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

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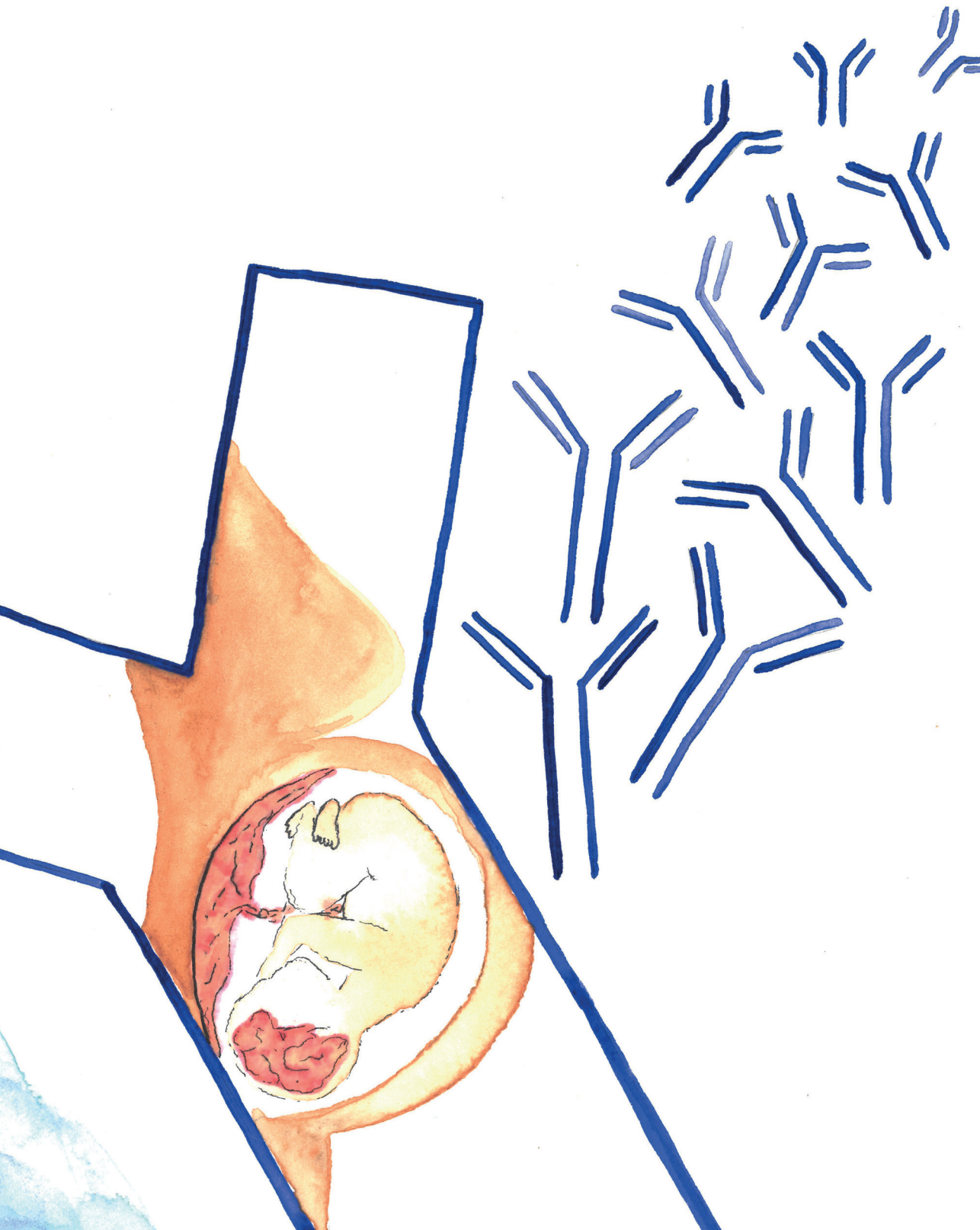

CHAPTER 3

The natural history of human platelet antigen (HPA)-1a alloimmunised pregnancies: a prospective observational cohort study

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ABSTRACT

Objective
To assess the incidence of clinically detectable severe fetal and neonatal alloimmune thrombocytopenia (FNAIT) in human platelet antigen-1a (HPA-1a) immunised pregnancies.

Design
Prospective observational study

Setting
The Netherlands

Participants
Between 1-3-2017 and 1-5-2020, 153 106 women, routinely screened for red cell antibodies in the 27th week of pregnancy, were eligible and were typed for HPA-1a.

Study outline
Clinical data were collected in HPA-1a negative women and in HPA-1a positive women (ratio 1:3). Participants' HPA-1a status was not reported to caregivers and researchers. HPA-1a antibody screening was performed in HPA-1a negative women and antibody quantitation, HLA-DRB3*0101, and fetal HPA-1a typing was done in HPA-1a immunised women.

Main outcome measure
The proportion of neonates with severe FNAIT (major bleeding and/or bleeding-related death) within HPA-1a immunised and incompatible pregnancies without intervention. Secondary outcomes included mild FNAIT (minor bleeding and/or treated thrombocytopenia), pregnancies and neonatal outcomes.

Results
Of the pregnant women, 2.43% (3722/153 106) were HPA-1a negative. Antibody screening was performed in samples from 913 pregnancies of 881 HPA-1a negative women (32 were included twice). Anti-HPA-1a was detected in 85 pregnancies, 82 of which concerned HPA-1a positive fetuses. One pregnancy was excluded because the previous child had been diagnosed with FNAIT. Eighty-one HPA-1a immunised and incompatible pregnancies, 820 HPA-1a negative non-immunised pregnancies, and 2704 pregnancies of HPA-1a positive women were included. One neonate (1.2%, 1/81) was diagnosed with severe HPA-1a mediated FNAIT (severe intracranial haemorrhage) and three neonates (3.7%, 3/81) had mild FNAIT (two with haematomas and one with mucosal bleeding). Major bleeding was observed in 0.1% (3/2749) of neonates of HPA-1a positive women. The incidence of clinically detectable severe anti-HPA-1a mediated FNAIT was 2.6 in 100 000 pregnancies. Of the neonates of HPA-1a-

immunised pregnancies, 15% (12/81) were born preterm (< 37 weeks' gestation) compared to 5% (132/2749) of neonates of HPA-1a positive women (*P*<0.001). Median birthweight percentile of neonates of immunised pregnancies was 0.46 (IQR 0.21 to 0.70) compared to 0.52 (IQR 0.26 to 0.77) in neonates of HPA-1a positive women. Hypertensive disorder during pregnancy was reported in 11% (9/81) of the immunised women compared to 4% (120/2704) in HPA-1a positive pregnant women.

Conclusion

The incidence of major bleeding in FNAIT is 11 in 10 000 HPA-1a negative pregnancies. Preterm delivery, low birthweight, and hypertensive disorders occur more frequently in HPA-1a immunised pregnancies.

Trail registration

Clinicaltrails.gov NTC04067375

SUMMARY BOX

What is already known on this topic

- Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare disease resulting in an increased risk of bleeding in fetus and neonate, with life-threatening and/or damaging intracranial haemorrhage as severe outcome.
- The incidence of major bleeding or perinatal death related to fetal and neonatal alloimmune thrombocytopenia (FNAIT) is between 13 and 20 in 10 000 HPA-1a negative pregnancies. This number is considered an underestimation.
- Besides fetal bleeding and thrombocytopenia, lower birthweights of neonates born to HPA-1a immunised women have been reported

What this study adds

- Our observational screening study showed the incidence of major bleeding-related FNAIT to be 11 in 10 000 HPA-1a negative pregnancies.
- Preterm delivery, reduced birthweight, and hypertensive disorders occur more frequently in HPA-1a immunised and incompatible pregnancies compared to controls. This underlines the association of placental pathology and HPA-1a immunisation.
- The presence of anti-HPA-1a is not associated with the number of previous pregnancies or deliveries.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT), the platelet equivalent of haemolytic disease of the fetus and neonate (HDFN), can cause major intracranial haemorrhage (ICH) and organ bleeding during pregnancy and shortly after delivery.[1, 2] FNAIT may develop during pregnancies in case of incompatibility between fetal and maternal human platelet antigens (HPA). During a first pregnancy such incompatibility can result in the formation of HPA-directed IgG antibodies. These antibodies are actively transported to the fetus. The HPA-1a epitope, targeted in most FNAIT cases in the white population,[3] is carried by the β3 integrin, which is expressed in many tissues. It is, for example, expressed by blood cells with relatively high levels by platelets, the outer layer of the placenta (syncytiotrophoblast),[4] and endothelial cells.[5] The HPA-1a alloantibodies cause platelet destruction.[6] *In vitro* studies have shown their potency to interfere with endothelial cell function, which may contribute to an increased risk of bleeding in fetuses and neonates.[7, 8] Besides thrombocytopenia and bleeding, the classic features of FNAIT, HPA-1a alloimmunisation is also associated with reduced birthweight.[9] Additionally, signs of immunological damage in the placenta of FNAIT cases were observed.[10-12]

Intracranial haemorrhage and its associated neurodevelopmental injury can be prevented by timely treatment during pregnancy. Researchers consider intravenous immunoglobulin (IVIg) infusions as highly effective in this respect.[13-15] Currently, almost all FNAIT cases are diagnosed postnatally in neonates with either thrombocytopenia detected by chance or in infants with bleeding symptoms. Antenatal treatment can be provided in subsequent pregnancies only. Interest in the prevention of the adverse outcome of HPA-1a-mediated FNAIT has increased.[16-20] Prevention may be achieved through population-based screening, which could be added to the widely implemented HDFN prevention programmes. According to the Wilson and Jungner criteria (W&J)[21] used to assess screening programmes, the introduction of an anti-HPA-1a-FNAIT prevention programme is hampered by a lack of knowledge on the natural history of FNAIT (W&J Principle 7) and the risk factors involved in selecting pregnancies for antenatal treatment (W&J Principle 8).

Previous prospective studies provided insight into the frequency of HPA-1a negativity and HPA-1a immunisation.[17, 22-30] In most of these studies, caregivers were informed about the maternal HPA status and the presence of HPA-1a antibodies and interventions were part of the study design. The data therefore might not truly represent the natural history of FNAIT.[17, 22-27] Our primary aim was to determine the incidence of clinically detectable severe FNAIT within HPA-1a immunised and incompatible pregnancies in the absence of any intervention. Secondarily, we aimed to determine the incidence of clinically detectable mild FNAIT and to describe pregnancy and neonatal outcomes within HPA-1a immunised pregnancies.

METHODS

The study protocol was published[31] and registered with www.clinicaltrails.gov (NTC04067375). The Medical Ethical Committee Leiden-The Hague-Delft approved the study protocol (P16.002). Analyses were performed in accordance with the predefined statistical analysis plan, which the research team approved internally before final data collection for this study ended. In addition, we determined maternal HLA DRB3*01:01 carrier status[32] and antibody quantitation[33] following two systematic reviews on this subject.

STUDY DESIGN

We performed an observational screening study in pregnant women in the Netherlands between 1 March 2017 and 1 May 2020. As part of nationwide prenatal screening for infectious diseases and erythrocyte immunisation, RhD and Rhc negative pregnant women were offered red cell antibody screening and fetal *RHD* typing (if RhD negative) at 27 weeks' gestation at one central laboratory. The uptake of this screening was >99%.[34] All women who were able to make an informed decision regarding their participating in this screening study were eligible. Caregivers obtained pregnant women's informed consent and their consent was reported on the laboratory request forms. Left-over material from the ethylenediamine tetra-acetic acid anticoagulated blood tubes was used. If insufficient material was available to perform serological HPA-1a typing the woman concerned was excluded. Immunised cases with known HPA-1a immunisation from a previous pregnancy were excluded from clinical follow-up.

PATIENT AND PUBLIC INVOLVEMENT STATEMENT

The Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) published a report[35] stating that knowledge on the natural history of FNAIT should be obtained so antenatal screening for FNAIT could be considered. Subsequently, the current study was designed with involvement of and approved by the RIVM. Patients were not involved in the design or conduct of the study.

LABORATORY ANALYSES

As previously described, plasma containing maternal platelets (3 to 6 days after drawing the blood sample) was used for serological HPA-1a typing with an enzyme-linked immunosorbent assay.[36] If the optic density (OD) was below 0.160, genotyping (allelic discrimination assay based on Taqman chemistry) was performed to confirm HPA-1a negativity with leukocyte-derived DNA.[36]

Antibody screening and clinical data collection was performed not earlier than three weeks after the due date. Antibody screening was performed with the Pak Lx assay, a bead-based glycoprotein (GP) specific HPA-antibody detection method (LIFECODES Pak Lx Assay,

Immucor GTI Diagnostics, Norcross, Georgia, USA). Anti-HPA-1a reactivity was concluded if the median of the mean fluorescence intensity (MFI) of three different beads coated with HPA (1a+; 1b-) typed GPIIb/IIIa was at least 2-fold higher than the MFI of both HPA (1a-; 1b+) typed beads. To reach optimal sensitivity, no minimal MFI for the HPA (1a+; 1b-) typed beads was set. Of all HPA-1a immunised women, the antibody quantitation was performed in the modified monoclonal antibody immobilisation of platelet antigens (MAIPA) with an international anti-HPA-1a standard (NIBSC product code 03/152, Hertfordshire, UK).[37] To save plasma, the MAIPA was tested with platelets of only one HPA (1a+; 1b-) typed platelet donor and one-third of plasma (40 µL) compared to the routine MAIPA. The MAIPA was negative if the OD was below the OD found in 95% of HPA antibody negative sera from controls (healthy donors, typed blood group AB) (0.092). Quantitation was possible above 0.3 IU/mL.

HLA types were imputed from genotyping with the UKBBv2 array by using the Applied Biosystems HLA Analysis v1.1 algorithm.[38] The HLA DRB3*01:01 frequencies of an ethnically equivalent cohort were derived from a population of 3364 Dutch blood donors who were included in either the DISIII[39] or bloodTyper study[38] and typed with this platform.

To prove fetal-maternal incompatibility, fetal HPA typing was performed in HPA-1a negative immunised women. Cell-free fetal DNA (cffDNA) was extracted from 300 µL maternal plasma (QIAmp circulating nucleic acid kit; Qiagen, Hilden, Germany). Fetal typing assays were performed with droplet digital polymerase chain reaction (ddPCR) using the Digital PCR System from BioRad (Hercules, California, United States of America) and analysed using Quantasoft Software.[40] Fetal DNA markers were used to quantify the amount of isolated fetal DNA: *RHD* was used if the pregnancy concerned an *RHD* positive fetus, *SRY* in case of a male fetus, and methylated *RASSF1A* in all other cases. An HPA-1a negative fetus was only concluded if the fetal DNA marker demonstrated the presence of fetal DNA, and a HPA-1a positive fetus only if the ratio between the HPA-1a and the fetal DNA marker was within the expected range.

CLINICAL DATA COLLECTION

For every HPA-1a negative woman, 3 HPA-1a positive women were selected at random and included in the control group. Researchers requested their obstetric caregivers to provide clinical data through an online digital case record form in ProMISe, an online data management system. Clinical follow-up of the women started from inclusion in the 27th week of pregnancy until one week after delivery. Clinical follow-up of the neonates contained data of their first week after birth. Maternal HPA-1a status was stored in a database separate from the clinical database and both researchers and caregivers were blinded for this information. We asked the obstetric caregivers to provide clinical data on gravidity, parity, (induced) miscarriages, obstetrical complications in participants' history or current pregnancy, maternal diabetes, hypertensive disorders during pregnancy, preeclampsia in current pregnancy, neonates

gestational age at delivery, mode of delivery, neonatal outcome, sex, birthweight (including percentile according to sex and gestational age[41]), Apgar scores, paediatric consultation, admission to neonatology ward and reason for admission, admission to neonatal intensive care unit (NICU), postnatal treatment, skin or organ bleeding, ICH (including neuroimaging reports), and perinatal mortality. Researchers kept reminding the caregivers by telephone and e-mail until they had entered the clinical data into the database. If a caregiver indicated that a woman had been referred to another caregiver, data were requested from the second caregiver. If clinical data were not available the participant concerned was considered as lost to follow-up. We asked the treating paediatrician and neonatologist to provide additional information on infants admitted to the hospital. A letter of discharge of infants was also requested if: (i) information concerning admission was incomplete, (ii) the infant was born before 34 weeks' gestation, (iii) it had an Apgar score of less than 7 at 5 minutes after birth, (iv) weighed less than 1000 grams at birth, or (v) in case of bleeding symptoms or a platelet count of less than 150×10^9/L.

DEFINITIONS

We divided the study population into three categories: (i) HPA-1a negative pregnant women with HPA-1a antibodies and an HPA-1a positive fetus were termed immunised women, (ii) HPA-1a negative pregnant women without HPA-1a directed antibodies were termed non-immunised women, (iii) HPA-1a positive women were termed controls.

Clinically detectable FNAIT was defined as thrombocytopenia (platelet count < 150×10^9/L), and/or minor or major bleeding, and/or death likely caused by bleeding, after 27 weeks' gestation until 28 days after delivery, in HPA-1a immunised and HPA-1a incompatible pregnancies. Severe FNAIT was defined as clinical detectable FNAIT with major bleeding and/or perinatal death likely caused by bleeding. Mild FNAIT was defined as clinically detectable FNAIT with minor bleeding and/or thrombocytopenia, making clinical observation and/or treatment necessary.

Major bleeding was defined as intraventricular haemorrhage (IVH) grade III, intraventricular haemorrhage of any grade with parenchymal involvement, parenchymal haemorrhage, cerebellar haemorrhage, and/or extra-axial haemorrhage visible on cranial ultrasound. Any other bleeding than intracranial haemorrhage was considered major if any bleeding-related therapy had been given. Minor bleeding was defined as petechiae, haematoma, mucosal bleeding, germinal matrix haemorrhage grade I, IVH grade II, or increased bleeding tendency as reported by the caregiver.

Two independent and experienced neonatologists specialised in neonatal neurology, Sylke Steggerda, MD, PhD and Linda de Vries, MD, PhD, reviewed and discussed all the neuroimaging reports. They classified the reports blinded for maternal HPA-1 status.

OUTCOMES

The primary objective of our study was to determine the incidence of clinically detectable severe FNAIT within HPA-1a immunised and incompatible pregnancies. Secondary outcomes were the incidence of clinically detectable mild FNAIT, perinatal death, pregnancy outcome (hypertensive disorder this pregnancy, preeclampsia, and mode of delivery), and neonatal outcome (prematurity, birthweight related to gestational age, paediatric consultation, neonatal admission, and neonatal treatment). Tertiary outcomes were risk factors for immunisation and severe disease: maternal HLA DRB3*01:01 status, antibody quantitation, clinical risk factors, and maternal red blood cell blood group.

STATISTICAL ANALYSIS

Clinical and laboratory data were merged to form one database and experienced data managers assisted to check for inaccuracies and incomplete data. Analyses were conducted using Stata, version 16, and IBM SPSS Statistics, version 26.0. Figures were made with GraphPad Prism, version 9.

Immunised cases were compared to HPA-1a positive controls. Data are presented as number of cases with percentages, means with standard deviations, and medians with interquartile ranges (IQRs) as appropriate. Categorical data were compared using Fisher's exact test or the chi-square test. Continuous variables were compared using the unpaired *t* test, the *t* test, or the Mann-Whitney test, as applicable. Risk ratios (RR) and absolute risk differences are presented with 95% confidence intervals (CI). The incidence of severe clinical detectable FNAIT was calculated as the number of neonates with severe FNAIT born to HPA-1a negative pregnant women and as the number of fetuses with severe FNAIT within the Dutch pregnant population.

Besides the analyses in the predefined analysis plan, we also examined risk factors for immunisation and severe disease. Risk factors for RBC alloimmunisation are exposure to the RBC antigen because of previous pregnancies, miscarriage, abortion, and complications during pregnancy with a higher risk of FMH,[42] which is why we investigated these factors in our cohort. Major ABO incompatibility has a protective effect against D alloimmunisation.[43,44] Maternal red blood groups were compared using the chi-square test followed by Dunn's pairwise comparison with adjustment for multiple comparisons using the Bonferroni method.

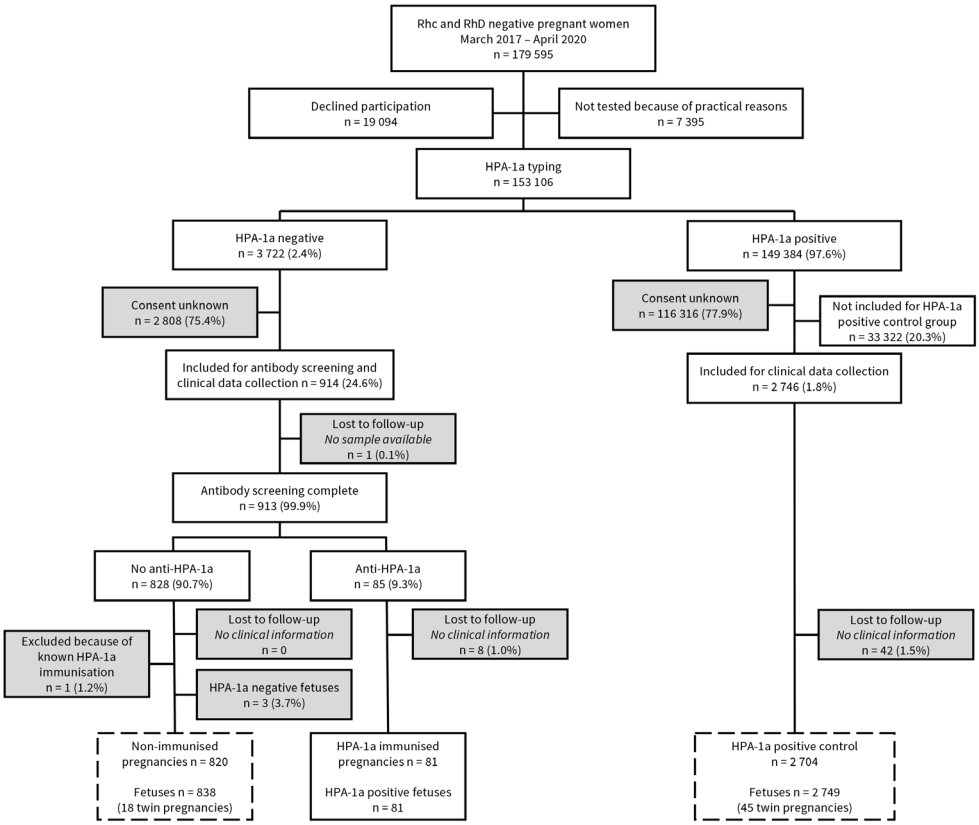

FIGURE 1. Study population
Flowchart of the study population.
Abbreviations: RhC, rhesus c; RhD, rhesus D; HPA-1a, human platelet antigen-1a.

RESULTS

STUDY POPULATION

During their 27th week of pregnancy, 179 595 RhD and Rhc negative women were screened for red cell antibodies (Figure 1). HPA-1a typing was performed in 153 106 (85.2%) of the women (19 094 women declined to participate and 7395 were not tested for logistic reasons). A total of 3722 (2.4%) women were HPA-1a negative. Antibody screening was done in 913 samples from 881 HPA-1a negative women. Thirty-two HPA-1a negative women were included who had two pregnancies during the study period. In 28 of these women no antibodies were detected in either pregnancy. In three women, anti-HPA-1a was detected in both pregnancies and in one woman, antibodies were detected in her second pregnancy and none in her third pregnancy. In 85 out of 913 (9.3%, 95% CI, 7.5% to 11.4%) HPA-1a negative pregnancies,

we detected anti-HPA-1a at 27 weeks' gestation. Borderline HPA antibody test results were observed in five cases: study numbers 31129 (G4P2, fetus typed as HPA-1a negative), 32859, 53786, 63488, and 127689) and considered as positive.

Fetal-maternal incompatibility was tested in all 85 HPA-1a immunised pregnancies and 82 (96.5%) were found to be incompatible (clinical characteristics of the excluded cases are shown in Supplemental Table 1). For comparison, fetal HPA-1a type was also determined in non-immunised HPA-1a negative cases and, in concordance with a random distribution of HPA-1a positive/negative neonates of HPA-1a-negative women, 82 out of 94 (87.2%) were found to be incompatible.

One immunised woman was excluded from analysis as antenatal IVIg was administered because a previous child of hers had been diagnosed with FNAIT (clinical data in Supplemental Table 1). In total, 81 fetuses from 81 immunised pregnancies of 78 different women were included. As controls, 2749 fetuses from 2704 HPA-1a positive pregnancies were included (45 twin pregnancies).

Baseline characteristics were not different between the immunised pregnancies, non-immunised pregnancies, and HPA-1a positive pregnancies (Table 1). The percentage of women in being pregnant for the first time was similar in immunised, non-immunised HPA-1a negative women, and HPA-1a positive women (32.1%, 36.7% and, 33.9% respectively). The proportion of nullipara in immunised, non-immunised HPA-1a negative women, and HPA-1a positive controls was highly comparable (45.2%, 43.2%, and 43.2%, respectively). There were no differences between the proportion of women included by primary and secondary caregivers. The proportion of RhD and Rhc negative women was equal between immunised and non-immunised women (Supplemental Table 2).

TABLE 1. Baseline characteristics

	HPA-1a negative women		HPA-1a positive women	
	immunised women	non-immunised women	controls	Total
	(n = 81) †	(n = 820)	(n = 2704)	(n = 3609)
Maternal age, y, mean (SD)	30.6 (5.1)	31.1 (4.6)	31.1 (4.5)	31.1 (4.5)
Gravidity, median (IQR)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)
Primigravidae, n (%)	26 (32)	301 (37)	917 (34)	1244 (35)
Parity, median (IQR)	1 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)
Nulliparae, n (%)	35 (43)	371 (45)	1168 (43)	1574 (44)
Maternal ITP, n (%)	0	1 (0)	3 (0)	4 (0)
Diabetes mellitus, n (%)	0	7 (1)	19 (1)	26 (1)
Gestational diabetes, n (%)	2 (3)	46 (6)	163 (6)	211 (6)
Sex neonate (male), n (%)‡	45/81 (56)	421/838 (50)	1378/2747 (50)	1844/3670 (50)

All statistics and percentages were calculated by use of valid numbers, excluding participants with missing data.
† Only HPA-1a incompatible pregnancies were included.
‡ Missing for one (dichorionic) twin that ended with fetal demise (their mother was HPA-1a positive).
Abbreviations: HPA, human platelet antigen; y, years; ITP, immune thrombocytopenia.

TABLE 2. Clinical detectable fetal and neonatal alloimmune thrombocytopenia

	HPA-1a negative group		HPA-1a positive group		
	Neonates of immunised women†	Neonates of non-immunised women	Controls	*P* value‡	Risk difference (95% CI) ‡
	(n = 81)	(n = 838)	(n = 2749)		
Severe FNAIT, n (%)	1 (1)	-	-	-	
Mild FNAIT, n (%)	3 (4)	-	-	-	
Bleeding, n (%)	4 (4.9)	18 (2.1)	57 (2.1)	0.095	2.9 (-1.9-7.6)
Of which major bleeding	1 (1.2)	0	3 (0.1)	0.110	1.1 (-1.3-3.5)
Of which minor bleeding	3 (3.7)	18 (2.3)	54 (2.0)	0.222	1.7 (-2.4-5.9)
Thrombocytopenia n/N (%) §	2/8 (25)	7/37 (19)	18/116 (16)	0.614	9.5 (-21.7-40.2)
Severe thrombocytopenia n/N (%) §	1/8 (13)	2/37 (5)	1/116 (1)	0.125	10.2 (-10.4-30.8)
Perinatal death, n (%)	1 (1)	3 (0)	11 (0)	0.295	0.8 (-1.6-3.3)
Of which due to bleeding	1 (1)	0	0	-	
Of which due to other causes	0	1 (0)	7 (0)	-	
Unknown cause of death	0	2 (0)	4 (0)	-	

† Only HPA-1a incompatible pregnancies were included.
‡ HPA-1a immunised cases were compared with HPA-1a positive cases.
§ Platelet count only known if determined by clinician. Platelet was count known in 8 immunised neonates, 37 non-immunised neonates and 114 controls. Thrombocytopenia was defined as a platelet count < 150×10^9/L, severe thrombocytopenia was defined as a platelet count < 50×10^9/L.
Abbreviations: FNAIT, fetal and neonatal alloimmune thrombocytopenia; HPA, human platelet antigen

INCIDENCE OF CLINICAL DETECTABLE FNAIT

One neonate (1/81, 1.2%, 95% CI, 0 to 6.7%) was diagnosed with severe FNAIT and three other neonates (3/81, 3.7%, 95% CI, 0.8% to 10.4%) were diagnosed with mild FNAIT. The case of severe FNAIT concerned a fetus with ICH detected at 29 weeks' gestation. Ultrasound examination was performed because of reduced movements. Magnetic resonance imaging showed extensive damage to the brain with large cysts. After a multidisciplinary meeting and parent counselling, the pregnancy was terminated at 34 weeks' gestation. The three neonates with mild FNAIT were diagnosed after birth: two had haematomas and one had mucosal bleeding visible upon intubation, without signs of pulmonary haemorrhage on chest X-rays (details of immunised cases in Supplemental Table 3). The incidence of clinically detectable severe FNAIT was 1 in 913 HPA-1a negative pregnancies. This extrapolates to 11 in 10 000 HPA-1a negative pregnancies or 2.6 in 100 000 pregnancies in the Netherlands. Bleeding symptoms are reported in Table 2. The absolute risk difference of major bleeding between HPA-1a immunised women and HPA-1a positive women was 1.1% (*P* = 0.110, 95% CI, -1.3 to 3.5). Three neonates in the control group of 2749 fetuses of HPA-1a positive mothers had major bleeding, one infant had an IVH grade III related to premature delivery at 27 weeks' gestation and the second infant was diagnosed with IVH grade III and subdural haemorrhage related to congenital abnormalities. The third infant was born at 30 weeks' gestation and suffered from perinatal asphyxia. This neonate had a gastrointestinal bleeding for which histamine H-2 receptor antagonist was given. Minor bleeding was detected in 3.7% (3/81) of the neonates of immunised mothers compared to 2.0% (54/2749) of neonates of HPA-1a positive women.

PREGNANCY OUTCOMES

Pregnancy outcomes are summarised in Table 3. Hypertensive disorder during pregnancy was diagnosed in 11% (9/81) of the immunised women and in 4% (120/2704) of the HPA-1a positive pregnant women (RR 2.4, 95% CI, 1.3 to 4.8). The proportion of cases with preeclampsia did not differ between HPA-1a immunised women and HPA-1a positive women. Pregnancy outcomes of the HPA-1a negative non-immunised and HPA-1a positive women were similar. In the obstetric history of multigravida women, no significant differences in proportions of miscarriages, abortions, or intrauterine fetal demise were reported (Supplemental Table 4).

NEONATAL OUTCOMES

Neonatal outcomes are presented in Table 4. The median gestational age at delivery was similar between the groups. The proportion of preterm births (< 37 weeks' gestational age) was 15% (12/81) in the HPA-1a immunised group compared to 5% (132/2745) in the HPA-1a positive group (RR 3.1, 95% CI, 1.8 to 5.3). The mean birthweight was 3271 (± 631) grams in the immunised group compared to 3459 (± 545) grams in the HPA-1a positive group (*P* = 0.002, mean difference 187, 95% CI, 66 to 308). As shown in Figure 2, the birthweight percentile of the immunised neonates (median 0.46, IQR 0.21 to 0.70) was lower compared to the neonates of HPA-1a positive women (0.52, IQR 0.26 to 0.77). The median birthweight percentile was not different between male and female neonates in the immunised group (0.44, IQR 0.24 to 0.70 versus 0.46, IQR, 0.18 to 0.72). The birthweight percentiles of neonates of immunised primigravida women were lower compared to the median birthweight percentiles of neonates of non-immunised women or HPA-1a positive controls (Supplemental Figure 1). The birthweight percentiles of multigravida women did not differ between the groups. Supplemental Figure 2 shows the relationship between the presence of preterm delivery, reduced birthweight, and hypertensive disorder during pregnancy.

TABLE 3. Pregnancy outcome

	HPA-1a negative women		HPA-1a positive women		
	immunised women†	non-immunised women	controls	*P* value‡	Risk difference (95% CI) ‡
	(n = 81)	(n = 820)	(n = 2704)		
Hypertensive disorder this pregnancy, n (%)	9 (11)	44 (5)	120 (4)	0.011	6.7 (-0.2-13.6)
Pre-eclampsia this pregnancy, n (%)	2 (3)	24 (3)	68 (3)	1	0 (-3.5-3.4)
Delivery mode, n (%)				-	-
Vaginal	70 (87)	641 (78)	2053 (76)		
Vaginal instrumental	1 (1)	52 (6)	184 (7)		
Primary caesarean section	3 (4)	51 (6)	220 (8)		
Secondary caesarean section	5 (6)	86 (8)	205 (8)		

All statistics and percentages were calculated by use of valid numbers, excluding participants with missing data.
† Only HPA-1a incompatible pregnancies were included.
‡ HPA-1a immunised cases were compared to HPA-1a positive cases.
Abbreviation: HPA, human platelet antigen.

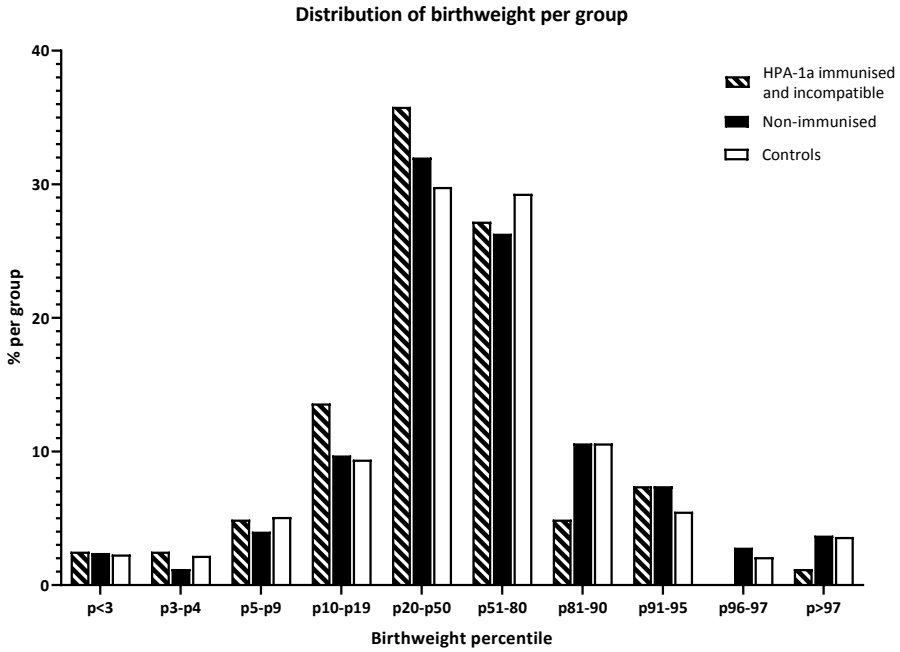

FIGURE 2. Birthweight distribution
Distribution of the birthweight percentile per study group according to the birthweight charts for the Dutch population.
Abbreviations: HPA, human platelet antigen; p, percentile

There was no significant difference in the proportion of neonates admitted to the neonatology ward (16%, 13/81 versus 10%, 281/2745). However, within the group of neonates admitted, a significantly higher percentage of neonates of immunised mothers were admitted to the NICU (37%, 5/13 versus 10%, 25/259, *P* = 0.008). Three neonates of the immunised mothers were admitted to the NICU because of prematurity, one because of perinatal asphyxia, and one because of early-onset sepsis. The most frequent reasons for admission in the HPA-1a positive group were prematurity (n = 86) and (suspected) early-onset sepsis (n = 49). The clinical outcome of neonates of non-immunised HPA-1a negative women was similar to the clinical outcome of neonates of HPA-1a positive women.

TABLE 4. Neonatal outcome

	HPA-1a negative women		HPA-1a positive women		
	Neonates of immunised women†	Neonates of non-immunised women	controls	*P* value‡	Risk difference (95% CI) ‡
	(n = 81)	(n = 838)	(n = 2749)		
GA at delivery, (weeks^{+days}), median (IQR)	39^{+5} (38^{+2}–40^{+5})	39^{+6} (38^{+6}–40^{+5})	39^{+6}(38^{+5}–40^{+4})	0·172	-
Preterm birth (< 32 weeks), n (%)	2 (3)	4 (1)	10 (0)	0·045	2·1 (-1·3-5·5)
Preterm birth (< 37 weeks), n (%)	12 (15)	46 (6)	132 (5)	<0·001	10·0 (2·2-17·8)
Birthweight (g), mean (SD)	3271 (631)	3477 (558)	3459 (545)	0·002	-
SGA (birthweight < p10), n (%)	8 (10)	63 (8)	266 (10)	0·851	0·2 (-6·4-6·8)
Apgar score < 7 at 5 minutes, n (%)	4 (5)	15 (2)	42 (2)	0·041	3·5 (-1·3-8·3)
Consultation with paediatrician, n (%)	27 (33)	342 (41)	977 (36)	0·682	-2·2 (-12·6-8·2)
Admission to neonatology ward, n (%)	13 (16)	97 (12)	281 (10)	0·096	5·8 (-2·2-13·9)
Admission to NICU, n (%)	5/13 (39)	8/85 (9)	25/259 (10)	0·008	28·8 (2·1-55·5)
Days in NICU, median (IQR; min-max)	0 (0 – 6; 0 – 26)	0 (0 – 0; 0 – 31)	0 (0 – 0; 0 – 22)	0·002	-
Postnatal treatment, n (%)	13 (16)	57 (7)	201 (7)	0·008	8·7 (6·8-16·7)

All statistics and percentages were calculated by use of valid numbers, excluding participants with missing data.
† Only HPA-1a incompatible pregnancies were included.
‡ HPA-1a immunised cases were compared with HPA-1a positive cases.
Abbreviations: HPA, human platelet antigen; CI, confidence interval; GA, gestational age; IQR, interquartile range; g, gram; SD, standard deviation; SGA, small for gestational age; p, percentile; NICU, neonatal intensive care unit.

TABLE 5. Risk factors for immunisation

	HPA-1a immunised pregnant women HPA-1a incompatible	HPA-1a negative non-HPA-1a immunised pregnant women	HPA-1a positive pregnant women	Total	Healthy blood donors
	(n = 81)†	(n = 820)	(n = 2704)	(n = 3609)	(n = 3364)
Clinical risk factors					
Abortion or miscarriage in history‡	31 (56)	218 (42)	831 (46)	1079 (46)	
Twin pregnancy	0	18 (2)	45 (2)	63 (2)	
Abdominal trauma current pregnancy§	0	1 (0)	9 (0)	10 (0)	
Fetal maternal haemorrhage in history‡§	0	5 (1)	25 (1)	30 (1)	
Caesarean section in obstetric history‡§	1 (2)	11 (2)	44 (3)	56 (2)	
Placental abruption in obstetric history‡§	1 (2)	0	5 (0)	6 (0)	
Laboratory parameters					
Blood group‖					
A	35 (45)	333 (41)	1128 (42)	1496 (42)	
B	10 (13)	79 (10)	285 (11)	374 (10)	
O	25 (32)	363 (45)	1176 (43)	1564 (43)	
AB	8 (10)	33 (4)	108 (4)	149 (4)	
Maternal HLA DRB3*01:01					
Negative	8 (10)				2247 (67)
Heterozygous positive	64 (79)				1024 (30)
Homozygous positive	9 (11)				93 (3)

All statistics and percentages were calculated by use of valid numbers, excluding participants with missing data.
† Only HPA-1a incompatible pregnancies were included.
‡ Primigravidae excluded.
§ Based on information from open text field in case report form.
‖ Missing values for 22 cases; 3 immunised cases, 12 non immunised cases, 7 HPA-1a positive cases
Abbreviation: HLA, human leukocyte antigen.

RISK FACTORS FOR IMMUNISATION AND SEVERE DISEASE

Table 5 shows risk factors for HPA-1a immunisation. The proportion of women who had a history of abortion or miscarriage was slightly higher among the HPA-1a immunised women (38%, 31/81) compared to non-immunised women (27%, 218/838) or the HPA-1a positive controls (31%, 831/2704).

In the immunised group, significantly more immunised pregnant women had blood group AB (10%, 8/78) compared to the HPA-1a positive women (4%, 108/2697). In addition, significantly fewer immunised pregnant women had blood group O (32%, 25/78) compared to the HPA-1a positive women (43%, 1176/2697, $P = 0.0082$). The distribution of blood groups of the non-immunised women was equal to the distribution in the HPA-1a positive women. HPA-1a immunisation was associated with maternal HLA-DRB3*01:01 status, 89% (72/81) of the immunised women were positive for HLA-DRB3*01:01, 13% (9/72) of whom homozygously. In the control group (3364 Dutch blood donors), 33% was found to be positive for HLA DRB3*01:01, 8% (93/1117) of whom homozygously. Thus 28.2% of the HLA-DRB3*01:01 positive women (with a HPA-1a incompatible pregnancy) versus 1.5% of the HLA-DRB3*01:01 negative women became immunised.

Antibody levels were quantitated using a modified MAIPA assay in 80 of the 81 immunised cases. In seven cases no anti-HPA-1a antibodies were detectable in the modified MAIPA and in 31 cases antibody levels appeared to be too low for quantitation. Median anti-HPA-1a quantitation was 1 IU/mL (range 0 to 90 IU/mL). In 22% (18/80) of the immunised women with an HPA-1a incompatible child, anti-HPA-1a quantitation was > 3 IU/mL. In the case with severe FNAIT antibody quantitation was highest: 90 IU/mL. For the other three cases with mild FNAIT, two with haematomas and one with mucosal bleeding, antibody quantitation was 2 IU/mL, 5 IU/mL, and 3 IU/mL, respectively. Figure 3 shows antibody quantitation stratified by maternal HLA-DRB3*01:01 status.

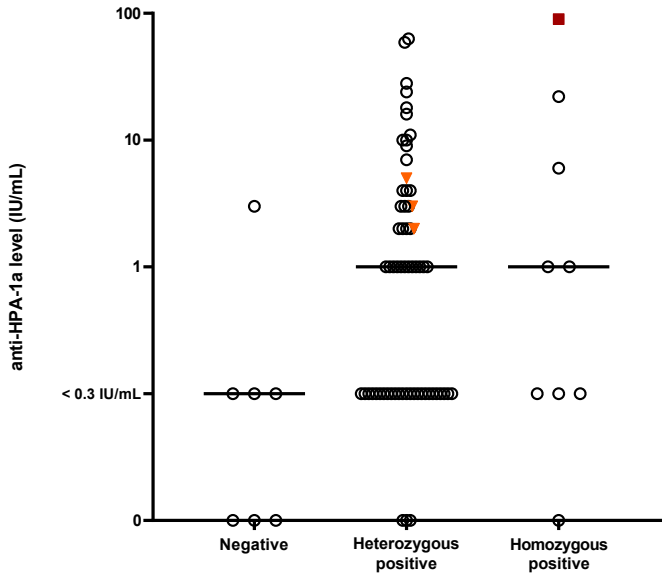

FIGURE 3. Maternal HLA DRB3*01:01 status and antibody level
Alloantibody levels as determined by the modified MAIPA stratified by maternal HLA DRB3*01:01 status. The red square dot represents the case with major bleeding. The orange triangles represent the cases with minor bleeding.
Abbreviations: HLA, human leukocyte antigen; IU/mL, international units/mililitre; MAIPA, monoclonal antibody immobilisation of platelet antigens.

DISCUSSION

We performed an observational study to determine the incidence of severe FNAIT in neonates of HPA-1a negative women. Researchers as well as caregivers were blinded for HPA-1a status and antibody test results. This allowed us to describe the clinical outcomes of HPA-1a immunised pregnancies without interference of medical interventions. Of the pregnant women, 2.4% were HPA-1a negative. HPA-1a antibodies were detected in 9.3% of the HPA-1a negative women at 27 weeks' gestation. The incidence of clinical detectable severe FNAIT was 1 in 81 HPA-1a alloimmunised pregnancies, which translates to 2.6 in 100 000 Dutch pregnancies. The HPA-1a positive neonates of HPA-1a alloimmunised mothers were born preterm more often and had lower birthweights than the control group of neonates of HPA-1a positive mothers and neonates of non-immunised HPA-1a negative women. The pregnancies of HPA-1a-immunised women were complicated more often by hypertensive disorder during pregnancy. Maternal HLA DRB3*01:01 status was a risk factor for HPA-1a immunisation, and maternal blood group O was protective.

HPA-1A NEGATIVITY AND ALLOIMMUNISATION

The proportion of HPA-1a negative women in our study population (2.4%) is in line with results from previous large screening studies in Europe (2.1 to 2.5%).[24, 26, 29, 30] We screened for HPA-1a antibodies at 27 weeks' gestation and detected antibodies in 9.3% (95% CI, 7.5% to 11.4%) of the HPA-1a negative pregnant women. This percentage is similar to findings by Williamson and colleagues[30] in the United Kingdom (9.4%) and Kjeldsen-Kragh and colleagues[26] in Norway (8.6%). Nevertheless, there are important differences between our study and theirs. Both the British and Norwegian studies started with antibody screening early in pregnancy and repeated measurements two to five times. Because we only measured once, during the 27th week of gestation, we may have missed pregnancies in which immunisation occurred later. However, in the previously mentioned studies in only 4 out of 37[30] and 3 out of 154[26] immunised pregnancies was anti-HPA-1a first detected after 27 weeks' gestation and before delivery. This suggests that we possible missed only a few late HPA-1a immunisations. Both studies reported disappearance of HPA-1a antibodies around the 27th week of pregnancy in considerable percentages of 22%[37] and 25%,[30] respectively.

Another difference was the platform we used to detect HPA-1a antibodies, which may have influenced detection of HPA-1a antibodies. Previous studies used the MAIPA assay or platelet fluorescence test.[26, 30] Contrastingly, we used a platform with platelet GPIIb/IIIa (αIIbβ3) glycoprotein-coated beads and Luminex technology for antibody detection (PAK Lx assay), because we considered our approach more suitable for high throughput screening. The disadvantage of this screening test was that the software algorithm did not pick up weak antibodies and therefore required additional assessment of MFI values of the different beads.[45] Previously, samples identified by the MAIPA showed that the PAK Lx assay is at least as sensitive as the MAIPA assay.[46] We observed that in comparison to the MAIPA assay the PAK Lx assay was even more sensitive compared with the MAIPA assay (data not shown). Others found that HPA-1a antibodies can be present in sera tested negative in the MAIPA by using purified GPIIb/IIIa with surface plasmon resonance technology.[47]

RISK OF MAJOR BLEEDING

In contrast to other screening studies, we did not report the maternal HPA status to the caregivers and we included a large control group. Thus, an important strength of our study is that we were able to estimate the true incidence of FNAIT compared to a control group, without any interference. Previously, it was argued that the incidence of major bleeding might have been underestimated in some screening studies on account of the interventions.[17, 22, 23, 25-27, 29, 30, 48] Our data, however, suggest that this was not the case. Even though the proportion with major bleeding found in our study (1.2%) was lower, it was in line with the combined figures from previous studies.[49] In total, four major bleedings were reported in all previous studies among 278 antenatally HPA-1a immunised pregnant women.[22, 26, 27, 30] A germinal matrix bleeding detected postnatally in the Norwegian screening study, and which resolved

spontaneously, was not considered as major.[26] In addition, major ICHs and two cases of fetal demise, possibly FNAIT-related, were reported.[26, 30] The combined proportion of severe FNAIT found in these studies was between 1.4% (4/278), excluding fetal demise, and 2.2% (6/278), including fetal demise. Interestingly, major ICHs were predominantly diagnosed during pregnancy in screening studies: at 34 weeks[26], at 37 weeks[30], at 48 hours after birth,[22] and at 29 weeks' gestation in our study (one study did not report the time of detection[27]). Probably, the effect on the occurrence of major bleeding of study-related perinatal treatment, such as near-term caesarean section of readily available postnatal platelet transfusion, may have been less high than expected because ICH had already occurred earlier on during pregnancy.[2] Two large screening studies reported the proportions of neonates with skin bleeding as 12% (5/25)[29] and 18% (7/36).[30] We found skin bleeding in only 4% (3/81) of the neonates at risk compared to 2% (54/2749) in neonates of HPA-1a positive pregnant women. It is conceivable that minor skin bleeding might have been underreported in our study because, in contrast to the other studies, caregivers were blinded for HPA status, and the presence of antibodies and platelet counts were not routinely determined.

BROADENING THE SPECTRUM OF CLINICAL FNAIT

In previous screening studies, thrombocytopenia and risk of bleeding were the most important outcome measures reported. In recent years, immunohistochemical analysis of FNAIT placentas and several animal studies suggested that HPA-1a immunisation could also involve pathology of the placenta.[9-12, 50-52] Placental dysfunction is associated with various symptoms including preeclampsia, pregnancy induced hypertension, prematurity, and growth restriction.[53] The results of our study emphasised that placenta pathology may be part of the FNAIT syndrome, because HPA-1a immunisation was associated with premature delivery, reduced birthweight, and hypertensive disorder during pregnancy. Intriguingly, this effect of reduced birthweight was seen mainly in first pregnancies. These effects may be related to a direct functional or an indirect immunological effect of the anti-HPA 1a antibodies on the syncytiotrophoblast. Future clinical studies should focus not only on the occurrence of bleeding symptoms but should include the analysis of placental pathology.

RISK FACTORS FOR IMMUNISATION

In accordance with previous work,[32] we observed that HPA-1a immunisation is strongly associated with maternal HLA DRB3*01:01 positivity. Previous studies on red cell alloimmunisation found that the ABO blood group is associated with alloimmunisation, showing a protective effect of naturally occurring anti-A and anti-B.[44, 54] We found an overrepresentation of women with group AB in the immunised women while women with group O were underrepresented. This may be explained by the protective effect of anti-A and anti-B antibodies in HPA-1a immunisation. In an additional analysis of the Norwegian screening study,[55] the ABO distribution of HPA-1a immunised women is comparable to that of the comparable general Swedish population.[55] They reported an association between group O of the HPA-1a

immunised mothers and a smaller risk of the neonates developing thrombocytopenia.[55] The different design of our study, with only one screening relatively late in pregnancy to determine the presence of HPA-1a antibodies, and the lack of data on platelet counts in neonates, all make our study difficult to compare to that of the Norwegian researchers.

In contrast to the foregoing prospective screening studies, we did not find that maternal parity or gravidity was a risk factor for HPA-1a alloimmunisation. The percentage of primigravida women with antibodies detected before 27 weeks' of gestation was lower in other studies: 4% (1/25),[29] 9% (14/154),[26] and 14% (4/28).[30] We had 32% (26/81) women in their first pregnancy, which was in the same range as in the control population: 34% (917/2704), suggesting that most women get immunised during their first pregnancy at risk. This is in agreement with the observations that antibodies in multigravida were – in contrast to in primigravidae - often found in first trimester[37] and that maternal immunisation in a series of severe FNAIT with ICH occurred in the majority of cases in their first born child.[2, 56] The higher proportion of primigravida in our study may have been influenced by the type and sensitivity of assay and the timing of antibody determination and used. In our study, we may have missed HPA-1a antibodies in multigravida, because in this group up to 20% of the antibodies disappear and are no longer detectable at week 27 (Kjeldsen-Kragh, personal communication). However, this evanescence rate is too low to explain the observed differences with the Norwegian cohort. Hence, the difference in assay used for HPA-1a antibody detection is most likely the most important factor to explain the observed differences.

Currently, there is no consensus on laboratory assays to identify, within the group of anti-HPA-1a immunised pregnancies, those at risk of severe neonatal outcomes. The Norwegian screening study showed that anti-HPA-1a quantitation is inversely correlated with platelet count.[37] In our study, the case with major bleeding had the highest antibody quantitation test result in our cohort. Also, the three cases with minor bleedings had anti-HPA-1a levels above the median of 1 IU/mL. These findings suggest that anti-HPA-1a quantitation in a screening programme could be used to determine whether antenatal IVIg is offered to immunised women or not. With the current assays, endothelium reactive αVβ3-specific anti-HPA-1a is not detected.[57] The question whether detection of this specific subtype of antibodies would increase the positive predictive value of identifying pregnancies with a high risk of bleeding associated with ICH,[8] should be addressed in future studies. Moreover, to date it is unknown whether this type of HPA-1a antibodies can be solely present and thus missed in our study. Nevertheless, it should be noted that severe bleeding did not occur in any of the HPA-1a negative pregnancies without αIIbβ3 antibodies.

STRENGTHS AND LIMITATIONS

This study has two important strengths. One was the blinded observational study design that allowed us to establish the natural history of HPA-1a immunised pregnancies without any

interference, either antenatally or after birth. The other strength was the inclusion of a large control group. This unique feature gave us the opportunity to compare pregnancies and neonatal outcomes between HPA-1a immunised women and controls. The disadvantage, however, of this observational design was that it did not allow us to perform platelet counts and routine cranial ultrasound examinations in all neonates. We cannot exclude that ICHs were missed as a result of the lack of these routine examinations. Nevertheless, we consider it a strength of our study that we focused on a clinical detectable, and thereby clinically relevant disease.

For practical reasons this study was performed in a cohort of RhD and Rhc negative women, representing one third of all pregnant women in the Netherlands. It is unlikely that RhD or Rhc negativity has a direct effect on the risk of anti-HPA-1a immunisation. Indeed, in this study we did not observe a difference in immunisation risk between RhD and Rhc negative women. An indirect effect of this selection, however, may be that non-white pregnant women were underrepresented in our cohort, because they have a lower frequency of RhD or Rhc negativity. And because these women also have a lower frequency of HPA-1a negativity and HLA DRB3*01:01 positivity,[58] we may have slightly overestimated the frequency of FNAIT in the total Dutch pregnant population.

IMPLICATIONS

Brain haemorrhage can have a significant impact on neonates' chances of survival and their short-term outcomes. If screening were to be implemented, assessment of the neurodevelopment of children should be included to assess the effect of antenatal and postnatal treatment.

The implementation of a population-based screening of platelet alloantibodies during pregnancy has been debated for decades. Due to insufficient knowledge on the incidence of severe haemorrhage in HPA immunised pregnancies, the impact of the disease at population level could not be properly estimated, complicating the introduction of a screening programme.[19, 20] This prospective observational study provides important insight into the natural history of FNAIT. We were able to estimate the incidence of severe clinically detectable FNAIT without interference of perinatal treatment. Our data support the proposal to restrict HPA-1a antibody screening in HLA DBR3*01:01 positive women and to only perform interventions in pregnancies with higher antibody levels. It also raises awareness for the other possible clinical features of FNAIT associated with placenta-related pathology. Prophylaxis is thought to be the solution to prevent all cases of anti-HPA-1a mediated FNAIT.[16] Our data suggest that prophylaxis should be administered already early in pregnancy to prevent immunisation. However, considering that HPA-1a is expressed by the placenta,[4] it can be questioned whether administration of anti-HPA-1a prophylaxis might damage the placenta.

CONCLUSIONS

The incidence of severe haemorrhage in fetuses and neonates as a result of HPA-1a immunisation in pregnancy is 11 in 10 000 HPA-1a negative women. This study emphasises that HPA-1a immunisation may be associated with placenta-related pathology leading to hypertensive disorder during pregnancy, reduced birthweight and, preterm delivery.

ETHICAL APPROVAL

The Medical Ethical Committee Leiden-The Hague-Delft approved the study protocol (P16.002) on 14 July 2016.

DATA AVAILABILITY STATEMENT

Detailed information of the immunised cases is provided in the supplemental data. Requests for data can be sent to the corresponding author and will be reviewed by the scientific committee. If approval is given, data will be shared via a secure portal.

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AUTHOR CONTRIBUTIONS

Conceptualisation, DO, JGvdB, EL, MdH and CEvdS; Data curation, TWdV and DW; Formal analysis, TWdV and DW; Funding acquisition, DO, MdH and CEvdS; Investigation, TWdV and DW, LP, MB, GO; Methodology, TWdV and DW, JGvdB, MdH and CEvdS; Resources, LP; Supervision, DO, MdH and CEvdS; Visualisation, TWdV and DW; Writing – original draft, TWdV and DW; Writing – review and editing, LP, MB, JGvdB, EL, DO, MdH, and CEvdS.

TRANSPARENCY

The first joint authors, TWdV and DW, and the last joint authors, MdH and EvdS, affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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COMPETING INTERESTS

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SUPPLEMENTAL TABLE 1. Clinical characteristics of the excluded cases

HIP	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Clinical course	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
Excluded because the fetus was HPA-1a negative.											
31129	G4P2	Abortion (1)		38+6	SVD	Male	2500 (2)	No bleeding	2160	1	1
47738	G2P1			40+2	SVD	Male	3715 (56)	No bleeding	134	ND	0
134460	G3P2			39+5	SVD	Female	4400 (99)	No bleeding	17082	25	1
Excluded because the mother was known with HPA-1a antibodies.											
133734	G4P2	FNAIT	IVIg treatment	38+1	SVD	Female	3210 (59)	Admission for observation. (PLT 40) cUS: normal imaging.	11946	4	1

Abbreviations: G, gravidity, P, parity; GA gestational age; BW birthweight; p, percentile; MFI, mean fluorescence index; Quant., quantitation; IU, international units; mL, millilitre; HLA, human leukocyte antigen; SVD, spontaneous vaginal delivery; ND, not detectable; cUS, cranial ultrasound.

Supplemental Table 2. Sensitivity analysis

	HPA-1a immunised pregnant women HPA-1a incompatible-	HPA-1a negative non-HPA-1a immunised pregnant women	HPA-1a positive pregnant women	Total
	(n = 81)	(n = 820)	(n = 2704)	(n = 3605)
Included by primary caregiver	71 (88)	697 (85)	2302 (85)	3070 (85)
Included by secondary caregiver	10 (12)	123 (15)	402 (15)	535 (15)
RhD negative	37 (46)	379 (46)	1156 (43)	1572 (44)
Rhc negative	44 (54)	439 (54)	1544 (57)	2027 (56)

Abbreviations: HPA, human platelet antigen; RhD, rhesus D; Rhc, rhesus c.

SUPPLEMENTAL TABLE 3. HPA-1a immunised cases

HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
43732	Severe	G3P1	Miscarriage (1)		34+0	TOP	Male	2482 (71)	Massive ICH, haematoma. (PLT 12)			17916	90	2
1136	Mild	G3P2		GD	33+5	Planned CS	Female	2016 (28)	Haematomas on both hands and thorax. (PLT 102)	Admission NICU because of prematurity. Respiratory distress.		9296	5	1
24231	Mild	G1P0			40+6	SVD	Female	3140 (13)	Cephalic haematoma			6420	2	1
122996	Mild	G2P0	Miscarriage (1)	PIH	37+0	Emergency CS	Male	3460 (89)	Blood visible upon intubation (PLT 190)	Admission NICU because of meningitis. Respiratory insufficiency.		3019	3	1
3967	No	G1P0			39+5	SVD	Male	2980 (8)	None			603	<0.3	1
7317	No	G2P0	Miscarriage (1)	PE, IUGR	34+1	Vaginal delivery after induction	Female	1715 (3)	None	Admission because of prematurity. Tube feeding.	HPA-5b, HLA	1384	<0.3	1
9321	No	G2P0	Miscarriage (1)		39+6	SVD	Male	3655 (57)	None			3285	<0.3	1
10752	No	G4P2	Miscarriage (1)		39+3	SVD	Female	2970 (16)	None		HLA	2933	1	2
11937	No	G1P0			40+1	SVD	Female	3410 (43)	None			147	ND	2
12287	No	G1P0			31+4	Forceps	Male	1750 (39)	None. cUS: normal imaging. (PLT 247)	Admission NICU because of prematurity. Phototherapy.		502	<0.3	
12661	No	G3P2			40+2	SVD	Female	3245 (26)	None			1006	<0.3	1
13042	No	G1P0			38+5	SVD	Female	2790 (12)	None			595	<0.3	1
14380	No	G1P0			41+2	SVD	Male	3405 (18)	None			390	ND	1
15187	No	G7P4	Miscarriage (1), IUFD		37+0	Vaginal delivery after induction	Male	2950 (45)	None		HLA	18526	16	1

SUPPLEMENTAL TABLE 3. Continued

HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
16995	No	G1P0	Miscarriage (1)		39+1	SVD	Male	3045 (16)	None			1898	<0.3	1
19164	No	G3P2	Miscarriage (1)		37+3	Planned CS	Male	2975 (38)	None			995	<0.3	2
19642	No	G1P0			39+6	SVD	Male	3320 (26)	None			5648	1	1
20801	No	G3P1	Miscarriage (1)	PIH	39+6	SVD	Female	3400 (46)				9117	1	1
21188	No	G4P2	Miscarriage (1)		41+0	SVD	Male	4410 (93)	None			443	ND	1
21232	No	G2P1			40+5	SVD	Male	4360 (93)	None			5694	1	1
30782	No	G5P1	Miscarriage (3)		39+3	SVD	Female	3125 (27)	None			642	<0.3	1
32027	No	G3P1	Miscarriage (1)		36+3	SVD	Male	2700 (32)	None. MRI: no ICH. (PLT 225)	Admission NICU because of perinatal asphyxia. Therapeutic hypothermia.		450	<0.3	1
32852	No	G7P1	Miscarriage (4)		39+2	SVD	Female	3435 (60)	None			16978	10	1
32859	No	G3P2			39+3	SVD	Female	3660 (77)	None			176	NA	0
33005	No	G3P2			40+1	SVD	Male	3384 (28)	None	Paediatrician was consulted because of meconium-stained amniotic fluid.	HLA	631	<0.3	0
38826	No	G4P1	Miscarriage (2)		40+6	SVD	Male	3460 (26)	None	Admission because of jaundice. Phototherapy.		18676	63	1
45711	No	G1P0			41+0	SVD	Male	3460 (24)	None			889	<0.3	1
47783	No	G3P2			39+0	SVD	Female	3535 (74)	None	Paediatrician was consulted because of medication mother.		1519	<0.3	1

SUPPLEMENTAL TABLE 3. Continued

HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
52473	No	G3P1	Miscarriage (2), IUGR in history		39+3	SVD	Male	3490 (49)	None			8678	3	0
52619	No	G4P2			39+5	SVD	Male	3525 (47)	None		HLA	17070	18	
53786	No	G4P2	Miscarriage (1)		38+6	SVD	Male	3160 (28)	None			295	ND	0
54090	No	G1P0		PIH, IUGR	36+6	SVD	Male	2150 (2)	None	Paediatrician was consulted because of low birthweight.	HLA	950	<0.3	0
56102	No	G2P1			37+1	SVD	Female	2610 (20)	None		HLA	13434	10	1
57183	No	G1P0			38+2	SVD	Male	3410 (63)	None			2353	1	1
58841	No	G3P2			38+2	Emergency CS	Male	3670 (84)	None	Paediatrician was consulted because of CS.		314	ND	1
59934	No	G1P0			38+4	SVD	Male	3180 (35)	None		HLA	16190	24	1
60102	No	G1P0			40+2	SVD	Female	4148 (94)	None			857	<0.3	2
63488	No	G4P0	Miscarriage (1)		28+3	Emergency CS	Male	1360 (74)	None. cUS: no ICH. (PLT 225)	Admission NICU because of prematurity.		608	<0.3	0
65095	No	G1P0			41+1	SVD	Female	2924 (4)	None			2762	3	1
65706	No	G3P1	Miscarriage (1)		39+1	SVD	Female	2865 (12)	None			6426	2	1
68109	No	G4P2	Miscarriage (1)		39+4	SVD	Male	3025 (11)	None		HLA	11698	22	2
73821	No	G2P1			40+6	SVD	Female	3990 (83)	None		HLA	1624	<0.3	1
74356	No	G2P1			39+5	SVD	Male	3540 (49)	None		HLA	16714	28	1
75777	No	G2P1			39+3	SVD	Female	3385 (53)	None		HLA	2690	1	1
76137	No	G4P1	Miscarriages (2)		39+2	SVD	Female	2825 (9)	None	Paediatrician was consulted because of LQTS mother.	HLA	10294	3	1
79666	No	G1P0			36+1	SVD	Male	2570 (25)	None	Admission because of prematurity.		3270	1	1

SUPPLEMENTAL TABLE 3. Continued

HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
81761	No	G3P2			40+6	SVD	Male	3128 (8)	None			16780	59	1
82163	No	G1P0		PIH	33+1	SVD	Female	1765 (13)	None, cUS: no ICH. (PLT 287)	Admission because of prematurity.		6799	3	1
82713	No	G2P1			41+3	SVD	Male	3740 (43)	None		HLA	14480	7	1
92273	No	G4P2	Miscarriage (1)		41+0	SVD	Male	4076 (77)	None	Paediatrician was consulted because of meconium-stained amniotic fluid.	HLA	932	<0.3	1
94907	No	G3P1	Miscarriage (1)		41+0	SVD	Female	3810 (69)	None	Paediatrician was consulted because of suspected perinatal infection.		1257	<0.3	1
96244	No	G1P0		GD	38+1	Induced VD	Male	3215 (47)	None. (PLT 206)	Admission because of suspected infection. Antibiotics and oxygen therapy.		4612	2	1
96863	No	G1P0			36+4	SVD	Male	2806 (40)	None	Paediatrician was consulted because of prematurity.		3093	1	1
103046	No	G2P1			38+1	Planned CS	Female	3380 (75)	None	Paediatrician was consulted because of CS.	HLA	528	<0.3	0
106832	No	G2P1			39+0	SVD	Male	3420 (50)	None			853	<0.3	1
107570	No	G1P0			40+5	SVD	Male	3390 (22)	None			1643	<0.3	1
109677	No	G1P0			40+6	SVD	Female	3100 (11)	None			2144	<0.3	1

SUPPLEMENTAL TABLE 3. Continued

HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
115382	No	G2P1			40+2	SVD	Female	4400 (98)	None			2154	1	1
117588	No	G4P1	Miscarriage (1)	PIH	41+1	SVD	Male	3920 (63)	None	Admission because of bad start.		14096	9	1
118095	No	G3P1	Miscarriage (1)		41+0	SVD	Female	3320 (24)	None			387	<0.3	1
119692	No	G2P0	Miscarriage (1)		41+0	SVD	Male	4370 (91)	None			962	1	1
127689	No	G2P0	Miscarriage (1)	PE	36+6	SVD	Female	2814 (46)	None	Paediatrician was consulted because of PE mother.	HLA	327	ND	0
128856	No	G3P2	CS in history		39+6	SVD	Male	3750 (66)	None			2927	1	2
129814	No	G1P0			40+2	Emergency CS	Female	3845 (79)	None	Paediatrician was consulted because of CS.		3208	1	1
130949	No	G3P1	Miscarriage (1)		39+1	SVD	Male	3670 (71)	None			389	<0.3	1
131963	No	G2P1			40+4	SVD	Male	4060 (80)	None			4188	2	1
134475	No	G2P0	Miscarriage (1)		40+2	SVD	Male	3040 (7)	None			4834	4	1
136032	No	G1P0	DVT previous pregnancy		39+5	SVD	Female	3510 (60)	None		HLA	374	<0.3	1
138890	No	G3P1	Miscarriage (1)		38+6	SVD	Female	3830 (92)	None	Paediatrician was consulted because of bilateral clubfeet.	HLA	1574	<0.3	2
140069	No	G1P0			42+0	SVD	Male	3920 (53)	None			1985	<0.3	1
141102	No	G1P0		PIH	38+0	SVD	Female	3135 (55)	None	Admission because of jaundice. Phototherapy.		10610	4	1
141933	No	G2P1		IUGR	36+6	Vaginal delivery after induction	Male	1885 (1)	None	Admission because of prematurity. Glucose IV.		8668	4	1

SUPPLEMENTAL TABLE 3. Continued

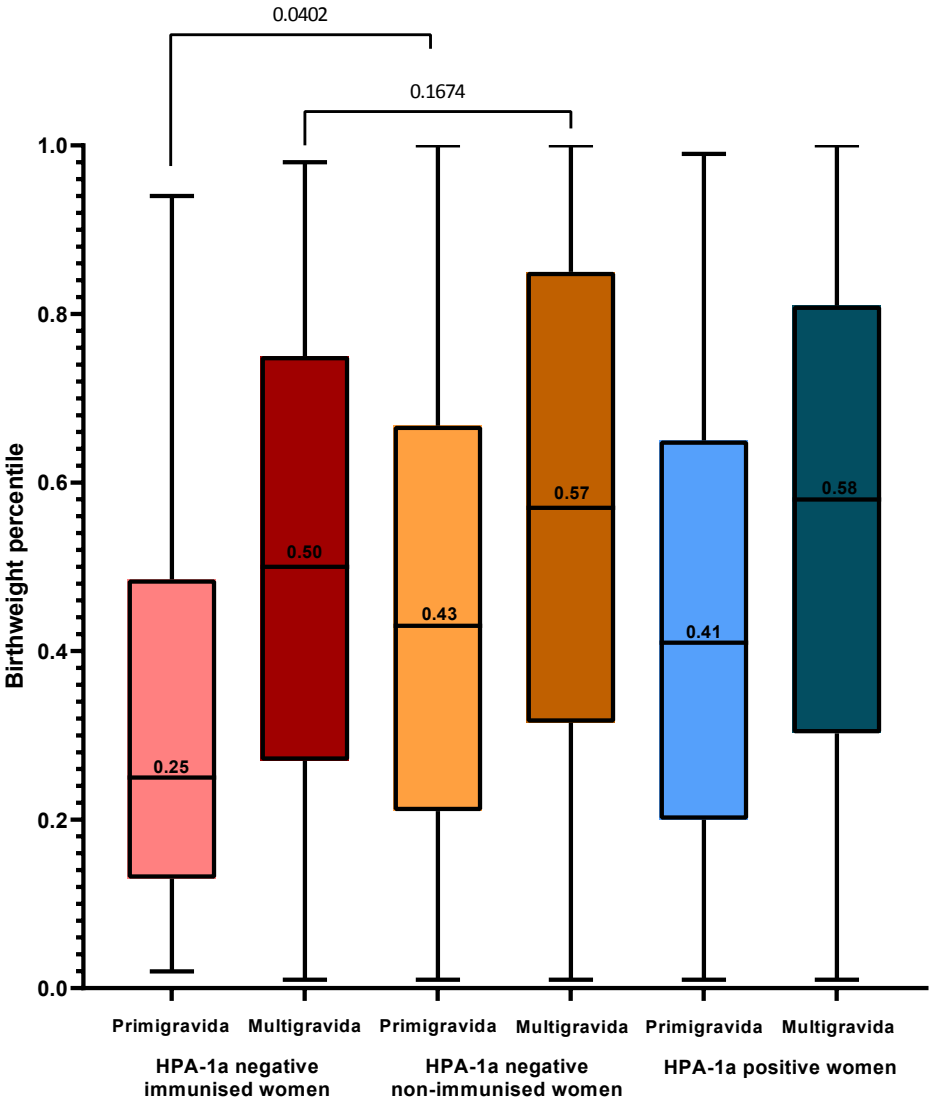
HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
145974	No	G1P0		PIH	40+4	SVD	Female	3280 (25)	None			2816	1	1
148643	No	G3P1	Miscarriage (1)		41+5	SVD	Female	3718 (53)	None			788	<0.3	1
149153	No	G2P1			40+5	SVD	Female	4000 (85)	None			6822	2	1
149368	No	G2P0			38+3		Male	2960 (19)	None			3708	1	1
155119	No	G2P0	Miscarriage (1)		39+6	SVD	Male	4150 (91)	None			768	<0.3	1
157366	No	G3P2			40+2	SVD	Female	3475 (47)	None			4663	6	2
159359	No	G2P1			40+6	SVD	Female	3660 (57)	None		HLA	12989	11	1

Abbreviations: FNAIT, fetal neonatal alloimmune thrombocytopenia; G, gravidity, P, parity; GA gestational age; BW birthweight; p, percentile; MFI, mean fluorescence index; Quant., quantitation; IU, international units; mL, millilitre; HLA, human leukocyte antigen; TOP, termination of pregnancy; ICH, intracranial haemorrhage; PLT, platelet count [× 10^9/L]; GD, gestational diabetes; CS, caesarean section; NICU, neonatal intensive care unit; SVD, spontaneous vaginal delivery; PIH, pregnancy induced hypertension; PE, pre-eclampsia; IUGR, intrauterine growth restriction; cUS, cranial ultrasound; ND, not detectable; IUFD, intrauterine fetal death; MRI, magnetic resonance imaging, NA, not available; LQTS, long QT syndrome; VD, vaginal delivery; DVT, deep venous thrombosis

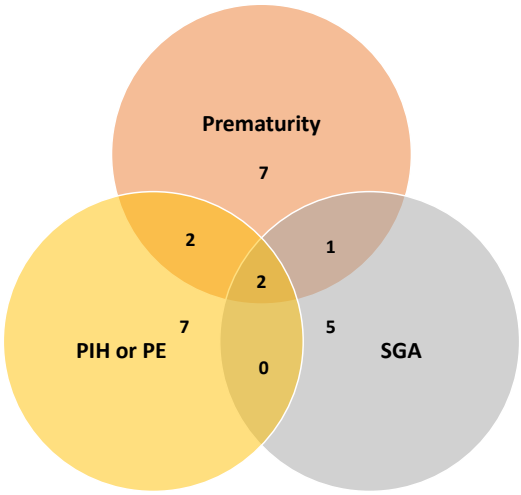
SUPPLEMENTAL TABLE 4. Obstetric history

	HPA-1a negative women		HPA-1a positive		
	Immunised women	Non immunised women	Controls	*P* value†	Risk difference (95% CI) †
	(n = 81)	(n = 820)	(n = 2704)		
Miscarriage in obstetric history ‡, n (%)	29/55 (53)	195/533 (37)	746/1818 (41)	0.095	11.8 (-1.6-25.2)
IUFD in obstetric history §, n (%)	1/46 (2)	9/462(2)	33/1558 (2)	1	0 (-4.3-4.3)

† HPA-1a immunised cases were compared with HPA-1a positive cases
‡ Primigravidae excluded
§ Nulliparae excluded
Abbreviations: HPA, human platelet antigen; IUFD, intrauterine fetal demise.



SUPPLEMENTAL FIGURE 1. Birthweight percentile stratified for primigravida and multigravida women
This figure shows percentile according to the Dutch growth charts. Median birthweight percentile of HPA-1a immunised cases were compared to non-immunised cases using the Kruskal-Wallis Test.
Abbreviation: HPA, human platelet antigen



SUPPLEMENTAL FIGURE 2. Overlap in clinical associated with placenta pathology
Cases born before 37 weeks' gestational age were defined as preterm. Small for gestational age, SGA, was defined as birthweight below 10th percentile. In 10/24 cases with one of or more symptoms antibody quantitation was > 3 IU/mL. In 14/24 cases with one or more symptoms antibody quantitation was ≤ 3 IU/mL.
Abbreviations: PIH, pregnancy induced hypertension; PE, preeclampsia; mL, mililitre.