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Vos, T.W. de; Porcelijn, L.; Hofstede-van Egmond, S.; Pajkrt, E.; Oepkes, D.; Lopriore, E.; ...
; Haas, M. de

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Clinical characteristics of human platelet antigen (HPA)-1a and HPA-5b alloimmunised pregnancies and the association between platelet HPA-5b antibodies and symptomatic fetal neonatal alloimmune thrombocytopenia

Thijs W. de Vos,^{1,2,3} 
 Leendert Porcelijn,⁴ 
 Suzanne Hofstede-van Egmond,⁴
 Eva Pajkr, ⁵ Dick Oepkes,³
 Enrico Lopriore,¹ C. Ellen van der
 Schoot,⁶ Dian Winkelhorst^{3,6} and
 Masja de Haas^{2,3,7}

¹Department of Pediatrics, Division of Neonatology, Leiden University Medical Centre, ²Centre for Clinical Transfusion Research, Sanquin Research, ³Department of Obstetrics and Gynecology, Leiden University Medical Centre, Leiden, ⁴Department of Immunohematology Diagnostics, Sanquin, ⁵Department of Obstetrics and Gynaecology, Amsterdam University Medical Centre, ⁶Department of Experimental Immunohematology, Sanquin, Amsterdam, and ⁷Department of Hematology, Leiden University Medical Centre, Leiden, the Netherlands

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Correspondence: Thijs W. de Vos, Department of Pediatrics, Leiden University Medical Centre, Post zone J6-P, P.O. Box 9600, 2300 RC Leiden, Leiden, the Netherlands
 E-mail: t.w.de_vos@lumc.nl

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 a severe clinical outcome more often than 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 immobilisation 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 multi-gravida pregnant women with a 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.

Keywords: alloimmune thrombocytopenia, neonatology, alloimmunisation during pregnancy, human platelet antigen.

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 Caucasian 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 pregnant women⁹ 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.

Patients and 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 Leiden University Medical Centre (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 immobilisation of platelet antigens (MAIPA)¹³ and the platelet immunofluorescence test (PIFT) including crossmatching between maternal serum and paternal platelets.¹⁴ FNAIT was confirmed if there was clinical suspicion with neonatal thrombocytopenia ($<150 \times 10^9/l$) 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 human leucocyte-specific antigen (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, gender, birth weight, 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 congenital abnormalities and maternal idiopathic thrombocytopenic purpura (ITP).

Definitions

The following other factors possibly related to neonatal thrombocytopenia¹⁵ were examined; prematurity <32 weeks gestational age, small for gestational age (SGA, defined as birth weight <10 th percentile¹⁶), neonatal sepsis (defined as a clinical suspicion of infection and positive blood culture), perinatal asphyxia (defined as Apgar score <7 at 5 min 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 two weeks after birth.

Outcome modifiers

We report whether FNAIT was diagnosed before or after birth, and if FNAIT was diagnosed antenatally whether

treatment with maternal intravenous immunoglobulins (IVIg) 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 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 Fig 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 on these cases is presented in Table SI). 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.

Clinical characteristics

The clinical characteristics of the FNAIT cases are presented in Table I. HPA-1a and HPA-5b cases did not differ in terms

of the distribution of sex, gestational age at delivery and birth weight. 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.

Bleeding

The clinical outcome is reported in Table II. 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 ICH; 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 $>25 \times 10^9/l$. The median platelet count after birth was lower in the HPA-1a group than 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 II. In Table SII

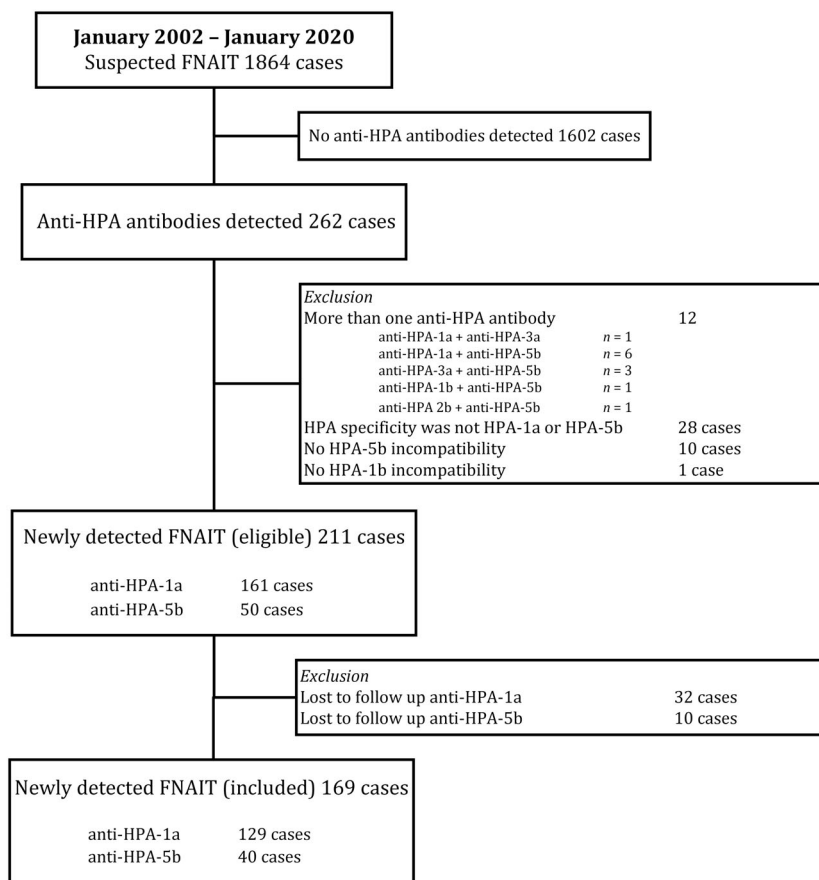


Fig 1. Flowchart study population. FNAIT, fetal neonatal alloimmune thrombocytopenia; HPA, Human platelet antigen.

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.

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 Table SIII. 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 11 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 Table SIV).^{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 III reports the observed *versus* expected fetal–maternal incompatibility

Table I. Clinical characteristics of the fetal neonatal alloimmune thrombocytopenia (FNAIT) cases.

	HPA-1a (<i>n</i> = 129)	HPA-5b (<i>n</i> = 40)
Sex (male),* <i>n</i> (%)	87 (69)	28 (70)
First pregnancy,† <i>n</i> (%)	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,‡ <i>n</i> (%)	3 (3)	1 (3)
Premature <37 weeks,‡ <i>n</i> (%)	26 (23)	12 (32)
Birth weight (g),§ mean (SD)	3031 (651)	2795 (692)
Small for gestational age (SGA),§ <i>n</i> (%)	23 (21)	8 (22)
Other risk factor for neonatal thrombocytopenia, <i>n</i> (%)	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, <i>n</i> (%)	5 (4)	11 (28)
Antenatal treatment, <i>n</i> (%)	1 (1)	11 (28)

HPA, human platelet antigen; IQR, interquartile range; ITP, immune thrombocytopenia; SD, standard deviation; SGA, small for gestational age.

*Assessed in 127/40 (99%) cases. Missing values for two cases.

†Assessed in 124/38 (98%) cases. Missing values for four cases.

‡Assessed in 111/37 (90%) cases. Missing values for 17 cases; four cases of antenatal death were excluded.

§Assessed in 111/36 (87%) cases. Missing values for 22 cases of which two due to antenatal death.

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 (Fig 1). Concerning the pregnancies of multigravida women in the HPA-5b group, 79% (38/48) of the children were HPA-5b-positive. Table SV 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).

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 in whom FNAIT is suspected, but who suffer from thrombocytopenia or 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 × 10⁹/l). Interestingly, in all 11 HPA-5b cases that received antenatal IVIg treatment because they were diagnosed prenatally, the platelet counts remained at >120 × 10⁹/l after birth. In multigravida pregnant women with a child in whom FNAIT is suspected, 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 than in 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

Table II. Clinical outcome of the FNAIT cases.

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

HPA, human platelet antigen; IQR, interquartile range; IVIg, intravenous immunoglobulin; l, litre; SD, standard deviation.

*Assessed in 122/38 (95%) cases. Missing values for nine cases of which four due to antenatal death.

[†]All HPA-1a cases are compared to all HPA-5b cases, categorical variables (bleeding status, thrombocytopenia <25 $\times 10^9/l$, treatment status) were compared with a chi-square test, perinatal death was compared with the Fisher's Exact Test, continuous variables (platelet counts) were compared using the 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).

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 Fig 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 in antibody-induced interference with the function of their target. Anti-HPA-1a can bind to $\alpha v\beta 3$ expressed on platelets and the endothelium and reduce vascular integrity.^{4,26} It has been shown that the $\alpha v\beta 3$ -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 $\alpha 2\beta 1$ 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 cases in which FNAIT is suspected. Prospective screening studies show that HPA-1a immunisation occurs in 0.2% of pregnant women⁹ 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 are 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 suspect for FNAIT.

All HPA-5b cases (*n* = 11) that were antenatally anticipated with maternal IVIg treatment were born with platelet counts above 120 $\times 10^9/l$. 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

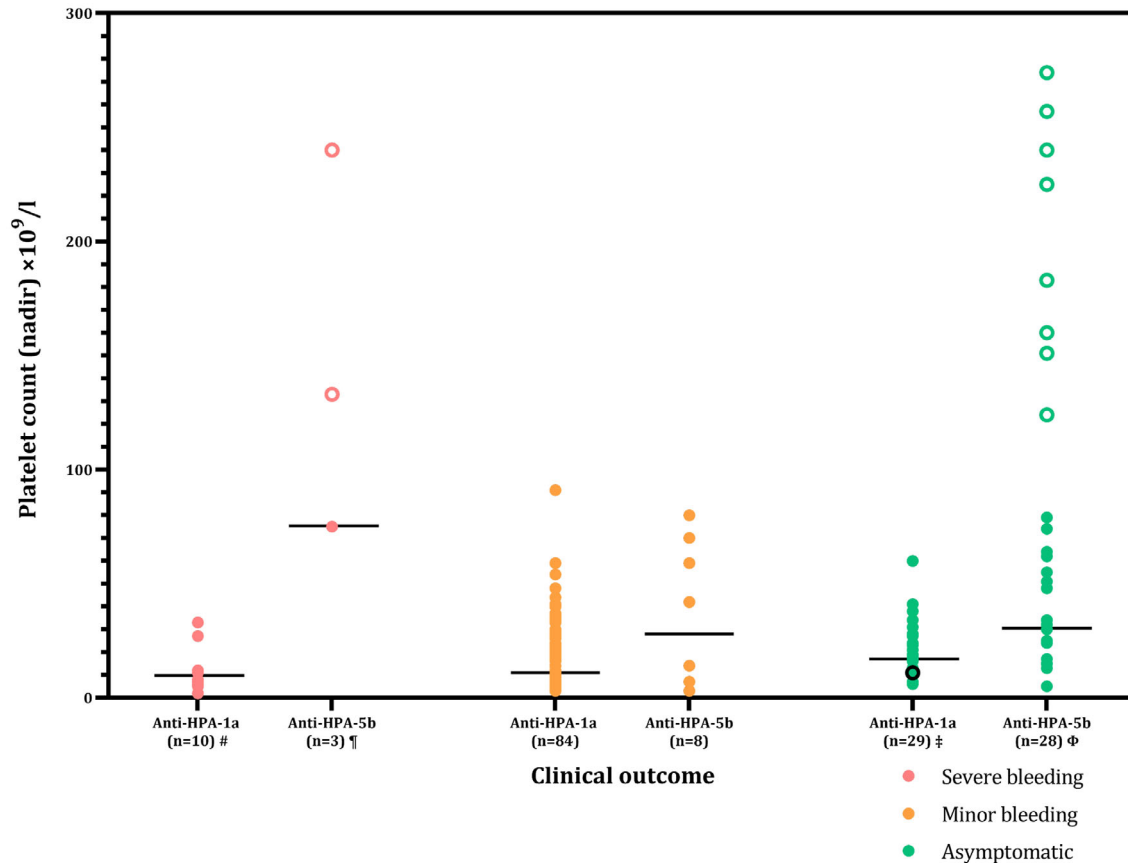


Fig 2. Platelet count and clinical outcome. HPA, Human platelet antigen; IVIg, intravenous immune globulins; Black lines represent median value per group, medians were calculated including cases that were not treated antenatally with IVIg. # Missing values for 4 cases due to mortality. ¶ Missing values 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 $364 \times 10^9/l$ not shown. [Colour figure can be viewed at wileyonlinelibrary.com]

Table III. 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, <i>n</i> (%)	81 (100)	0	38 (79)	10 (21)
Expected, <i>n</i> (%)	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

a true assessment of the differences between the clinical outcome of pregnancies affected by these antibodies. Currently, such a study is under way 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 anti-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.

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Details of ethical approval

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Author contributions

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.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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

Table SII. Clinical outcome stratified by presence of other risk factors for neonatal thrombocytopenia.

Table SIII. Description of antenatally detected fetal neonatal alloimmune thrombocytopenia (FNAIT) cases.

Table SIV. Risk of HPA-1a or HPA-5b incompatibility in multigravida pregnancies.

Table SV. Description of cases with detected HPA-5b antibodies without fetal–maternal incompatibility.

References

1. Winkelhorst D, Kamphuis M, Steggerda S, Rijken M, Oepkes D, Lopriore E, et al. Perinatal outcome and long-term neurodevelopment after intracranial haemorrhage due to fetal and neonatal alloimmune thrombocytopenia. *Fetal Diagn Ther.* 2019;**45**(3):184–91.
2. Santoso S, Wihadmadayati H, Bakchoul T, Werth S, Al-Fakhri N, Bein G, et al. Antiendothelial alphavbeta3 antibodies are a major cause of intracranial bleeding in fetal/neonatal alloimmune thrombocytopenia. *Arterioscler Thromb Vasc Biol.* 2016;**36**(8):1517–24.
3. Eksteen M, Heide G, Tiller H, Zhou Y, Nedberg NH, Martinez-Zubiaurre I, et al. Anti-human platelet antigen (HPA)-1a antibodies may affect

trophoblast functions crucial for placental development: a laboratory study using an in vitro model. *Reprod Biol Endocrinol.* 2017;**15**(1):28.

4. van Gils JM, Stutterheim J, van Duijn TJ, Zwaginga JJ, Porcelijn L, de Haas M, et al. HPA-1a alloantibodies reduce endothelial cell spreading and monolayer integrity. *Mol. Immunol.* 2009;**46**(3):406–15.
5. Winkelhorst D, Kamphuis MM, de Kloet LC, Zwaginga JJ, Oepkes D, Lopriore E. Severe bleeding complications other than intracranial hemorrhage in neonatal alloimmune thrombocytopenia: a case series and review of the literature. *Transfusion.* 2016;**56**(5):1230–5.
6. Van Loghem JJ, Dorfmeijer H, Van Hart M, Schreuder F. Serological and genetical studies on a platelet antigen (Zw). *Vox Sang.* 1959;**4**(2):161–9.
7. Versiti. HPA Database. 2020. Available from: <https://www.versiti.org/hpa-gene>
8. Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. *Transfusion.* 2004;**44**(8):1220–5.
9. Kamphuis MM, Paridaans N, Porcelijn L, De Haas M, van der Schoot CE, Brand A, et al. Screening in pregnancy for fetal or neonatal alloimmune thrombocytopenia: systematic review. *BJOG.* 2010;**117**(11):1335–43.
10. Panzer S, Auerbach L, Cechova E, Fischer G, Hostensteiner A, Kittl E-M, et al. Maternal alloimmunization against fetal platelet antigens: a prospective study. *Br J Haematol.* 1995;**90**(3):655–60.
11. Rousseau J, Goldman M, David M. HPA-5b (Bra) neonatal alloimmune thrombocytopenia in Quebec: incidence and clinical outcome in 31 cases. *Transfusion.* 2004;**44**(6):844–8.
12. Kaplan C, Morel-Kopp MC, Kroll H, Kiefel V, Schlegel N, Chesnel N, et al. HPA-5b (Br(a)) neonatal alloimmune thrombocytopenia: clinical and immunological analysis of 39 cases. *Br J Haematol.* 1991;**78**(3):425–9.
13. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C. Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. *Blood.* 1987;**70**(6):1722–6.
14. Porcelijn L, Huiskes E, de Haas M. Progress and development of platelet antibody detection. *Transfus Apher Sci.* 2020;**59**(1):102705.
15. Gunnink SF, Vlug R, Fijnvandraat K, van der Bom JG, Stanworth SJ, Lopriore E. Neonatal thrombocytopenia: etiology, management and outcome. *Exp Rev Hematol.* 2014;**7**(3):387–95.
16. Hoftiezer L, Hukkelhoven CW, Hogeveen M, Straatman HM, van Lingen RA. Defining small-for-gestational-age: prescriptive versus descriptive birthweight standards. *Eur J Pediatr.* 2016;**175**(8):1047–57.
17. Carlsson LE, Greinacher A, Spitzer C, Walther R, Kessler C. Polymorphisms of the human platelet antigens HPA-1, HPA-2, HPA-3, and HPA-5 on the platelet receptors for fibrinogen (GPIIb/IIIa), von Willebrand factor (GPIb/IX), and collagen (GPIa/IIa) are not correlated with an increased risk for stroke. *Stroke.* 1997;**28**(7):1392–5.
18. Metcalfe P. HPA allele frequencies. 2021 [cited 2021 Jan 21]. Available from: https://www.ebi.ac.uk/ipd/hpa/freqs_1.html
19. Ghevaert C, Campbell K, Walton J, Smith GA, Allen D, Williamson LM, et al. Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. *Transfusion.* 2007;**47**(5):901–10.
20. Santoso S, Kiefel V, Mueller-Eckhardt C. Immunochemical characterization of the new platelet alloantigen system Bra/Brb. *Br J Haematol.* 1989;**72**(2):191–8.
21. Pischel KD, Bluestein HG, Woods VL Jr. Platelet glycoproteins Ia, Ic, and IIa are physicochemically indistinguishable from the very late activation antigens adhesion-related proteins of lymphocytes and other cell types. *J Clin Invest.* 1988;**81**(2):505–13.
22. Wuhrer M, Porcelijn L, Kapur R, Koeleman CAM, Deelder AM, de Haas M, et al. Regulated glycosylation patterns of IgG during alloimmune responses against human platelet antigens. *J Proteome Res.* 2009;**8**(2):450–6.
23. Kapur R, Kustiawan I, Vestrheim A, Koeleman CAM, Visser R, Einarsdotir HK, et al. A prominent lack of IgG1-Fc fucosylation of platelet alloantibodies in pregnancy. *Blood.* 2014;**123**(4):471–80.
24. Sonneveld ME, Natunen S, Sainio S, Koeleman CAM, Holst S, Dekkers G, et al. Glycosylation pattern of anti-platelet IgG is stable during pregnancy and predicts clinical outcome in alloimmune thrombocytopenia. *Br J Haematol.* 2016;**174**(2):310–20.

25. Fustolo-Gunnink SF, Huisman EJ, van der Bom JG, van Hout F, Maki-neli S, Lopriore E, et al. Are thrombocytopenia and platelet transfusions associated with major bleeding in preterm neonates? A systematic review. *Blood Rev.* 2019;**36**:1–9.
26. Yougbare I, Zdravic D, Ni H. Angiogenesis and bleeding disorders in FNAIT. *Oncotarget.* 2015;**6**(18):15724–5.
27. Leeksa OC, Giltay JC, Zandbergen-Spaargaren J, Modderman PW, van Mourik JA, von dem Borne AE. The platelet alloantigen Zwa or PLA1 is expressed by cultured endothelial cells. *Br J Haematol.* 1987;**66**(3):369–73.
28. Li C, Piran S, Chen P, Lang S, Zarpellon A, Jin JW, et al. The maternal immune response to fetal platelet GPIIb/IIIa causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. *J Clin Invest.* 2011;**121**(11):4537–47.
29. Winkelhorst D, de Vos TW, Kamphuis MM, Porcelijn L, Lopriore E, Oepkes D, et al. HIP (HPA-screening in pregnancy) study: protocol of a nationwide, prospective and observational study to assess incidence and natural history of fetal/neonatal alloimmune thrombocytopenia and identifying pregnancies at risk. *BMJ Open.* 2020;**10**(7):e034071.