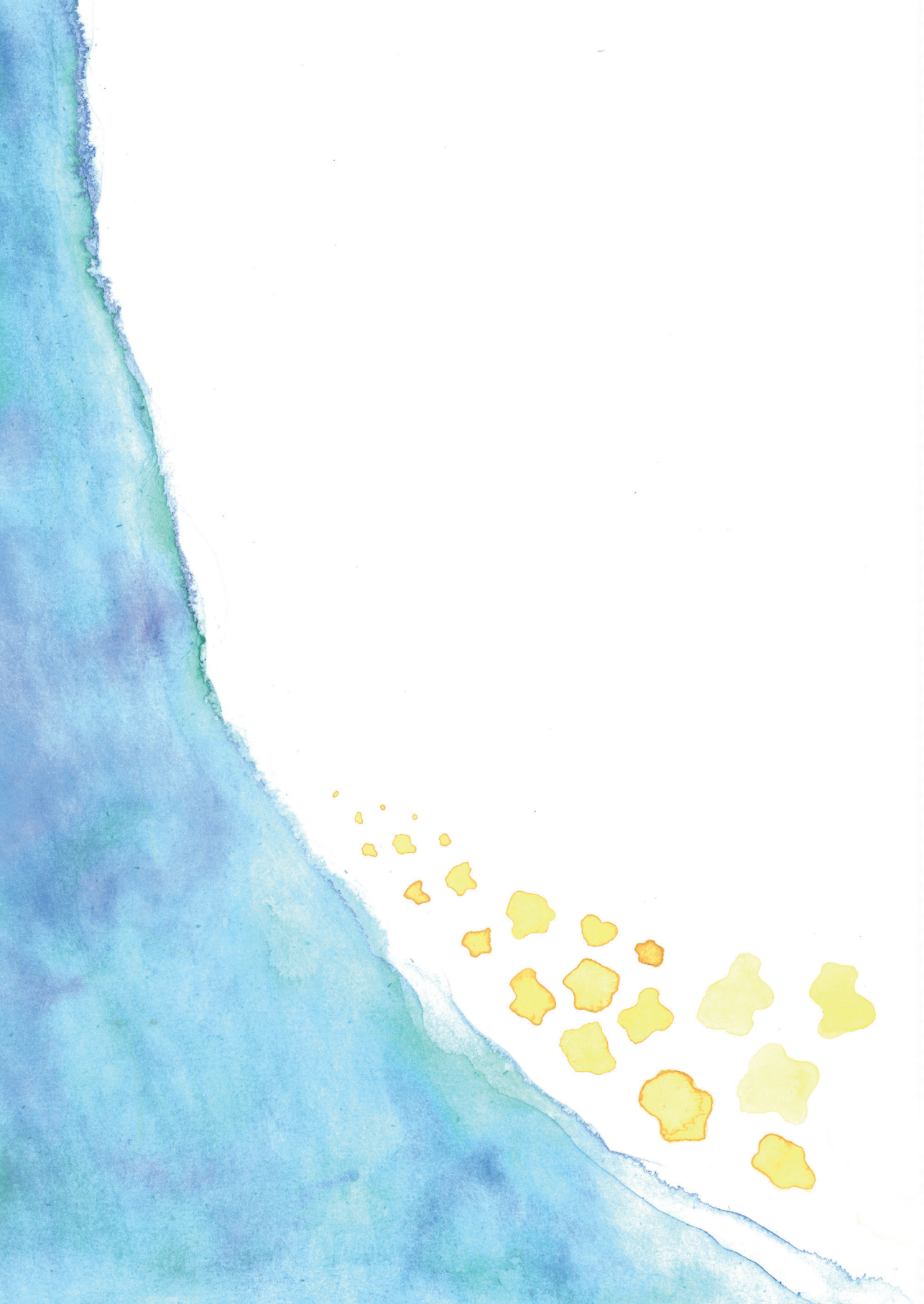
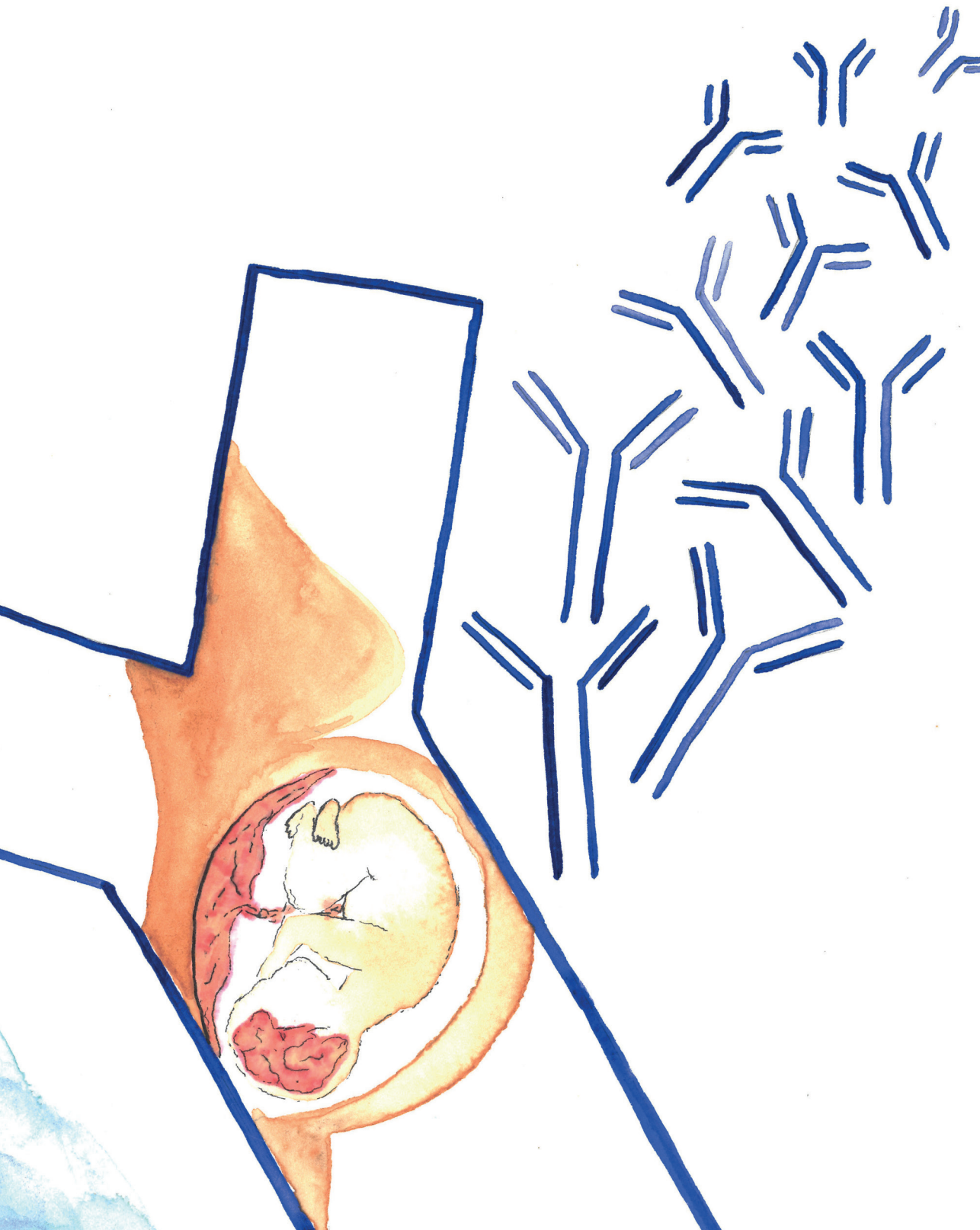


PART THREE

Clinical relevance of HPA-5b antibodies



CHAPTER 5

Clinical characteristics of HPA-1a and HPA-5b alloimmunised pregnancies and the association between platelet HPA-5b antibodies and symptomatic FNAIT: a retrospective cohort study

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SUMMARY

Fetal neonatal alloimmune thrombocytopenia (FNAIT) is caused by maternal alloantibodies directed against the human platelet antigens (mostly HPA-1a or HPA-5b) of the (unborn) child and can lead to severe bleeding. Anti-HPA-1a mediated FNAIT shows more often a severe clinical outcome compared to anti-HPA-5b mediated FNAIT. Given the relatively high prevalence of anti-HPA-5b in pregnant women, the detection of anti-HPA-5b in FNAIT-suspected cases may in some cases be an incidental finding. Therefore we investigated the frequency of anti-HPA-5b associated severe bleeding in FNAIT. We performed a retrospective nationwide cohort study in cases with clinical suspicion of FNAIT. HPA-antibody screening was performed using monoclonal antibody-specific immobilization of platelet antigens. Parents and neonates were typed for the cognate antigen. Clinical data were collected by a structured questionnaire. In 1,864 suspected FNAIT cases, 161 cases (8.6%) had anti-HPA-1a and 60 (3.2%) had anti-HPA-5b. The proportion of cases with severe bleeding did not differ between the cases with anti-HPA-1a (14/129; 11%) and anti-HPA-5b (4/40; 10%). In multigravida pregnant women with an FNAIT-suspected child, 100% (81/81) of anti-HPA-1a cases and 79% (38/48) of anti-HPA-5b cases were HPA-incompatible, whereas 86% and 52%, respectively were expected, based on the HPA-allele distribution. We conclude that anti-HPA-5b can be associated with severe neonatal bleeding symptoms. A prospective study is needed for true assessment of the natural history of anti-HPA-5b mediated FNAIT.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a potentially severe condition that can lead to intracranial haemorrhage (ICH) or organ bleeding in the fetus or neonate, with lifelong sequelae.[1] This condition results from an incompatibility between the fetal and maternal human platelet antigens (HPAs), leading to the formation of maternal alloantibodies. Immunoglobulin G (IgG) class antibodies are actively transported across the placenta during pregnancy. If the fetus is positive for the cognate antigen, HPA-reactive antibodies can bind to fetal platelets and to other cells expressing HPA, such as endothelial cells and trophoblasts. HPA antibodies may cause fetal thrombocytopenia and increased risk of bleeding, with the risk of bleeding being most prominent in anti-HPA-1a complicated pregnancies. The latter may be due to functional impairment of platelets and endothelial cells caused by the binding of different subtypes of anti-HPA-1a to the fibrinogen and vitronectin receptors of platelets and endothelial cells, respectively.[2-4] In all types of HPA antibody-complicated pregnancies, symptoms can vary from an asymptomatic thrombocytopenia to minor signs of bleeding such as skin bleeding or large ICH or organ haemorrhage.[5]

HPAs are encoded by single-nucleotide polymorphisms that result in amino acid changes of certain glycoproteins (GPs) expressed at the cell surface of platelets. The first HPA, HPA-1, was described in 1959 by Van Loghem *et al.*[6]. Currently, 41 HPAs have been described and named in order of discovery.[7] Based on the largest cohort of FNAIT cases so far, an estimated 78% of the FNAIT cases in the white population are caused by anti-HPA-1a antibodies and 9% are caused by anti-HPA-5b antibodies.[8] In contrast to the higher rate of anti-HPA-1a mediated FNAIT compared to the rate of anti-HPA-5b-mediated FNAIT reported in retrospective studies, in prospective screening studies anti-HPA-1a is found in 0.2% of the in pregnant women[9] and anti-HPA-5b in 1.8% of the pregnant women.[10] In about 10% of the HPA-1a incompatible pregnancies anti-HPA-1a is present and in approximately 27% of the HPA-5b incompatible pregnancies anti-HPA-5b is present. The discrepancy between the almost 10 times higher prevalence of anti-HPA-5b in pregnant women in screening studies but the lower number of symptomatic FNAIT cases in cohort studies led to the conclusion that HPA-5b antibodies are clinically less relevant.[11, 12] Given the high prevalence of anti-HPA-5b in pregnant women, it is difficult to determine if anti-HPA-5b can cause severe neonatal thrombocytopenia or bleeding and whether anti-HPA-5b in thrombocytopenic neonates is detected incidentally.
We performed a retrospective cohort study to describe the differences in the clinical characteristics between anti-HPA-1a-associated FNAIT and anti-HPA-5b-associated FNAIT and investigated if anti-HPA-5b can be associated with cases of severe neonatal bleeding.

METHODS

STUDY POPULATION

This was a retrospective cohort study that included all newly detected FNAIT cases identified at Sanquin Diagnostics in Amsterdam, the national reference laboratory for FNAIT, or at the Leiden University Medical Center (LUMC), the national clinical expertise centre on fetal medicine. Cases diagnosed with FNAIT between January 2002 and January 2020 were eligible. All mothers and fathers and/or cases were genotyped for HPA-1, -3, -5 and -15 to determine possible fetal maternal incompatibilities. Platelet antibodies were screened and identified with monoclonal antibody-specific immobilization of platelet antigens (MAIPA)[13] and platelet immunofluorescence test (PIFT) including crossmatching between maternal serum and paternal platelets.[14] FNAIT was confirmed if there was clinical suspicion with neonatal thrombocytopenia (< 150×10^9/L) and/or fetal/neonatal bleeding, confirmed HPA incompatibility between the mother and child and the presence of an HPA antibody in the maternal blood sample. If FNAIT was suspected, because of fetal/neonatal maternal HPA incompatibility but antibody screening was negative, postpartum testing was repeated six weeks later to ensure that HPA-1a or HPA-5b antibodies were not missed. All FNAIT cases that were based on HPA-1a *or* HPA-5b-directed antibodies were included. The exclusion criteria were cases with additional anti-HPA antibodies, presence of HPA-1a *and* HPA-5b antibodies or cases with incomplete follow-up data regarding bleeding symptoms. FNAIT cases mediated by alloantibodies directed against HPA-1a were compared to FNAIT cases with alloantibodies directed against HPA-5b. To improve the readability of this article, these cases will be referred to as 'HPA-1a cases' and 'HPA-5b cases', respectively. The occurrence of HLA antibodies was not considered in this study. The medical ethical committee Leiden-Delft-The Hague provided ethical approval (G17.007).

CLINICAL DATA COLLECTION

Clinical data were obtained by a structured questionnaire sent to the referring clinician and/or completed by telephone and from LUMC medical records. All collected data were de-identified before analysis. The following data were collected: HPA-alloantibody specificity, fetal/maternal HPA types, gravidity, parity, obstetric history, mode of delivery, gestational age at delivery, sex, birthweight, bleeding (including type of bleeding and cerebral imaging reports), platelet count at birth, platelet count nadir (lowest platelet count), postnatal treatment, type of postnatal treatment and perinatal death, neonatal sepsis, asphyxia and the presence of a congenital abnormalities and maternal idiopathic thrombocytopenic purpura (ITP).

DEFINITIONS

The following other factors possibly related to neonatal thrombocytopenia[15] were examined; prematurity <32 weeks gestational age, small for gestational age (SGA, defined as birth <10th percentile[16]), neonatal sepsis (defined as a clinical suspicion of infection *and* positive blood culture), perinatal asphyxia (defined as Apgar score <7 at 5 minutes or arterial blood pH<7),

severe congenital abnormalities and the presence of maternal thrombocytopenia/ITP. Severe bleeding was defined as ICH; intraventricular haemorrhage (IVH) grade III-IV, ICH with parenchymal involvement or major organ bleeding (requiring supportive care, e.g. red blood cell transfusion). Minor bleeding was defined as all other uncomplicated haemorrhages such as petechiae, haematoma and/or mucosal bleeding. Platelet count after birth was defined as the first platelet count measured after birth. Platelet count nadir was defined as the lowest platelet count in the first 2 weeks after birth.

OUTCOME MODIFIERS

We report whether FNAIT was diagnosed before or after birth, and if FNAIT was diagnosed antenatally whether maternal intravenous immunoglobulin (IVIg) treatment was started. Neonatal platelet counts were analysed with and without the inclusion of these antenatally treated cases, as administering maternal IVIg can influence neonatal platelet counts.

PRIMARY AND SECONDARY OUTCOME

The primary outcome was the prevalence of severe bleeding in children with newly detected anti-HPA-1a and anti-HPA-5b mediated FNAIT. The secondary outcomes were platelet count after birth, platelet count nadir, the proportion of cases that received postnatal treatment and mortality. We also investigated whether the presence of HPA-5b antibodies was associated with thrombocytopenia/neonatal bleeding or if HPA-5b antibodies are detected incidentally in platelet antibody screening. Therefore, we assessed the presence of other risk factors for thrombocytopenia and compared the rate of fetal–maternal HPA incompatibility in multigravida women to the expected rate of fetal–maternal HPA incompatibility calculated with gene frequencies in the general population while also considering the HPA positivity of the father.

STATISTICAL ANALYSIS

The HPA-1a group was compared to the HPA-5b group. Descriptive statistics were used to report proportions, medians with interquartile ranges and means with standard deviations as appropriate. Categorical variables between the groups were compared with the chi-square test; continuous variables were compared by using the Mann-Whitney U test. Data were analysed using IBM SPSS Statistics 25.0 (Chicago, IL, USA).

RESULTS

The study population is presented in Figure 1. Between 2002 and 2020, 1,864 cases with suspicion for FNAIT were referred for diagnostic assays; HPA antibodies were detected in 262 cases (14%). Six cases were excluded from further analysis because both anti-HPA-1a and anti-HPA-5b could be implicated (additional information of these cases is presented

in Supplemental Table 1). Six other cases were excluded because next to anti-HPA-1a/5b another HPA antibody was present, and the neonate was positive for the cognate antigen. Anti-HPA-1a or anti-HPA-5b was detected in 161 cases (8.6%) and 60 cases (3.2%), respectively. HPA incompatibility between mother and child was confirmed in all 161 HPA-1a cases and in 50/60 (83%) HPA-5b cases. Clinical follow-up was complete for 80% of cases for both the HPA-1a group and HPA-5b group.

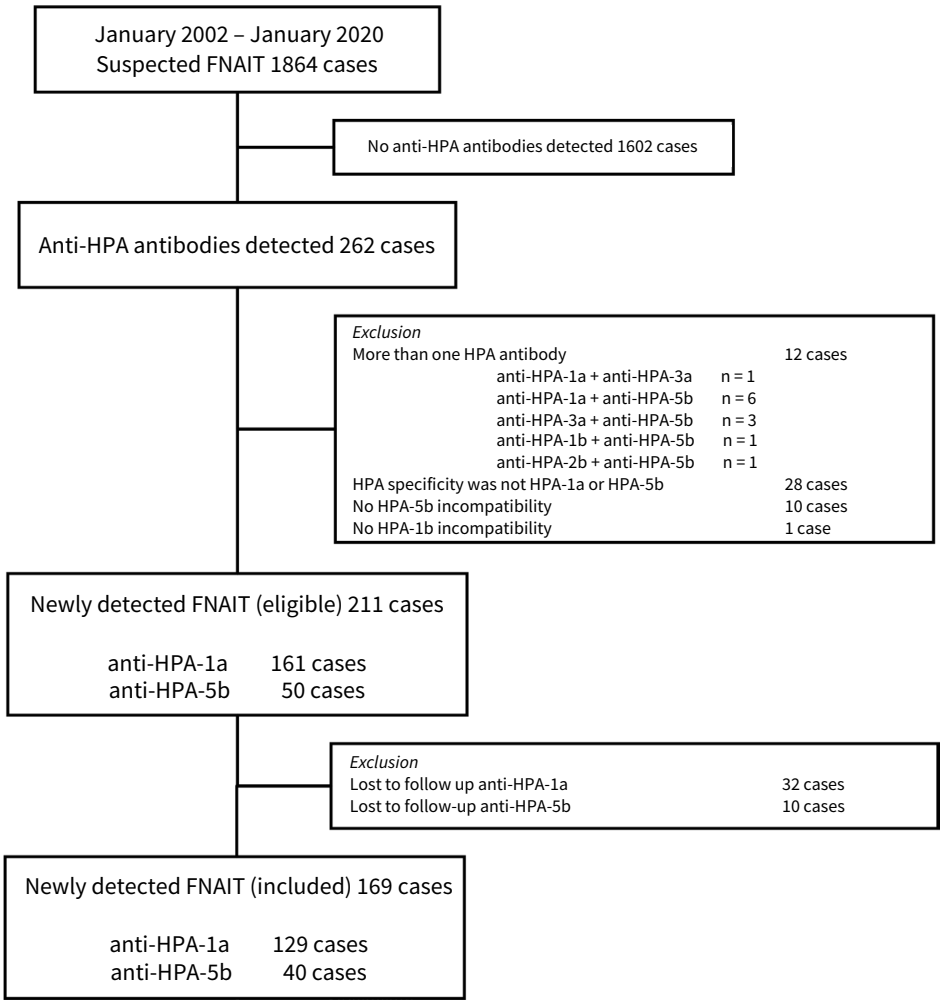

FIGURE 1. Flowchart study population

Abbreviations: FNAIT, fetal neonatal alloimmune thrombocytopenia; HPA, Human platelet antigen.

CLINICAL CHARACTERISTICS

The clinical characteristics of the FNAIT cases are presented in Table 1. HPA-1a and HPA-5b cases did not differ in terms of the distribution of sex, gestational age at delivery and birthweight. In both groups, SGA was the most frequent other risk factor for thrombocytopenia; neonatal sepsis was more frequent in the HPA-1a group and congenital abnormalities, asphyxia and maternal ITP were more frequently reported in the HPA-5b group. In 4% (n = 5) of the HPA-1a cases and 28% (n = 11) of the HPA-5b cases, FNAIT was strongly suspected during pregnancy due to the finding of HPA antibodies; subsequently, one (1%) HPA-1a case and 11 (11%) HPA-5b cases received antenatal IVIg treatment.

TABLE 1. Clinical characteristics of the FNAIT cases

	HPA-1a (n = 129)	**HPA-5b (n = 40)**
Sex (male)¶ - n (%)	87 (69)	28 (70)
First pregnancy§ - n (%)	46 (37)	8 (21)
Gestational age at delivery (weeks^{+days})† - median (IQR)	38^{+4} (37^{+0} – 40^{+1})	38^{+1} (36^{+0} – 39^{+2})
Premature <32 weeks† - n (%)	3 (3)	1 (3)
Premature <37 weeks† - n (%)	26 (23)	12 (32)
Birthweight (grams)‡ - mean (SD)	3031 (651)	2795 (692)
Small for gestational age (SGA) ‡ - n (%)	23 (21)	8 (22)
Other risk factor for neonatal thrombocytopenia - n (%)	27 (21)	18 (45)
SGA	16	5
SGA and premature birth <32 weeks	1	0
SGA and neonatal sepsis	3	1
SGA and asphyxia	2	0
Premature birth <32 weeks	1	0
Neonatal sepsis	1	1
Asphyxia	1	4
Congenital abnormalities	1	5
Maternal ITP	0	2
Antenatal diagnosis - n (%)	5 (4)	11 (28)
Antenatal treatment - n (%)	1 (1)	11 (28)

¶ Assessed in 127/40 (99%) cases. Missing values for 2 cases.
§ Assessed in 124/38 (98%) cases. Missing values for 4 cases.
† Assessed in 111/37 (90%) cases. Missing values for 17 cases, 4 cases of antenatal death were excluded.
‡ Assessed in 111/36 (87%) cases. Missing values for 22 cases of which 2 due to antenatal death.
Abbreviations: HPA, Human platelet antigen; IQR, Interquartile range; SD, Standard deviation; SGA, Small for gestational age; ITP, Immune thrombocytopenia; IVIg, intravenous immunoglobulin

TABLE 2. Clinical outcome of the FNAIT cases

	HPA-1a (n = 129)	HPA-5b (n = 40)	*P*-value*
Cases with bleeding - n (%)	98 (76)	12 (30)	*P* < 0.001
of which minor bleeding	84 (65)	8 (20)	
of which severe bleeding	14 (11)	4 (10)	
Platelet count after birth (×10^9/L) ¶ - median (IQR)			
all cases	17 (10-30)	80 (27-170)	*P* < 0.001
cases without antenatal treatment	17 (10-30)	48 (18-81)	*P* = 0.008
Platelet count nadir (×10^9/L) ¶ - median (IQR)			
*all cases***	14 (8-27)	55 (17-133)	*P* < 0.001
*cases without antenatal treatment***	14 (8-27)	31 (15-62)	*P* = 0.059
Thrombocytopenia <25x10^9/L ¶ - n (%)	90 (70)	11 (28)	*P* < 0.001
Postnatal treatment given ¶ - n (%)	85 (69)	8 (22)	*P* < 0.001
platelet transfusion	52	6	
IVIg	10	1	
platelet transfusion and IVIg	23	1	
Perinatal death - n (%)	6 (5)	1 (3)	*P* = 1.000

*All HPA-1a cases are compared to all HPA-5b cases, categorical variables (bleeding status, thrombocytopenia < 25x10^9/L, treatment status) were compared with Chi-square test, perinatal death was compared with by the Fisher's Exact Test, continuous variables (platelet counts) were compared by using Mann Whitney U-test.
** Platelet count nadir was shown for all cases (122/38) and for the cases without antenatal IVIg treatment only (121/27).
¶ Assessed in 122/38 (95%) cases. Missing values for 9 cases of which 4 due to antenatal death.
Abbreviations: HPA, Human platelet antigen; IQR, Interquartile range; SD, Standard deviation; L, Litre

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BLEEDING

The clinical outcome is reported in Table 2. In total, 76% of the HPA-1a infants had bleeding compared to 31% of the HPA-5b cases (relative risk (RR) 2.5, 95% confidence interval (CI) 1.6–4.1, *P* < 0.001). The proportion of cases with severe bleeding did not differ between the HPA-1a and HPA-5b groups (11% and 10%, respectively). In total, 14 (11%) HPA-1a cases had severe bleeding; these could be described as follows: intraparenchymal bleeding (n = 8), subdural bleeding (n = 2), IVH grade IV (n = 1) and subarachnoid bleeding (n = 1). Two HPA-1a cases had organ bleeding (one pulmonary bleeding that required mechanical ventilation and one gastrointestinal bleeding requiring red blood cell transfusion). In three HPA-1a cases, the ICH was classified as minor: IVH grade II (n = 2) and IVH grade I (n = 1). In four (10%) HPA-5b cases, the ICH diagnosed was described as severe: IVH grade IV (n = 3), and one other ICH could not be specified, but because the death of the fetus could be attributed to the bleeding, it was classified as severe. One anti-HPA-5b–associated FNAIT case had IVH grade II (minor bleeding). For both the HPA-1a group and HPA-5b group, severe bleeding was reported in all cases of perinatal death.

PLATELET COUNT, BLEEDING AND POSTNATAL TREATMENT

Figure 2 shows the lowest platelet count per case stratified for HPA specificity and bleeding symptom severity. The median platelet counts were lower in the cases with bleeding symptoms, although the relationship between severity of thrombocytopenia and bleeding risk was not linear; severely thrombocytopenic infants were observed in the group with

severe and minor bleeding but also in children without bleeding symptoms. In two HPA-1a cases and one HPA-5b case with severe bleeding that did not receive antenatal treatment, the platelet count was >25 × 10^9/L. The median platelet count after birth was lower in the HPA-1a group compared to that in the HPA-5b group ($P < 0.001$); however, the median platelet count nadir was not different between the groups ($P = 0.058$). Postnatal treatment was given in 85 HPA-1a cases and eight HPA-5b cases (RR 3.2, 95% CI 1.7–6.0, $P < 0.001$); specification of treatment strategies is listed in Table 2. In Supplemental Table 2 clinical outcome is presented stratified by the presence of other risk factors for neonatal thrombocytopenia. In the HPA-1a group, severe bleeding was observed in the subgroup with and without other risk factors for neonatal thrombocytopenia in 18% (5/27) and 9% (9/102) of the cases, respectively. In the HPA-5b group, severe bleeding was observed in the subgroup with and without other risk factors for neonatal thrombocytopenia in 6% (1/18) and 14% (3/22) of the cases, respectively.

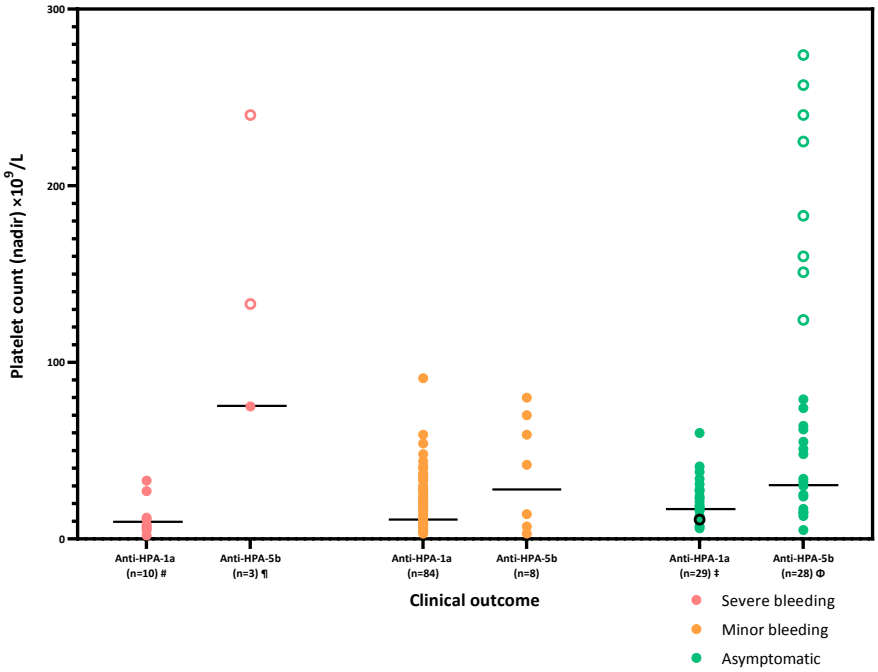

FIGURE 2. Platelet count and clinical outcome

Black lines represent median value per group, medians were calculated including cases that were not treated antenatally with IVIg.

Missing values for 4 cases due to mortality.

¶ Missing values for 1 case due to mortality, 2 cases were treated with IVIg during pregnancy (open dots).

‡ Missing values for 2 cases, 1 case was treated with IVIg during pregnancy (open dot, black border).

Φ Antenatal IVIg treatment was applied in 9 pregnancies (open dots) of which one case with platelet count 364 ×10^9/L not shown.

Abbreviations: HPA, Human platelet antigen; IVIg, intravenous immunoglobulin

ANTENATALLY SUSPECTED FNAIT CASES

Five cases in the HPA-1a group and 11 cases in the HPA-5b group were suspected antenatally; their clinical course is described in Supplemental Table 3. In each group, one case was diagnosed during a next pregnancy after the birth of a previous thrombocytopenic child. All other cases were detected at the end of the second trimester or beginning of the third trimester, as cerebral abnormalities were observed with routine ultrasound investigations during pregnancy. In all (4/4) of the HPA-1a cases with suspected antenatal bleeding, ICH was confirmed by radiography, whereas ICH was confirmed in only 25% (3/12) of the HPA-5b–associated antenatally suspected FNAIT cases with cerebral abnormalities. In all these cases, IVIg administration to the pregnant woman was started. The platelet count was not available for two HPA-1a cases due to fetal death. The two antenatally detected HPA-1a cases were born with platelet count $<30 \times 10^9$/L, whereas all eleven antenatally detected HPA-5b cases were born with high platelet counts of $>120 \times 10^9$/L.

OBSERVED VERSUS EXPECTED RATE OF FETAL–MATERNAL INCOMPATIBILITY

In HPA-immunised women, fetal–maternal incompatibility in pregnancy can be absent if the father is heterozygous for the HPA type and immunisation has occurred in earlier pregnancies. If anti-HPA is an incidental finding in cases with suspected FNAIT, the rate of HPA-1a- or HPA-5b–positive children in HPA-immunised pregnancies would be as calculated with the known allele frequencies and taking the HPA positivity of the father into account (see Supplemental Table 4[17, 18]). We calculated that in HPA-1a–immunised multigravida pregnancies, with per definition an HPA-1a–positive father, 86% of the infants will be HPA-1a–positive. In HPA-5b–immunised multigravida pregnancies, 52% of the infants will be HPA-5b–positive. Table 3 reports the observed versus expected fetal–maternal incompatibility rates. In all 161 cases (100%) with HPA-1a antibodies, FNAIT was reported as confirmed; all children were HPA-1a–positive. In the cases with anti-HPA-5b antibodies, 50 children were HPA-5b–positive and 10 children were HPA-5b–negative (Figure 1). Concerning the pregnancies of multigravida women in the HPA-5b group, 79% (38/48) of the children were HPA-5b–positive. Supplemental Table 5 contains additional information on the HPA-5b–compatible cases. In these cases there were various explanations for HPA-5b immunisation of the mother; earlier pregnancy with an HPA-5ab father (n = 3), earlier pregnancy from another father (n = 4), maternal platelet transfusions (n = 1) or it remained unknown (n = 2).

TABLE 3. Observed versus expected HPA-1a and HPA-5b incompatibility in multigravida women

	HPA-1a incompatibility Confirmed FNAIT	HPA-1a compatible FNAIT not confirmed	HPA-5b incompatible Confirmed FNAIT	HPA-5b compatible FNAIT not confirmed
Observed - n (%)	81 (100)	0	38 (79)	10 (21)
Expected – n (%)	70 (86)	11 (14)	25 (52)	23 (48)

Gravidity status unknown for 34 HPA-1a immunised women and 11 HPA-5b immunised women, numbers were extrapolated based on available data. Expected rates were calculated based on the German allele frequencies.[18]

DISCUSSION

MAIN FINDINGS

This retrospective cohort study describes the differences in the clinical characteristics between anti-HPA-1a–associated FNAIT and anti-HPA-5b–associated FNAIT. In addition we evaluated if anti-HPA-5b may be detected incidentally in children suspected for FNAIT but suffering from thrombocytopenia of bleeding because of other causes. In our cohort of 1,864 suspected FNAIT cases, anti-HPA-1a and anti-HPA-5b was detected in 8.6% and 3.2% of the cases, respectively. Clinical conditions possibly leading to thrombocytopenia were more frequently present in the HPA-5b group (45%) compared to the HPA-1a group (21%). We found no difference in the proportion of cases with severe bleeding between the HPA-1a and HPA-5b groups (11% and 10%, respectively). The nature of severe bleeding was different: organ bleeding was observed in the HPA-1a group only, ICHs in the HPA-1a group were predominantly parenchymal and were mostly of intraventricular origin in the HPA-5b group. Further, three cases (one HPA-1a- and two HPA-5b–mediated) had severe bleeding without severe thrombocytopenia (defined as platelet count $<25 \times 10^9$/L). Interestingly, in all eleven HPA-5b cases that received antenatal IVIg treatment because they were diagnosed prenatally, the platelet counts remained at $>120 \times 10^9$/L after birth. In multigravida pregnant women with a child suspected of FNAIT, all HPA-1a cases (n = 81) and 79% (n = 38) of the HPA-5b cases were HPA-incompatible, which is both higher than the percentages expected by chance of 86.2% and 52.2%, respectively.

INTERPRETATION

In the HPA-1a group, FNAIT was more often detected because of signs of bleeding and median platelet counts were lower compared to HPA-5b cases; these findings are in line with other retrospective studies.[12, 19] Those studies also reported similar proportions (8–16%) of severe bleeding in anti-HPA-1a- and anti-HPA-5b–mediated FNAIT. Similar to our HPA-5b cases with severe bleeding, platelet counts were normal in one and modestly low in two cases in this cohort study.[19] One retrospective study found no FNAIT cases with severe bleeding in the anti-HPA-5b–complicated group.[11] In that study, fetal–maternal HPA incompatibility was not confirmed. Possibly, this could have led to the inclusion of cases without HPA incompatibility and underestimation of the bleeding rates. The absence of HPA-5b incompatibility in 10 cases in our cohort underlines the importance of maternal, paternal and/or fetal HPA typing as part of the diagnostic workup in FNAIT.

Several factors could be related to the lower risk of bleeding in HPA-5b–incompatible neonates as compared to the HPA-1a group. It may be that HPA-5b antibodies can lead to thrombocytopenia and the relatively low level of expression of HPA-5b may require higher levels of anti-HPA-5b for platelet destruction.[20, 21] Perhaps differences in the glycosylation of the Fc tail, the effector part, of the HPA specific antibody can explain the variety in clinical outcome in HPA-5b mediated FNAIT.[22] Fc glycosylation influences the binding and affinity of

different Fc receptors on effector cells and it was shown that Fc afucosylation and increased Fc galactosylation are associated with severe disease in FNAIT.[23, 24] The lower platelet count in the HPA-1a group cannot fully explain the differences in clinical outcome between the HPA-1a and HPA-5b cases. As illustrated in Figure 2 and also shown by a study that estimated the risk of bleeding in premature thrombocytopenic infants, thrombocytopenia is a poor predictor of the risk for severe bleeding in infants.[25] It may therefore also be that variation in the pathogenicity of HPA antibodies can be explained by variation by antibody-induced interference with the function of their target. Anti-HPA-1a can bind to αvβ3 expressed on platelets and the endothelium and reduce vascular integrity.[4, 26] It has been shown that the αvβ3 specific subtype of anti-HPA-1a is a possible risk factor for occurrence of ICH in the child.[2] No such effect has been described for HPA-5b although HPA-5b is carried by α2β1 both on platelets and endothelial cells.[27, 28]

Given the relatively high proportion of cases with other risk factors for thrombocytopenia in our group of HPA-5b cases, together with the observation of the high platelet counts in neonates from antenatally IVIg-treated mothers with anti-HPA-5b, we examined if anti-HPA-5b is an incidental finding in FNAIT suspected cases. Prospective screening studies show that HPA-1a immunisation occurs in 0.2% of pregnant women[9] and that HPA-5b immunisation occurs in 1.8% of pregnant women.[10] We found a higher prevalence of anti-HPA-1a (8.6%) and anti-HPA-5b (3.2%) in our retrospective FNAIT cohort. In the HPA-5b group, but not in the HPA-1a group, there were cases referred because of suspicion of FNAIT but without HPA-5b compatibility. On the other hand, in children with thrombocytopenia and bleeding from multigravida women, fetal–maternal HPA-1a or HPA-5b incompatibility with the presence of HPA antibodies occurred more often than could be expected. Based on these findings, we conclude that HPA-5b antibodies in suspected FNAIT cases is not merely an incidental finding, but can be associated with neonatal thrombocytopenia, and although less often, with thrombocytopenia with bleeding. Our data underline the importance of screening for HPA-5b antibodies in cases suspected for FNAIT.

All HPA-5b cases (n = 11) that were antenatally anticipated with maternal IVIg treatment were born with platelet counts above 120 x 10^9/L. This could be due to a milder clinical scenario of anti-HPA-5b mediated FNAIT compared to anti-HPA-1a mediated FNAIT. It is, however, currently impossible to identify pregnancies at risk for severe neonatal outcome in both anti-HPA-1a and anti-HPA-5b mediated FNAIT. Therefore, based on these retrospective data, we would not (yet) recommend to adapt antenatal IVIg treatment regimens based on HPA antibody specificity.

STRENGTHS AND LIMITATIONS

One limitation of our study is the retrospective study design, which introduces selection bias, especially since there is a broad range in pathology associated with neonatal thrombocytopenia.

Our study extends insights into FNAIT by presenting a cohort of anti-HPA-1a- and anti-HPA-5b–associated FNAIT cases with a high level of information on clinical presentation. A prospective non-intervention study should be performed for a true assessment of the differences between the clinical outcome of pregnancies affected by these antibodies. Currently, such a study is underway in the Netherlands[29], providing the possibility to determine the incidence of neonatal bleeding in HPA-5b-incompatible and immunised pregnancies.

CONCLUSION

Anti-HPA-5b–mediated FNAIT often shows a less severe clinical course compared to HPA-1a-mediated FNAIT cases, with only moderate thrombocytopenia, but can be associated with severe bleeding. In a minor proportion of cases, anti-HPA-5b may be an incidental finding and HPA-incompatibility between the mother and child should be confirmed. Based on the low proportion and absolute number of severely affected anti-HPA-5b–mediated FNAIT cases, diagnostic tools for predicting the neonatal outcome seem indispensable before the introduction of antenatal screening for anti-HPA-5b can be considered. To truly assess the natural history of anti-HPA-5b FNAIT, a prospective screening study that focuses on the natural course of anti-HPA-5b–complicated pregnancies is needed.

DECLARATIONS

CONTRIBUTION TO AUTHORSHIP

DW, EL and MdH conceptualised and designed the research study; LP, SE, EP, TV and DW performed data collection; TV and DW analysed the data; TV drafted the manuscript; EP, ES, DO, DW, EL and MH critically revised the manuscript.

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DISCLOSURE OF INTERESTS

There are no conflicts of interest to disclose.

DETAILS OF ETHICAL APPROVAL

Ethical approval was provided by the medical ethical committee Leiden-Delft-The Hague for cases by study protocol G17.007.

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SUPPLEMENTAL TABLE 1. Description of cases with both anti-HPA-1a and anti-HPA-5b (excluded from further analysis)

Case	G/P	HPA	MAIPA OD C17*/10G11#	Genotype child/ father	Clinical features and reason suspicion	Bleeding	Platelet count (×10^9/L)	Other risk factors	Treatment
156	G2P1	1a + 5b	>3.000/2.032	1ab5ab/1aa5bb	Child born at 37 wks gestation BW 2140 gr. FNAIT suspected because of skin bleeding	Hematoma Petechiae	5	SGA	IVIg (postnatal)
185	G1P0	1a + 5b	>3.000/>3.000	1ab5ab/1aa5bb	Child born at 39 wks gestation BW 3225 gr. FNAIT suspected because of skin bleeding	Hematoma Petechiae minor ICH	0	None	PTx + IVIg (postnatal)
204	G2P0	1a + 5b	>3.000/>3.000	1ab5ab/1aa5ab	Child born at 32 wks gestation BW 1215 gr. Low platelet count found in routine full blood count	None	68	Pre-mature	PTx
231	G5P0	1a + 5b	>3.000/2.652	NT/1aa5ab	Cerebral hematoma at antenatal ultrasound and MRI scan	Major ICH	NT due to mortality	None	NA
232	G7P2	1a + 5b	>3.000/2.335	1ab5ab/1aa5ab	Child born at 38 wks gestation BW 3395 gr. Suspected because of hematoma and prolonged bleeding after heel prick	Hematoma	21	None	PTx
305	G2P0	1a + 5b	Not tested child was born abroad.		Child born at 38 wks gestation, FNAIT suspected because of petechiae	Hematoma	11	None	PTx + IVIg (postnatal)

*C17 is a monocolonal antibody used in the MAIPA to detect platelet antibodies directed at glycoprotein IIb/IIIa (HPA-1 is located at glycoprotein IIIa)
#10G11 is a monocolonal antibody used in the MAIPA to detect platelet antibodies directed at glycoprotein Ia/IIa (HPA-5 is located at glycoprotein Ia)
The cut-off value for a positive antibody test was MAIPA OD > 0.150.
Abbreviations: G, gravidity; P, parity; HPA, human platelet antibody; MAIPA OD, monoclonal antibody-specific immobilization of platelet antigen optical density; L, litre; wks, weeks; BW, birthweight; gr., grams; FNAIT, fetal neonatal alloimmune thrombocytopenia, SGA, small for gestational age; IVIg, intravenous immune globulin; ICH, intracranial haemorrhage, PlateletTx, platelet transfusion; NT, not tested.

SUPPLEMENTAL TABLE 2. Clinical outcome stratified by presence of other risk factors for neonatal thrombocytopenia

Other risk factors for neonatal thrombocytopenia	HPA-1a			HPA-5b		
	Present n = 27	Absent n = 102	all HPA-1a n =129	Present n = 18	Absent n = 22	all HPA-5b n = 40
Cases with bleeding - *n* (%)	19 (70)	79 (78)	98 (76)	4 (22)	8 (36)	12 (30)
of which minor	14 (52)	70 (69)	84 (65)	3 (17)	5 (23)	8 (20)
of which severe	5 (18)	9 (9)	14 (11)	1 (6)	3 (14)	4 (10)
Platelet count after birth ($\times 10^9$/L) ¶- median (IQR)						
all index cases	17 (8-37)	17 (10-28)	17 (10-30)	35 (20-84)	128 (57-195)	80 (27-170)
index cases without antenatal treatment	17 (8-37)	17 (10-28)	17 (10-30)	34 (15-56)	65 (20-111)	48 (18-81)
Platelet count nadir ($\times 10^9$/L) ¶- median (IQR)						
all index cases	13 (6-30)	13 (8-26)	14 (8-27)	31 (15-65)	74 (41-172)	55 (17-133)
index cases without antenatal treatment	13 (6-30)	13 (8-26)	14 (8-27)	28 (14-47)	53 (19-69)	31 (15-62)
Thrombocytopenia < 25x10^9/L ¶ - *n* (%)	17 (63)	73 (72)	90 (70)	7 (39)	4 (18)	11 (28)
Postnatal treatment given ¶ - *n* (%)	18 (69)	67 (69)	85 (69)	6 (35)	2 (10)	8 (22)
platelet transfusion	11	41	52	5	1	6
IVIg	3	7	10	0	1	1
platelet transfusion and IVIg	4	19	22	1	0	1
Perinatal death - *n* (%)	2 (7)	4 (4)	6 (5)	0	1 (5)	1 (3)

¶ Assessed in 122/38 (95%) cases. Missing values for 9 cases of which 4 due to antenatal death
Abbreviations: HPA, Human platelet antigen; IQR, Interquartile range; SD, Standard deviation; l, Litre

SUPPLEMENTAL INFORMATION TABLE 3. Description of antenatally detected FNAIT cases

	Case	G/P	GA suspicion (weeks)	Reason suspicion	Course of pregnancy	Other risk factors	Postnatal course	Platelet count (× 10^9/L)
HPA-1a	171	G2P1	22	ICH; intraparenchymal fetal hydrops	IUFD	No	NA	NT
	203	G3P1	19	ICH; IVH grade III-IV	IUFD	No	NA	NT
	155	G1P0		ICH; right temporal lobe	TOP	No	NA	27
	243	G3P1	29	ICH bilateral intraparenchymal	TOP at 34 weeks	No	NA	12
	127	G2P1	34	Sibling with neonatal thrombocytopenia	IVIg from 36 weeks	No	PlateletTx, no skin bleeding	13
HPA-5b	87	G2P1	28	ICH; IVH grade IV, twin pregnancy one child: ventriculomegaly and ICH	IVIg from 30 weeks	No	No therapy	133
	174	G2P1	33	ICH; IVH grade IV	IVIg from 33 weeks	No	No therapy, no skin bleeding	240
	228	G3P2	20	ICH; IVH grade I-II	IVIg after diagnosis	No	No therapy, no skin bleeding	183
	227	G2P1	29	Isolated ventriculomegaly	IVIg from 30 weeks	No	No therapy, no skin bleeding	160
	246	G2P1		Isolated ventriculomegaly	IVIg	No	No therapy, no skin bleeding	364
	146	G5P4	32	Isolated ventriculomegaly	IVIg	No	No therapy, no skin bleeding	274
	182	G4P2	32	Isolated ventriculomegaly	IVIg	CMV infection, drugs and medication	No therapy, no skin bleeding	225
	97	G1P0	20	Ventriculomegaly and agenesis of corpus callosum	IVIg from 36 weeks	Other cerebral abnormalities.	No therapy, no skin bleeding	240
	136	G2P1	22	Isolated ventriculomegaly that disappeared spontaneously	IVIg	No	No therapy, no skin bleeding	257
	86	G2P1	28	Twin brother of case 87, no ICH	IVIg from 30 weeks	No	No therapy, no skin bleeding	151
	107	G2P1	28	Sibling with neonatal thrombocytopenia	IVIg from 28 weeks	No	No therapy, no skin bleeding	124

Abbreviations: G, gravidity; P, parity, GA, gestational age; L, litre; ICH, intracranial haemorrhage; IUFD, intrauterine fetal demise; NA, not applicable, NT, not tested; IVH; intraventricular haemorrhage; TOP, termination of pregnancy; IVIg, intravenous immune globulin; PlateletTx, platelet transfusion; CMV, cytomegalovirus.

SUPPLEMENTAL TABLE 4. Risk of HPA-1a or HPA-5b incompatibility in multigravida pregnancies

HPA-1		HPA-5	
Genotype		Genotype	
HPA-1aa	= 0.7056	HPA-5aa	= 0.84
HPA-1ab	= 0.2688	HPA-5ab	= 0.1472
HPA-1bb	= 0.0256	HPA-5bb	= 0.0064
Calculation or rates of paternal genotypes assuming that father is HPA-1a or HPA-5b positive.*			
HPA-1aa	= 0.7056 / (0.7056 + 0.2688) = 0.7241	HPA-5ab	= 0.1472 / (0.1472 + 0.0064) = 0.9583
HPA-1ab	= 0.2688 / (0.7056 + 0.2688) = 0.2759	HPA-5bb	= 0.0064 / (0.1472 + 0.0064) = 0.0417
Chance of HPA-1a or HPA-5b positive fetus			
Fetus HPA-5ab	= (Father HPA-1ab × 0.5) + (Father HPA-1aa × 1) = 86%	Fetus HPA-5ab	= (Father HPA-5ab × 0.5) + (Father HPA-5bb × 1) = 52.1%

*We assumed that fathers were HPA-1a or HPA-5b positive because alloimmunisation during pregnancy took place, mothers were diagnosed with either anti-HPA-1a or anti-HPA-5b.

SUPPLEMENTAL TABLE 5. Description of cases with detected HPA-5b antibodies without fetal-maternal incompatibility

#	G/P	HPA antibody	MAIPA OD 10G11#	Genotype child/ father	Clinical features and reason suspicion	Bleeding	Platelet count (×10⁹/L)	Other risk factors	Treatment
I		5b	>3.000	5aa/5aa*	Cerebral abnormalities, deformities of extremities on antenatal ultrasound	ICH possible?	-	Congenital abnormalities	TOP
II	G2P1	5b	2.425	5aa/5ab	Thrombocytopenia (tested because of suspected infection)	None	60	Suspected infection	None
III		5b	0.424	5aa/5aa	Thrombocytopenia (tested because of maternal ITP)	None	6	Maternal ITP	PlateletTx
IV	G2P1	5b	>3.000	5aa/5aa	Thrombocytopenia and skin bleeding	Petechiae	70	None	
V	G2P1	5b	1.421	5aa/5ab	Isolated ventriculomegaly (unilateral)	None	NT	None	None
VI	G2P0	5b	>3.000	5aa/5aa	Severe hydrocephalus, obduction ICH	ICH	NT	None	TOP
VII		5b	2.707	5aa/5ab	Thrombocytopenia	None	10	Asphyxia	Platelet Tx
VIII	G3P1	5b	2.100	-/5aa	Mild ventriculomegaly	None	NT	None	None
IX		5b		5aa/5aa	No information				
X		5b		-/5aa	No information				

* Genotyping father not certain, possibly sample change, another sample was requested but never sent in.
# #10G11 is a monocolonal antibody used in the MAIPA to detect platelet antibodies directed at glycoprotein Ia/IIa (HPA-5 is located at glycoprotein Ia)
The cut off value for a positive antibody test was set at MAIPA OD > 0.150.
Abbreviations: G, gravidity; P, parity, MAIPA OD, monoclonal antibody-specific immobilization of platelet antigen optical density; ICH, intracranial haemorrhage; TOP, termination of pregnancy; ITP, immune thrombocytopenia, PlateletTx, platelet transfusion; NT, not tested.