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Natural history of human platelet antigen 1a-alloimmunised pregnancies: a prospective observational cohort study

Thijs W de Vos, Dian Winkelhorst*, Leendert Porcelijn, Mila Beaufort, Gonda Oldert, Johanna G van der Bom, Enrico Lopriore, Dick Oepkes, Masja de Haas†, Ellen van der Schoot†*

Summary

Background Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare disease that untreated can lead to intracranial haemorrhage or death. The natural history of FNAIT is still unclear; therefore, the benefits of screening cannot be estimated and no routine screening is yet in place. We aimed to assess the incidence of clinically detectable FNAIT among pregnancies in human platelet antigen-1a (HPA-1a)-immunised women.

Methods We did a prospective observational cohort study of pregnant women negative for rhesus D (RhD) and rhesus c (Rhc) antigens, without age limits, who underwent routine antenatal screening for red cell antibodies at 27 weeks' gestation and were typed for HPA-1a between March 1, 2017, and May 1, 2020. HPA-1a-negative women were tested for HPA alloantibodies. Health-care professionals were masked to all test results. The main outcome was the proportion of neonates with severe, clinically detectable FNAIT, defined as having an intracranial bleed, organ bleed, or bleeding-related death observed during pregnancy or within the first week of life. Cases of clinically detectable FNAIT not categorised as severe were categorised as mild. This study is registered with ClinicalTrials.gov (NCT04067375).

Findings Of 153 106 women typed for HPA-1a, 3722 (2·4%) were negative for HPA-1a. 913 HPA-1a-negative women gave informed consent, underwent HPA-1a antibody screening, and were included in the study. Anti-HPA-1a antibodies were detected in 85 HPA-1a-negative participants, among whom three with HPA-1a-negative fetuses and one with a previous child with FNAIT were excluded. As controls, 820 HPA-1a-negative, non-immunised pregnancies and 2704 randomly selected pregnancies of women negative for RhD and Rhc who were typed HPA-1a positive were included. Of 81 fetuses included, one (1·2%) was diagnosed with severe HPA-1a-mediated intracranial haemorrhage and three (3·7%) had mild FNAIT. Gravidity and parity did not seem to be risk factors for HPA-1a immunisation. 73 (90·1%) of 81 HPA-1a-immunised women were positive for HLA-DRB3*01:01.

Interpretation Our data suggest that, without intervention, the incidence of major clinically detectable bleeding in FNAIT is estimated as 11 (95% CI 0–32) per 10 000 HPA-1a-negative pregnancies. These findings imply that severe bleeding is a rare event that potentially could be prevented by a screening programme.

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Introduction

Fetal and neonatal alloimmune thrombocytopenia (FNAIT), the platelet equivalent of haemolytic disease of the fetus and neonate, can cause major intracranial haemorrhage and organ bleeding in fetuses and neonates. The condition can develop when there is incompatibility between fetal and maternal human platelet antigens (HPAs) and leads to destruction of the fetal platelets if maternal anti-HPA antibodies are produced. During pregnancy, all types of IgG class antibodies are actively transported by the neonatal IgG-Fc receptor, FcRn, via the placenta from the mother to the fetus. Anti-HPA-1a IgG antibodies are also transported. The HPA-1a epitope, targeted in most cases of FNAIT among White populations,[1] is carried on integrin β3 expressed by platelets, syncytiotrophoblasts,[2] and endothelial cells.[3] It has been postulated that the combination of platelet destruction and interference with endothelial cell function are both important to bleeding tendency in fetuses and neonates in HPA-1a alloimmunisation.[4] Besides thrombocytopenia and bleeding, HPA-1a alloimmunisation can be associated with placental damage.[5–9]

Intravenous immunoglobulin appears to be an effective preventive antenatal therapy.[10] However, because FNAIT is most often diagnosed after birth, antenatal treatment can be provided only in subsequent pregnancies. Prevention of the first occurrence of FNAIT requires population-based screening, which could be added to widely implemented prevention programmes for haemolytic disease of the fetus and neonate. The introduction of an FNAIT screening and prevention programme is hampered by insufficient knowledge of the natural history of the condition and of the risk factors that could inform the identification of pregnancies requiring antenatal treatment.

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*Joint first authors

†Joint last authors

Willem-Alexander Children's Hospital, Department of Pediatrics, Division of Neonatology, Leiden University Medical Center, Leiden, Netherlands (T W de Vos MD, M Beaufort BSc, Prof E Lopriore MD); **Department of Experimental Immunohematology, Sanquin Research, Amsterdam, Netherlands** (T W de Vos, D Winkelhorst PhD, Prof M de Haas MD, Prof C E van der Schoot MD); **Department of Obstetrics and Gynecology, Leiden University Medical Center, Leiden, Netherlands** (D Winkelhorst, Prof D Oepkes MD); **Department of Immunohematology Diagnostics, Sanquin Diagnostic Services, Amsterdam, Netherlands** (L Porcelijn MD, G Oldert BSc, Prof M de Haas); **Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands** (Prof J G van der Bom MD); **Department of Hematology, Leiden University Medical Center, Leiden, Netherlands** (Prof M de Haas)

Correspondence to: Prof Dr C E van der Schoot, Department of Experimental Immunohematology, Sanquin Research, 1066 CX, Amsterdam, Netherlands **e.vanderschoot@sanquin.nl**

Research in context

Evidence before this study

We searched PubMed without language restrictions between database inception and Nov 24, 2022, for antenatal screening studies reporting on the incidence of severe bleeding in HPA-1a immunised pregnancies, using the terms "HPA-1a" and "foetal neonatal alloimmune thrombocytopenia". One systematic review, published in 2010, reported on the incidence of severe bleeding in HPA-1a-immunised pregnancies. On the basis of combined figures from previous screening studies, the incidence of major bleeding or perinatal death related to fetal and neonatal alloimmune thrombocytopenia (FNAIT) is between 13 and 20 per 10 000 HPA-1a-negative pregnancies. This number is considered to be an underestimation as, in most of these studies, health-care professionals were informed about the maternal HPA status and the presence of HPA-1a antibodies and interventions were part of the study design. Moreover, interpretation of previous data has been hampered by a lack of control groups in the study design.

Added value of this study

Our observational screening study showed the incidence of clinically overt major bleeding-related FNAIT to be 11 per 10 000 HPA-1a-negative pregnant women, indicating that previous studies with interventions were not underestimating the incidence of severe FNAIT. Our study underlines the association of placental pathology and HPA-1a immunisation, since we found higher rate of hypertensive disorders in HPA-1a immunised pregnant women and a lower birth weight in HPA-1a immunised women in their first pregnancy. The presence of anti-HPA-1a was not found to be associated with the number of previous pregnancies or deliveries.

Implications of all the available evidence

Our results imply that if HPA screening is added to the existing screening programmes during pregnancy, the occurrence of major bleeding in the fetus could be prevented in 11 per 10 000 HPA-1a-negative pregnant women, and suggest that antibody concentration can be used to guide preventive measures. Further studies on the associations between HPA-1a immunisation and pathology related to placental damage are warranted. Our finding that immunisation occurs mainly before the 27th week of a first pregnancy with an HPA-1a-positive fetus, has direct implications for immunoprophylactic approaches.

We aimed to assess the incidence of clinically detectable, severe FNAIT in the absence of any intervention in a screening study of pregnant women. In addition, we aimed to evaluate the pregnancy and overall neonatal outcomes of HPA-1a immunised pregnancies compared with controls.

Methods

Study design and participants

We conducted an observational screening study in pregnant women in the Netherlands between March 1, 2017, and May 1, 2020. See appendix (pp 13–24) for a list of participating sites. As part of nationwide prenatal screening programme, pregnant women negative for rhesus D (RhD) and rhesus c (Rhc) antigens are offered red cell antibody screening and fetal *RHD* genotyping (if RhD-negative) at 27 weeks' gestation at a central laboratory. The women were informed of the study by their health-care professional for obstetric care, and consent to participate was indicated by checking a box on the laboratory request form. All RhD-negative and Rhc-negative pregnant women were eligible to participate in this study, with no age limit. Leftover material from all blood samples was used for HPA-1a typing, as described previously (appendix pp 2–3). Women already treated with intravenous immunoglobulin because of FNAIT in a previous pregnancy were excluded. For every HPA-1a-negative woman, three HPA-1a-positive women were randomly selected to serve as controls by use of random numbers generated by Microsoft Excel software. Clinical data collection from HPA-1a-positive pregnant women was done to allow the comparison of neonatal and pregnancy outcomes of HPA-1a-immunised women with a large group that, per definition, were not immunised against HPA-1a. Antibody screening and clinical data collection were done at least 3 weeks after the delivery due date. Health-care professionals were not informed of any test results (ie, maternal HPA-1a status or antibody test results).

See Online for appendix

The study protocol was published previously.[11] The Medical Ethical Committee Leiden–The Hague–Delft approved the study on July 14, 2016. Analyses were done in accordance with the predefined statistical analysis plan.

Procedures

Antibody screening was done with a bead-based glycoprotein-specific HPA-antibody detection assay (Lifecodes Pak Lx Assay; Immucor GTI Diagnostics, Norcross, GA, USA). Among HPA-1a-immunised participants, antibody quantitation was done with a modified monoclonal antibody immobilisation of platelet antigens (MAIPA) assay (appendix pp 2–3).

HLA types were imputed from genotyping with the UKBBv2 array using the Applied Biosystems HLA Analysis v1.1 algorithm. The HLA DRB3*01:01 frequencies in the Dutch population were derived from a cohort of 3364 Dutch blood donors that had been HLA-typed with the same UKBBv2 array (appendix pp 2–3).

Fetal HPA typing was done with cell-free fetal DNA analysis (QIAmp Circulating Nucleic Acid Kit; Qiagen, Hilden, Germany). Fetal typing assays were done with

droplet digital PCR using a BioRad Digital PCR System (Hercules, CA, USA) and analysed with Quantasoft Software (appendix p 3).

Researchers requested obstetric health-care professionals to provide clinical data through an online data management system. Clinical follow-up of the participants started from inclusion in the 27th week of pregnancy until 1 week after delivery. Neonatal data from the first week of life were used. Researchers and health-care professionals were masked to maternal HPA-1a status. Clinical data were collected from medical records and included gravidity, parity, (induced) miscarriages, obstetric complications, diabetes, hypertensive disorders, gestational age of neonates at delivery, mode of delivery, sex, birthweight, Apgar scores, need for paediatric consultation, admission to a neonatology ward or neonatal intensive care unit (NICU) and reason for admission, postnatal treatment, skin or organ bleeding, intracranial haemorrhage (including neuroimaging reports), and perinatal mortality. If clinical data were not available, the participant was considered lost to follow-up. We requested health-care professionals to provide a letter of discharge if the infant was admitted to the hospital, was born before 34 weeks' gestation, had an Apgar score of less than 7 at 5 min, weighed less than 1000 g at birth, or had bleeding symptoms or a platelet count of less than 150×10^9 platelets per L.

We divided the study population into three categories: (1) HPA-1a-negative women with detectable HPA-1a antibodies and an HPA-1a-positive fetus were termed immunised women; (2) HPA-1a-negative pregnant women without detectable HPA-1a antibodies were termed non-immunised women; and (3) HPA-1a positive women were termed controls.

Clinically detectable FNAIT was defined as thrombocytopenia (platelet count $<150\times10^9$ platelets per L), or minor or major bleeding, or death likely caused by bleeding, or a combination of these, after 27 weeks' gestation until 28 days after delivery in HPA-1a-immunised women with an HPA-1a-positive fetus. FNAIT was defined as severe in cases of major bleeding or perinatal death likely caused by bleeding. All FNAIT cases that were clinically detectable and not defined as severe were defined as mild. Classifications of major and minor bleeding are provided in the appendix (p 4). Two independent neonatologists specialised in neonatal neurology, who were masked to maternal HPA-1 status, classified the neuroimaging reports. Pre-eclampsia and hypertension during pregnancy were defined as present when identified by the health-care professional; no blood pressure findings were checked in the case report forms.

Outcomes

The primary objective of our study was to assess the incidence of clinically detectable, severe FNAIT in HPA-1a-immunised and incompatible pregnancies. Secondary outcomes were the incidence of clinically detectable mild FNAIT, perinatal death, pregnancy outcomes (hypertensive disorders and mode of delivery), and neonatal outcomes (preterm birth, birthweight, requirement for paediatric consultation, admission to neonatology ward or NICU, and neonatal treatment). A post-hoc analysis was done to determine risk factors for immunisation and severe disease using maternal HLA-DRB3*0101 status,[12] antibody quantification,[13] and clinical variables.

Statistical analysis

To achieve a power of 80% at an α level of 5%, we calculated that a total study population of 2400 pregnant women would be needed, assuming that 5% of our total study population would be defined as HPA-1a-immunised.[11] Clinical and laboratory data were merged into a single database. Analyses were done with Stata (version 16) and IBM SPSS Statistics (version 26.0).

Immunised cases were compared with HPA-1a-positive controls. In twin pregnancies, each child was included in the analysis as an individual. Women could be included twice in the study, with each pregnancy analysed separately. Data are presented as number of cases with percentages, means with SDs, and medians with IQRs, as appropriate. Categorical data were compared by Fisher's exact test or the χ^2 test. Continuous variables were compared using an unpaired *t* test, *t* test, or Mann-Whitney test, as applicable. Risk ratios (RR) and absolute risk differences are presented with 95% CIs. The incidence of severe, clinically detectable FNAIT was calculated using the number of neonates with severe FNAIT born to HPA-1a-negative pregnant women and the number of fetuses with severe FNAIT within the Dutch pregnant population. To calculate this incidence, it was assumed that the overall incidence of HPA-1a immunisation in the Dutch pregnant population is similar to that found in our study. 95% CIs were calculated with SPSS.

In addition, we examined risk factors for immunisation and severe disease, based on the pathophysiological similarity of FNAIT with red cell alloimmunisation.[14]

This study is registered with ClinicalTrials.gov (NCT04067375).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

During their 27th week of pregnancy, 179 595 RhD-negative and Rhc-negative women were screened for red cell antibodies (figure 1). HPA-1a typing was done in 153 106 (85·3%) women, among whom 3722 (2·4%) were negative for HPA-1a. 914 (24·6%) HPA-1a-negative women gave informed consent and were included in the study. Antibody screening was done in all but one HPA-1a-negative women.

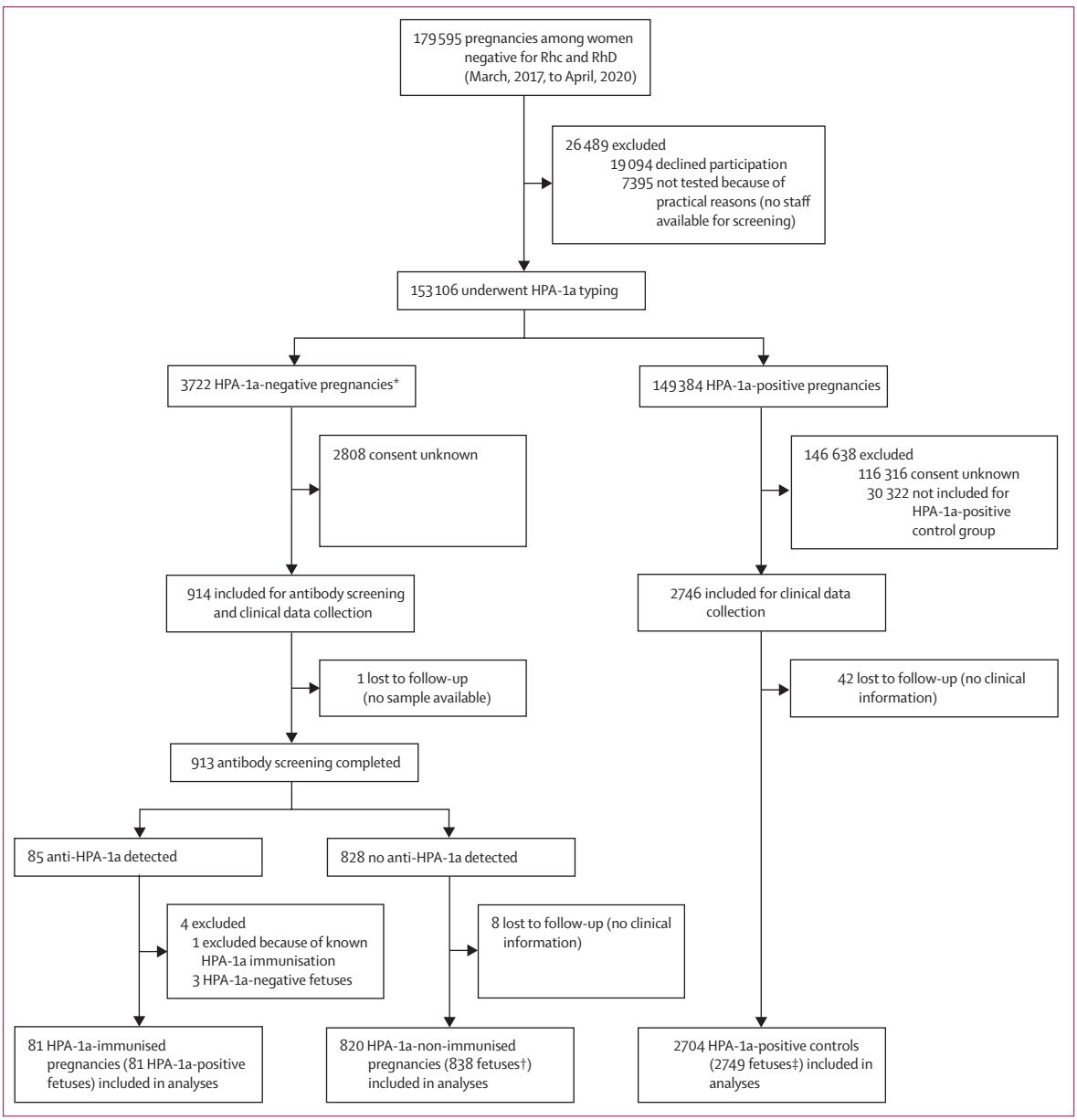

Figure 1: **Study profile**
Rhc=Rhesus c. RhD=Rhesus D. HPA-1a=human platelet antigen-1a. *32 HPA-1a negative women were included who had two pregnancies during the study period: 28 with no anti-HPA-1a antibodies detected in either pregnancy, three with anti-HPA-1a detected in both pregnancies, and one with anti-HPA-1a antibodies detected in her second pregnancy but not in her third pregnancy. †Includes 18 twin pregnancies. ‡Includes 45 twin pregnancies.

In 85 (9·3% [95% CI 7·5–11·4]) of 913 HPA-1a-negative pregnancies, we detected anti-HPA-1a antibodies at 27 weeks' gestation. Among these HPA-1a-immunised pregnancies, 82 (96·5%) were incompatible (had an HPA-1a-positive fetus). One HPA-1a-immunised woman was excluded from analyses as antenatal intravenous immunoglobulin had been administered because of a previous child with FNAIT. In total, 81 fetuses from 81 HPA-1a-immunised pregnancies (of 78 different women, with three women having two successive pregnancies) were included. From a random selection of 94 non-immunised HPA-1a-negative women, 82 (87·2%) of their fetuses were genotyped as HPA-1a positive.

Baseline characteristics did not differ between the HPA-1a-immunised pregnancies, non-immunised pregnancies (n=820), and the control group of 2704 HPA-1a-positive pregnancies (table 1). The proportions of women who were pregnant for the first time were similar across the three groups (32·1%, 36·7%, and 33·9%, respectively). The proportions of RhD-negative and Rhc-negative women were equal between HPA-1a-immunised

and non-immunised women (appendix p 5). Median follow-up by health-care professionals after delivery was 8 days (IQR 7–8).

Among the 81 HPA-1a-positive fetuses and neonates from HPA-1a-incompatible pregnancies, one (1·2% [95% CI 0·0 to 6·7]) was diagnosed with severe FNAIT and three others (3·7% [0·8 to 10·4]) had mild FNAIT. The fetus with severe FNAIT had intracranial haemorrhage. After multidisciplinary counselling, the parents requested termination of pregnancy. The three neonates with mild FNAIT were diagnosed after birth via clinical features. The incidence of clinically detectable severe FNAIT was one per 913 HPA-1a-negative pregnancies, which extrapolates to 11 (95% CI 0 to 32) per 10 000 HPA-1a-negative pregnancies, or 2·6 per 100 000 pregnancies in the Netherlands. Bleeding symptoms are reported in table 2. The absolute risk difference for major bleeding between HPA-1a-immunised women and HPA-1a-positive women was 1·1% (95% CI –1·3 to 3·5; p=0·110). Three neonates in the control group of 2749 fetuses of HPA-1a-positive mothers had major bleeding: one had a grade 3 intraventricular haemorrhage related to premature delivery at 27 weeks' gestation, the second infant was diagnosed with a grade 3 intraventricular haemorrhage and subdural haemorrhage related to congenital abnormalities, and the third neonate had asphyxia and gastrointestinal bleeding.

Pregnancy outcomes are summarised in the appendix (p 6). Hypertensive disorder during pregnancy was diagnosed in nine (11·1%) of 81 HPA-1a-immunised women and in 120 (4·4%) of 2704 HPA-1a-positive controls (RR 6·7 [95% CI –0·2 to 13·6], p=0·011). The proportion of pregnancies in which pre-eclampsia was diagnosed did not differ between HPA-1a-immunised women and HPA-1a-positive women.

Neonatal outcomes are presented in table 3. The median gestational age at delivery was similar between the groups. The proportion of preterm births (at <37 weeks' gestational age) was 12 (14·8%) of 81 in the HPA-1a immunised group and 132 (4·8%) of 2749 in the HPA-1a-positive group (RR 10·0 [95% CI 2·2–17·8], p<0·001). The mean birthweight was 3271 g (SD 631) in the HPA-1a-immunised group compared with 3459 g (545) in the HPA-1a positive group (mean difference 187 g [95% CI 66–308]; p=0·002). To correct for gestational age and sex, the birthweight percentiles of neonates were compared and were found to be differently distributed among HPA-1a immunised women than among HPA-1a positive women; appendix p 8). This finding could be largely attributed to the lower birthweight percentiles of neonates born to HPA-1a-immunised primigravid women (median 25th percentile [IQR 13–49]) compared with those of neonates from non-immunised HPA-1a-negative primigravid women (43rd percentile [21–67], p=0·040; appendix p 9). The median birthweight percentile was not different between male and female neonates in the HPA-1a-immunised group (44th percentile [24–70] *vs* 46th percentile [18–72]). The percentage of neonates born small for gestational age did not differ between the groups. The association between the presence of preterm delivery, reduced birthweight, and hypertensive disorder during pregnancy is shown in the appendix (p 10).

	HPA-1a-negative women		HPA-1a-positive women (n=2704)
	Immunised* (n=81)	Non-immunised (n=820)	
Maternal age, years	30·8 (27·2–34·4)	31·1 (28·1–34·3)	31·2 (28·1–34·1)
Gravidity			
Median	2 (1–3)	2 (1–3)	2 (1–3)
Primigravid	26/81 (32·1%)	301/820 (36·7%)	917/2704 (33·9%)
Parity			
Median	1 (0–1)	1 (0–1)	1 (0–1)
Nulliparous	35/81 (43·2%)	371/820 (45·2%)	1168/2704 (43·2%)
Maternal immune thrombocytopenia	0/81	1/820 (0·1%)	3/2704 (0·1%)
Diabetes	0/81	7/820 (0·9%)	19/2704 (0·7%)
Gestational diabetes	2/81 (2·5%)	46/820 (5·6%)	163/2704 (6%)
Sex of neonate†			
Male	44/81 (54·3%)	421/838 (50·2%)	1378/2747 (50·2%)
Female	37/81 (45·7%)	417/838 (49·8%)	1369/2747 (49·8%)

Data are median (IQR) or n/N (%). The group of immunised HPA-1a-negative women includes three women who had two successive pregnancies and were thus included twice; the group of non-immunised HPA-1a-negative women includes 28 women who had two successive pregnancies; and no women in the HPA-1a positive group were included more than once. *Only pregnancies with an HPA-1a-positive fetus were included. †Data missing for one HPA-1a-positive woman with a (dichorionic) twin that ended with fetal demise.

Table 1: **Baseline characteristics**

There was no statistically significant difference in the proportion of neonates admitted to the neonatology ward (13 [16·1%] of 81 *vs* 281 [10·2%] of 2749; p=0·096; RR 1·6 [95% CI 0·9–2·6]). However, within the group of neonates admitted, a significantly higher proportion of neonates of HPA-1a-immunised mothers were admitted to the NICU (five [38·5%] of 13 *vs* 25 [9·7%] of 259; p=0·008; RR 3·7 [95% CI 1·7–8·2]). Three neonates of HPA-1a-immunised mothers were admitted to the NICU because of preterm birth, one because of asphyxia, and one because of early-onset sepsis. The most frequent reasons for NICU admission in the HPA-1a positive group were preterm birth (n=12) and respiratory distress (n=8). Clinical outcomes of neonates born to non-immunised HPA-1a-negative and HPA-1a-positive women were similar.

Risk factors for HPA-1a immunisation are shown in the appendix (p 7). HPA-1a-immunised women had slightly more often had a previous miscarriage or abortion (31 [38·3%] of 81) than non-immunised women (218 [26·6%] of 820; p=0·039). Gravidity and parity were not found to be risk factors.

HPA-1a immunisation was associated with maternal HLA-DRB3*01:01 status: 73 (90·1%) of 81 HPA-1a-immunised women were positive for HLA-DRB3*01:01, of whom nine (12·3%) were homozygous. Among controls (3364 blood donors), 1117 (33·2%) were HLA-DRB3*01:01

	Neonates of HPA-1a-immunised women* (n=81)	Neonates of non-immunised women (n=838)	Controls (n=2749)	p value†	Risk difference (95% CI)†‡
Severe FNAIT	1/81 (1·2%)	..	..	..	..
Mild FNAIT	3/81 (3·7%)	..	..	..	..
Bleeding	4/81 (4·9%)	18/838 (2·1%)	57/2749 (2·1%)	0·095	2·9 (–1·9 to 7·6)
Major bleeding	1/81 (1·2%)	0/838	3/2749 (0·1%)	0·11	1·1 (–1·3 to 3·5)
Minor bleeding	3/81 (3·7%)	18/838 (2·1%)	54/2749 (2·0%)	0·22	1·7 (–2·4 to 5·9)
Thrombocytopenia§	2/8 (25·0%)	7/37 (18·9%)	18/116 (15·5%)	0·61	9·5 (–21·7 to 40·2)
Severe thrombocytopenia§	1/8 (12·5%)	2/37 (5·4%)	1/116 (0·9%)	0·13	10·2 (–10·4 to 30·8)
Perinatal death	1/81 (1·2%)	3/838 (0·4%)	11/2749 (0·4%)	0·30	0·8 (–1·6 to 3·3)
Due to bleeding	1/81 (1·2%)	0/838	0/2749	..	..
Due to other causes	0/81	1/838 (0·1%)	7/2749 (0·3%)	..	..
Unknown cause	0/81	2/838 (0·2%)	4/2749 (0·1%)	..	..

Data are n/N (%) unless otherwise specified. All statistics and percentages were calculated by use of available data, excluding participants with missing data. FNAIT=fetal and neonatal alloimmune thrombocytopenia. HPA=human platelet antigen. *Only HPA-1a-incompatible pregnancies were included. †Comparison of HPA-1a-immunised pregnancies with HPA-1a-positive pregnancies (controls). ‡Percentage points difference in HPA-1a-immunised pregnancies compared with controls. §Denominators are neonates with a known platelet count (ie, determined by clinician); thrombocytopenia was defined as a platelet count <150×10⁹ platelets per L and severe thrombocytopenia as a platelet count <50×10⁹ platelets per L.

Table 2: **Clinically detectable fetal and neonatal alloimmune thrombocytopenia**

positive. HPA-1a immunisation occurred in 73 (28·2%) HLA-DRB3*01:01-positive women (with an HPA-1a incompatible pregnancy) and eight (1·5%) HLA-DRB3*01:01-negative women.

Antibody concentrations were too low for quantitation (<0·3 IU/mL) in 38 cases. In 18 HPA-1a-immunised women with an HPA-1a-incompatible infant, anti-HPA-1a concentration was greater than 3 IU/mL.[15] In the remaining 25 cases, antibody concentrations were between 0·3 and 3 IU/mL. The highest antibody concentration (90 IU/mL) was detected in the woman whose fetus had severe FNAIT. For the three cases of mild FNAIT, maternal antibody concentrations were 2, 3, and 5 IU/mL. Figure 2 shows antibody concentrations stratified by maternal HLA-DRB3*01:01 status. The plot of antibody concentrations against PAK Lx data are provided in the appendix (p 11).

Discussion

This observational study was designed to assess the incidence of severe FNAIT in neonates of HPA-1a-negative women. Researchers and health-care professionals were masked to HPA-1a status and antibody test results, allowing assessment of the outcomes of pregnancies with HPA-1a immunisation and risk factors for immunisation without interference by medical interventions. 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 (2·1–2·5%).[16–19] Additionally, the proportion of 9·3% HPA-1a-allo-immunised women was similar to those previously reported in the UK[19] (9·4%) and Norway[17] (8·6%). These studies, however, started with early antibody screening and two to five repeated measurements. Because we only measured once, at 27 weeks' gestation, we might have missed later immunisations. In the aforementioned studies, only in 11%[19] and 2%[17] of cases were antibodies first detected after 27 weeks.

Another difference was the platform to detect HPA-1a antibodies. Previous studies used the MAIPA assay or platelet fluorescence test.[17,19] We used a platform with platelet GPIIb/IIIa (αIIbβ3) glycoprotein-coated beads for antibody detection (PAK Lx assay), which has been shown to have at least equal sensitivity to MAIPA[20] and was considered to be more suitable for high-throughput screening. In fact, we observed that the PAK Lx assay tended to be slightly more sensitive than the MAIPA assay (data not shown).

An important strength of our study is that, in contrast to previous studies,[16–19,21–25] we were able to estimate the true incidence of clinically FNAIT compared with a control group, without any interference. Compared with previous studies,[26] we found a slightly lower incidence of major bleeding (1·2% *vs* 1·4%). In contrast to our assumption, interventions done in the other screening studies might not have had a major effect on the incidence of intracranial haemorrhage, as our estimated incidence of 11 per 10 000 HPA-1a-negative pregnant women is in line with the combined results of these studies. The absence of interventions is in part understandable since in the largest study, only near-term caesarean section and rapid postnatal treatment were offered, whereas most cases of severe bleeding occur before 35 weeks' gestation.[27]

We found skin bleeding in only three (4%) of the 81 neonates at risk compared with 54 (2%) of 2749 neonates born to HPA-1a-positive pregnant women. Two large screening studies reported the proportions of neonates with skin bleeding to be as high as 20% (five of 25)[18] and 19% (seven of 36).[19] Minor skin bleeding might have been under-reported in our study because, in contrast to the other studies, health-care professionals were masked to HPA-1a status and antibody presence.

In previous screening studies, thrombocytopenia and risk of bleeding were the most important outcome measures reported. Recently, several studies have suggested that HPA-1a immunisation could also involve pathology of the placenta[5–9] and lower birth weight.[28] Our study shows an association of FNAIT with preterm delivery, reduced birthweight, and hypertensive disorders, and warrants further investigations involving placental pathology. The effect of reduced birthweight was mainly seen in first pregnancies. Although the mean difference in birth weight of 187 g might not be clinically relevant, it

	HPA-1a negative women		HPA-1a positive controls (n=2749)	p value*	Risk difference (95% CI)†
	Neonates of immunised women‡ (n=81)	Neonates of non-immunised women (n=838)			
Median gestational age at delivery, weeks+days	39+5 (38+2–40+5)	39+6 (38+6–40+5)	39+6 (38+5–40+4)	0·17	..
Preterm birth at <32 weeks' gestation	2/81 (2·5%)	4/838 (0·5%)	10/2749 (0·4%)	0·045	2·1 (–1·3 to 5·5)
Preterm birth at <37 weeks' gestation	12/81 (14·8%)	46/838 (5·5%)	132/2749 (4·8%)	<0·001	10·0 (2·2 to 17·8)
Mean birthweight, g	3271 (631)	3477 (558)	3459 (545)	0·002	..
Small for gestational age (birthweight <10th percentile)	8/81 (9·9%)	63/838 (7·5%)	266/2749 (9·7%)	0·85	0·2 (–6·4 to 6·8)
Apgar score <7 at 5 min	4/81 (4·9%)	15/838 (1·8%)	42/2749 (1·5%)	0·041	3·5 (–1·3 to 8·3)
Consultation with paediatrician	27/81 (33·3%)	342/838 (40·8%)	977/2749 (35·5%)	0·68	–2·2 (–12·6 to 8·2)
Admission to neonatology ward	13/81 (16·1%)	97/838 (11·6%)	281/2749 (10·2%)	0·096	5·8 (–2·2 to 13·9)
Admission to NICU	5/13 (38·5%)	8/85 (9·4%)	25/259 (9·7%)	0·008	28·8 (2·1 to 55·5)
Days in NICU					
Median (IQR)	0 (0–6)	0 (0–0)	0 (0–0)	0·002	..
Range	0–26	0–31	0–22	..	..
Postnatal treatment	13/81 (16·1%)	57/838 (6·8%)	201/2749 (7·3%)	0·008	8·7 (6·8 to 16·7)

Data are median (IQR), n/N (%), or mean (SD) unless otherwise specified. All statistics and percentages were calculated by use of available data, excluding participants with missing data. HPA=human platelet antigen. NICU=neonatal intensive care unit. *Comparison of HPA-1a-immunised pregnancies with HPA-1a-positive pregnancies (controls). †Percentage points difference in HPA-1a-immunised pregnancies compared with controls. ‡Only HPA-1a-incompatible pregnancies were included.

Table 3: **Neonatal outcomes**

supports the hypothesis that anti-HPA-1a antibodies might have direct functional or indirect immunological effects on the syncytiotrophoblast. Future clinical studies should include the analysis of placental pathology.

We confirmed that HPA-1a immunisation is strongly associated with maternal HLA DRB3*01:01 positivity.[12] In contrast to other screening studies[17–19] we did not find that parity or gravidity were risk factors for HPA-1a alloimmunisation. The low number of HPA-1bb fetuses (three of 58) in immunised multigravida women suggests that antibody concentrations decline and become undetectable if not boosted by an HPA-1a-positive fetus in a subsequent pregnancy.[15] The number of women with a miscarriage or abortion was slightly higher in the group of immunised women, which was also noted in previous studies. The percentage of primigravida women with antibodies detected before 28 weeks' gestation was lower in other studies: 4%,[18] 9%,[17] and 14%.[19] In our study, we might have missed HPA-1a antibodies in multigravida women, as previous studies reported disappearance of HPA-1a antibodies around the 27th week of pregnancy in considerable percentages of women (22%[15] and 25%[19]). However, even if we take the evanescence of HPA-1a antibodies in multigravid women into account, we still have an over-representation of primigravid women. The difference in the assay used for HPA-1a antibody detection is probably the most important factor in explaining the observed differences.

In our study, the case with major bleeding had the highest anti-HPA-1a antibody concentration in our cohort, and the three cases with minor bleeding had anti-HPA-1a concentrations above the median of 1 IU/mL. In the Norwegian screening study, anti-HPA-1a quantitation was inversely correlated with platelet count.[15] These findings suggest that in a screening programme, anti-HPA-1a quantitation could be used to select

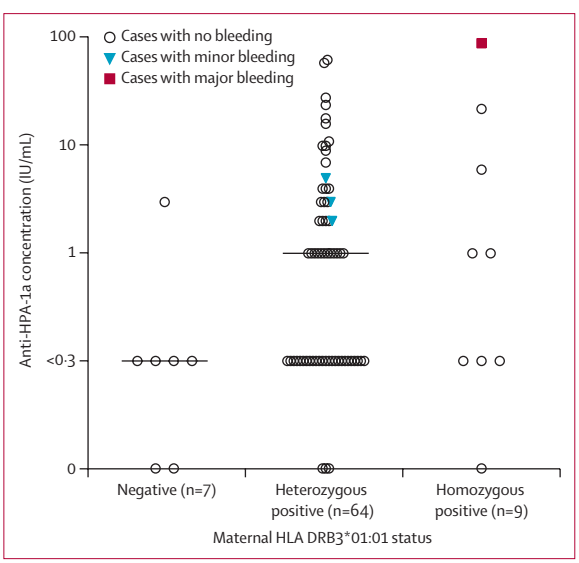

Figure 2: **Anti-HPA-1a antibody concentration by maternal HLA DRB3*01:01 status**

Alloantibody levels as determined by the modified monoclonal antibody immobilisation of platelet antigens assay. The horizontal lines indicate median anti-HPA-1a concentrations. Samples with values below 0·3 IU/mL were positive but had optical density values outside the quantitative range of the ELISA. Negative samples (shown at 0 on the graph) had optical density values below the cutoff value of 0·092 IU/mL.

HPA-1a-immunised women who will benefit from preventive therapy such as intravenous immunoglobulin.

Strengths of our study include the blinded observational design and 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 limitation, however, of this observational design was that it did not allow us to measure platelet counts and conduct routine cranial ultrasound examinations in all neonates. We cannot exclude that intracranial haemorrhages were missed as a result of the lack of these routine examinations. Moreover, intracranial haemorrhage might present simply with lethargy, poor feeding or vomiting, and no obvious signs of diathesis.[29] Nevertheless, we consider it a strength of our study that we focused on a clinically detectable, and thus clinically relevant, disease.

Another limitation of our study is that we included fewer women than anticipated, which reduced the statistical power of the study. As a result, the confidence interval for our estimate of the incidence (0–32 per 10 000 HPA-1a-negative women) is somewhat wider than anticipated, yielding an imprecise estimate of the incidence. The informed consent rate was lower than originally anticipated (50%), we assume because of logistical hurdles in this opt-in study. More than 400 health-care providers were involved. Informed consent was transferred by ticking a box on the laboratory request form, but this form was not used at all sites. Formally, we cannot exclude selection bias, but at time of inclusion it is difficult to envision which factors would have caused such a selection. Only pregnancy losses due to early fetal haemorrhages caused by FNAIT might have been missed. Inclusion in the present study was also mostly determined by whether the health-care professional had decided to participate and whether logistics to transfer the informed consent were in place. Thus, selection was independent of the characteristics of eligible individuals. We therefore believe it is unlikely that our findings were biased by selection.

The implementation of population-based screening of platelet alloantibodies during pregnancy has been debated for decades. Due to insufficient knowledge of the incidence of severe haemorrhage in HPA-1a-immunised pregnancies, the impact of the disease at the population level could not be properly estimated, complicating the introduction of a screening programme.[30] This prospective observational study provides important insight into the natural history of FNAIT, without a screening and intervention programme. 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 to HLA DBR3*01:01 positive women and to only give interventions in pregnancies with higher antibody concentrations. It also raises awareness of the other possible clinical features of FNAIT associated with placenta-related pathology. Prophylaxis is thought to prevent all cases of anti-HPA-1a-mediated FNAIT.[30] Our data suggest that prophylaxis should be administered early in pregnancy to prevent immunisation, although the question remains of whether administration of anti-HPA-1a prophylaxis might damage the placenta.

On the basis of our findings, the estimated incidence of clinically detectable severe haemorrhage in fetuses and neonates as a result of HPA-1a immunisation in pregnancy is around 11 in 10 000 HPA-1a-negative women. Furthermore, HPA-1a immunisation might be associated with hypertensive disorders during pregnancy, reduced birthweight, and preterm delivery.

Contributors

DO, JGvdB, EL, MdH, and CEvdS conceptualised the study. TWdV and DW accessed the raw data and did data curation. TWdV and DW did the formal analysis. DO, MdH and CEvdS acquired funding. TWdV, DW, LP, MB, and GO contributed to the investigation. WdV, DW, JGvdB, MdH, and CEvdS contributed to the methodology; LP contributed to resources. DO, MdH, and CEvdS supervised the study. TWdV and DW contributed to data visualisation. TWdV and DW wrote the original draft of the manuscript. LP, MB, JGvdB, EL, DO, MdH, and CEvdS reviewed and edited the manuscript.

Declaration of interests

JGvdB reports an unrestricted research grant from Novo Nordisk and a previous payment for teaching by Bayer (both paid to the institution). DO is funded as a research consultant by Janssen Pharmaceuticals and participates on the advisory board of Janssen Pharmaceuticals. EL reports a consultancy fee from Janssen Pharmaceuticals as member of the advisory board on FNAIT. All other authors declare no competing interest.

Data sharing

Tables with patient-level information or further details of the patients and their outcomes can be provided on request to the corresponding author. Requests for data will be reviewed by the scientific committee. If approval is given, data will be shared via a secure portal.

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