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Fetal and Neonatal Alloimmune Thrombocytopenia: evidence based screening

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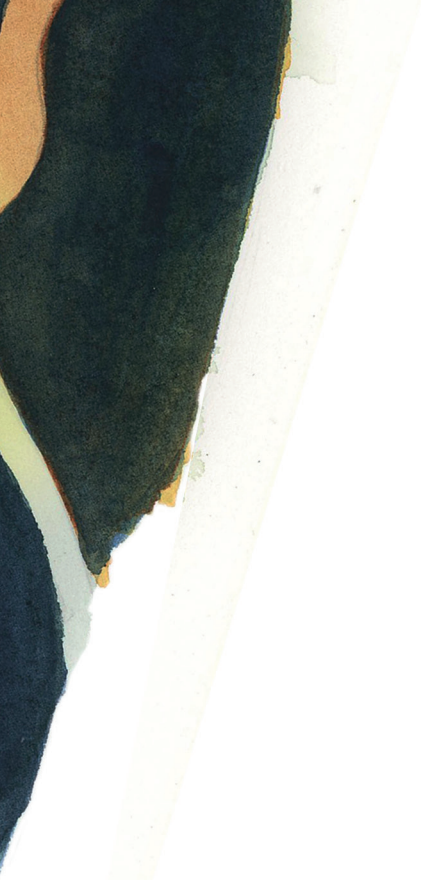

Chapter 9

HIP-study (HPA-screening In Pregnancy): interim-analysis

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Abstract

Introduction. Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a potentially life-threatening disease. In subsequent pregnancies, when immunization is known, devastating bleeding complications can be effectively prevented with weekly maternal intravenous immunoglobulins (IVIg) infusions. Population-based screening to identify alloimmunized pregnancies that would benefit from treatment is a highly debated topic. Missing knowledge on natural history and lack of diagnostic tools to select immunized pregnancies at high-risk is currently complicating implementation of such screening.

Materials and Methods. We performed a nation-wide, prospective, non-interventional cohort study. At 27 weeks' gestation serological HPA-1a typing was performed with enzyme-linked immunosorbent assay (ELISA) and antibody screening using a bead-based PAKLx assay. All women gave consent for antibody testing and clinical data collection.

Results. A total of 40,945 pregnant women were typed for HPA-1a, of which 986 (2.4%) were HPA-1a negative. Within 262 HPA-1a negative cases that gave consent for further testing, 24 immunizations were detected (9.2%). These resulted in 4 cases of clinical relevant FNAIT, 3 with minor hemorrhage and 1 with a severe ICH leading to late pregnancy termination at 34 weeks' gestation. Compared to a HPA-1a positive control group, HPA-1a immunized cases had a higher risk at premature delivery before 34 and 37 weeks' gestation (risk ratios, 8.1; 95% CI, 1.8 to 36.2 and 3.9; 95% CI, 1.7 to 8.9), a higher risk at hemorrhage (risk ratio, 13.0; 95% CI, 4.4 to 38.5), a higher risk at hypertensive disorders (risk ratio, 3.1; 95% CI, 1.4 to 7.0) and significantly more miscarriages in there obstetric history (risk ratio, 1.6; 95% CI, 1.1 to 2.4).

Conclusions. Incidence numbers of HPA-1a negativity and anti-HPA-1a alloimmunization are in line with numbers reported in literature. For careful extrapolation of incidence of clinical relevant FNAIT to the general pregnant population, data from the completed study have to be awaited.

Introduction

Bleeding problems and severe thrombocytopenia in otherwise healthy term-born infants are most likely to be caused by fetal and neonatal alloimmune thrombocytopenia (FNAIT).[1,2] Incompatibility between fetus and mother, for human platelet antigens (HPAs), might result in the formation of specific alloantibodies during pregnancy. These maternal alloantibodies can enter the fetal circulation by active transport across the placenta and lead to platelet destruction and possibly also endothelial damage.[3,4] Clinical consequences of FNAIT can vary from asymptomatic thrombocytopenia to minor skin hemorrhage, such as hematomas or petechiae, or ultimately result in severe internal organ hemorrhages and perinatal death.[5,6] FNAIT is considered to be the platelet counterpart of hemolytic disease of the fetus and newborn (HDFN), because of the similar pathophysiologic fundaments. In this comparison, HPA-1a, that causes the vast majority of the (severe) cases of FNAIT, is the equivalent of RhD in HDFN.[7] Accordingly, a lot of interest has been gone into the prevention of FNAIT by population-based screening, comparable to the RhD prophylaxis and screening program.[8-10] However, besides their resemblance, important differences complicate the design and implementation of such population-based screening, in order to timely detect, prevent and treat FNAIT.

First, despite a couple of large prospective cohort studies, the natural history of the disease is still undetermined. Most of these screening studies, were not completely observational and performed some kind of intervention, thereby prevent drawing any firm conclusion on the natural history of FNAIT.[11-15] Additionally, to implement a screening program in the Netherlands, more accurate estimates of incidence and prevalence of the disease in the Dutch population need to be known. Second, one of the most important differences from HDFN, making it even less achievable to implement population-based screening for prevention of FNAIT, is the lack of diagnostic tools to identify pregnancies at high risk for bleeding complications. In HDFN, alloimmunization is monitored by both laboratory and clinical assessment. High risk cases are identified by laboratory assessment (antibody titer and antibody-dependent cellular cytotoxicity), followed by ultrasound assessment of pre-selected cases (estimation of fetal anemia by Doppler-based assessment of flow velocity in the middle cerebral artery). These cases most likely benefit from fetal blood sampling (FBS), followed by an intrauterine transfusion (IUT).[16] In FNAIT, without the clinical applicability of such (non-invasive) diagnostic parameters, all known alloimmunizations are treated with non-invasive intravenous immunoglobulin (IVIg) infusions.[17,18] The vast majority of these alloimmunizations are currently detected because of the disease they've already caused. However, in a potential screening scenario, far more alloimmunized pregnancies will be detected, among which pregnancies that would have never resulted in fetal or neonatal bleeding problems. Treating all these alloimmunizations in a potential screening setting would result in considerable overtreatment.

To obtain insight in these two important features of the disease (natural history and risk assessment severe course of disease) and to facilitate assessment of the feasibility of screening, we designed the HIP-study (HPA-screening In Pregnancy). The study is completely observational. This way, besides the incidence of HPA-1a alloimmunizations and clinical manifestations, we will also be able to conclude on the natural history of FNAIT. Ultimately, by collecting both blood samples and testing for antibody characteristics, genetic markers and data on bleeding symptoms in children from alloimmunized and non-alloimmunized pregnancies, we aim to develop a diagnostic tool. This tool will allow the identification of immunized pregnancies at high risk for bleeding complications, that would benefit from treatment. Which would not only be beneficial in current management of FNAIT, but most of all in a potential future screening setting.

Materials and methods

Study population

From March 2017, all RhD or Rhc negative pregnant women in the Netherlands were eligible for enrolment in the study. As part the Dutch nationwide Prenatal Screening for Infectious diseases and Erythrocyte immunization (PSIE), these women are offered a free of charge red blood cell (RBC) antibody screening and fetal *RHD* typing at 27 weeks' gestation. Blood for this screening is drawn at certified, local laboratories all over the Netherlands ($n = \pm 90$) and transported to our laboratory.[19] Left-over material of the ethylenediamine tetra-acetic acid (EDTA) anticoagulated blood tubes was used. Pregnant women with known HPA alloimmunization, and that received IVIg treatment, were excluded. All pregnant women gave informed consent for inclusion in the HIP-study. The study was approved by the Committee of Medical Ethics at the Leiden University Medical Center (P16.002).

Laboratory analysis

After regular testing and reporting of these results, left-over material of the blood samples, mainly 3-6 days-old, was used. Plasma containing platelets was used for serological HPA-1a typing, using an enzyme-linked immunosorbent assay (ELISA), as described previously.[20] In short, 20µl of plasma was automatically pipetted onto 96-well microtiter plates coated with anti-CD61 (C17). Then, recombinant HRP-labeled anti-HPA-1a (B2G1) was added, plates were centrifuged and incubated for 45 minutes. After 15 minutes of incubation with anti-HRP, reaction was stopped with sulfuric acid (H_2SO^4) and results were obtained with an Anthos reader. HPA-1a typing was performed in all samples that did not decline consent. In all samples with an ELISA OD below 0.160, supportive genotyping using allelic discrimination polymerase chain reaction (PCR) assay was performed. Plasma and buffy-coat of samples that were typed HPA-1a negative were stored at -20°C. Additionally, for each HPA-1a negative case, one HPA-1a positive

control was stored as well. For further testing informed consent was needed. Well after the estimate due date, the incidence of alloimmunization was evaluated. With the stored, left-over plasma of all HPA-1a negative women a Pak Lx assay was a performed, a bead-based GP-specific HPA-antibody detection method (LIFECODES Pak Lx Assay, Immucor GTI Diagnostics, Norcross, United States of America) to screen for HPA-1a alloantibodies. Possible laboratory parameters that will be tested to assess risk at bleeding complications are: HLA-DRB3*0101 status, antibody level, Fc-core glycosylation and FcgRIII-binding index, endothelial cell binding and endothelial cell function.[4,21-26] Combining the clinical information with the laboratory results will enable the identification of two groups; immunized cases with disease and immunized cases without disease. The latter group will form a unique and optimal control group for identification of possible parameters to predict the development of (severe) bleeding complications.

Clinical data collection

Of all stored samples, obstetric care givers were contacted to obtain clinical information, through a digital case report form (CRF). This form contained data on maternal medical history, obstetric history, current pregnancy, delivery and neonatal period (first week of life). Primary outcome was clinically relevant HPA-1a mediated FNAIT, which was further classified into severe FNAIT (with severe bleeding, ICH or internal organ hemorrhage) or mild FNAIT (all other bleeding, petechiae, bruising, hematoma or mucosal bleeding, and/or treatment or clinical observation for thrombocytopenia).

Statistical analysis

Clinical data will be entered into a validated data capture system, called ProMISe, provided and designed by the LUMC. The system is protected by password and contains internal quality checks to identify inaccurate or incomplete data. Laboratory data will be entered in a separate password protected database by independent technicians, inaccessible to the researchers. Both clinical and laboratory data will be combined and further data management and analysis will be performed using SPSS and Graphpad. Included cases were divided into three groups: HPA-1a negative cases with anti-HPA-1a (referred to as immunized cases), HPA-1a negative cases without anti-HPA-1a (referred to as non-immunized cases) and HPA-1a positive controls. Comparisons between groups for categorical data were performed using chi-square or Fisher exact test. Risk ratios (relative risks) for immunized cases versus the HPA-1a positive control group were calculated for categorical data. For continuous variables groups were compared using student t-test or Mann-Whitney U, as applicable. A *p*-value smaller than 0.05 was considered statistically significant.

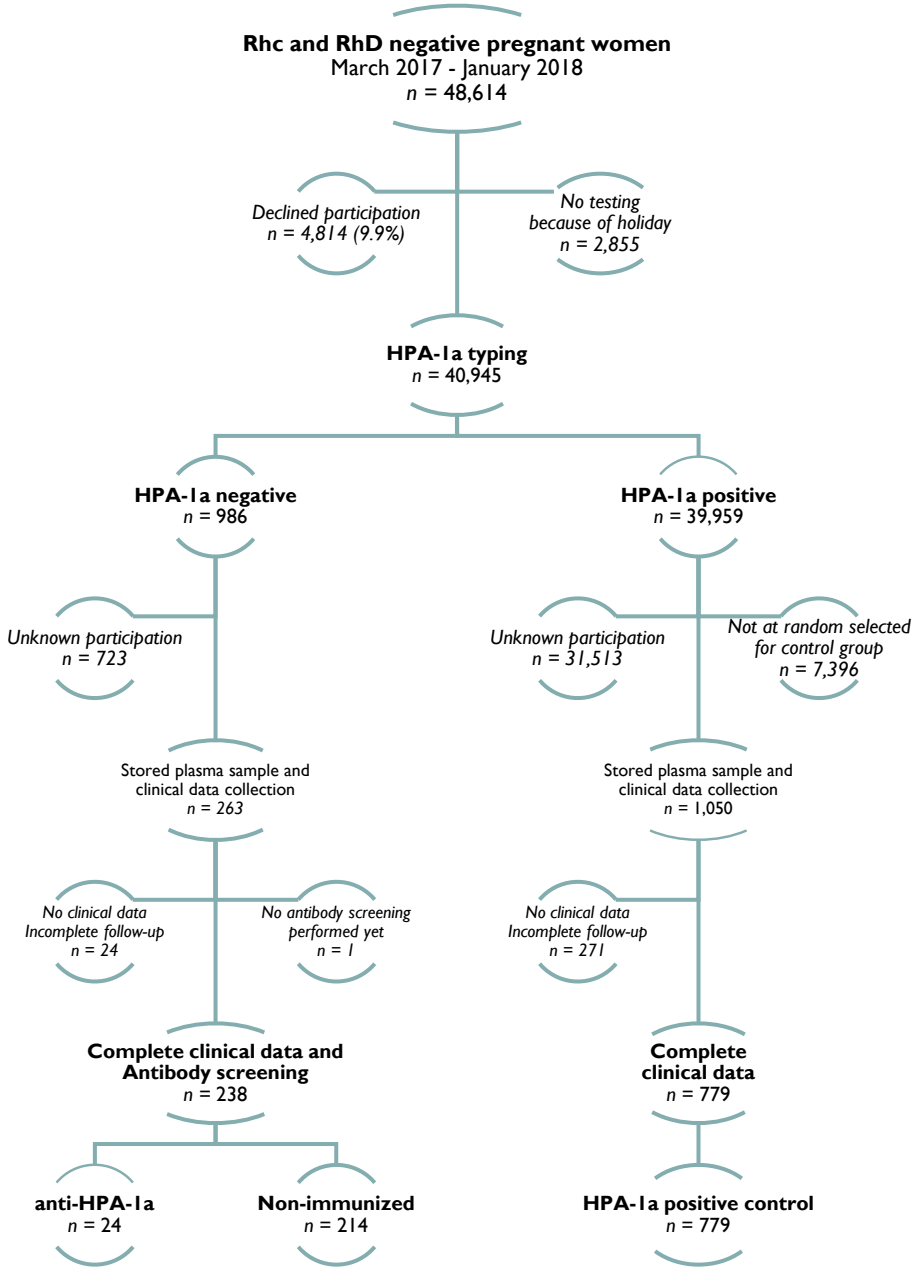

Figure 9.1 – Description of study population

Results

Participants

In a period of 10 months, from March 2017 to January 2018, 48,614 pregnant women were tested as part of the population-based screening program at 27 weeks' gestation for RBC antibodies or fetal *RHD* type because of their RhD-negative or Rhc-negative blood type (Figure 9.1). Of these, 4,814 (9.9%) declined participation to the HIP-study and in 2,855 serological HPA-1a typing was not performed due to holidays and/or understaffing. This leaves a total of 40,945 women that were serologically typed for HPA-1a. Of these, 986 (2.4%) were HPA-1a negative. For every HPA-1a negative sample a HPA-1a positive sample with consent was stored, in total we included 1,050 HPA-1a positive controls. Informed consent was registered for 27%, so a total of 263 HPA-1a negative samples were available for further testing. In all but one case antibody screening was performed, leading to the detection of 24 anti-HPA-1a immunizations (24/262, 9.2%).

Table 9.1 – Baseline Characteristics

	HPA-1a negative (*n* = 238)	**HPA-1a positive (*n* = 779)**	**Total (*n* = 1,017)**
Gravidity	2 (1-3)	2 (1-3)	2 (1-3)
Primigravidae	77 (32)	271 (35)	348 (34)
Parity	1 (0-1)	1 (0-1)	1 (0-1)
Nulliparae	95 (40)	342 (44)	437 (43)
Medical history ITP	0	0	0
Miscarriage in history	57 (24)	196 (25)	253 (25)
IUFD in history	2 (1)	6 (1)	8 (1)
Multiple gestation	4 (2)	13 (2)	17 (2)

Data presented as n (%) or median (interquartile range); HPA, human platelet antigen; ITP, immune thrombocytopenia; IUFD, intrauterine fetal demise.

Descriptive data

The clinical data collection was complete for 1,018 of 1,249 included cases (82%). We were able to analyze a total of 238 HPA-1a negative women and 779 HPA-1a positive controls. Baseline characteristics (gravidity, parity, medical history with ITP, obstetric history and multiple gestations) were comparable between both groups, no statistical significant differences were observed (Table 9.1).

Table 9.2 – Clinical Outcome

		HPA-1a negative (n = 238)	
		Immunized (n = 24)	Non-immunized (n = 214)
Perinatal	Gestational age at delivery	39^{+6} (37^{+3} – 40^{+5})*	39^{+6} (39^{+0} – 40^{+5})
	Premature delivery (< 37 weeks)	4 (17)*	6 (3)
	Premature delivery (< 34 weeks)	2 (9)*	2 (1)
Neonatal	Male sex	14 (58)	104 (49)
	Birth weight (g)	3195 (2950 – 3435)*	3480 (3194 – 3870)
	Small for gestational age (< p10)	2 (8)*	18 (9)
	Apgar score <7 at 5 minutes	1 (4)*	2 (1)
	Hemorrhage	4 (17)	2 (1)
	Of which severe hemorrhage	1 (4)	0
	Of which leading to perinatal death	1 (4)	0
	Perinatal death without bleeding	0	0

Data presented as n (%) or median (interquartile range); CI, confidence interval; HPA, human platelet antigen.

* one case of termination of pregnancy at 34 weeks' gestation excluded.

** risk ratio of immunized cases versus HPA-1a positive cases.

Clinical outcome

The clinical outcome of all three groups is presented in table 9.2. Median gestational age at delivery was similar between the three groups. A total of four pregnancies (17%) ended in premature delivery before 37 weeks' gestation in the immunized group and 42 (5%) in the HPA-1a positive control group (risk ratio, 3.9; 95% CI, 1.7 to 8.9). The proportion of premature deliveries before 34 weeks' gestation was 9% in the immunized cases and 1% in the HPA-1a positive controls (risk ratio, 8.1; 95% CI, 1.8 to 36.2). Median birth weight was lower in the immunized group (3195 gram) than in the non-immunized and HPA-1a positive control group (3480 gram and 3510 gram, respectively). There was no significant difference in the proportion of infants that were SGA (8% versus 9% and 11%, respectively; *p* 0.45). Four infants (17%) in the immunized group suffered from increased bleeding tendency and 10 infants in the HPA-1a positive control (1%) had any form of bleeding (risk ratio, 13.0; 95% CI, 4.4 to 38.5). One of the four infants with bleeding symptoms in the immunized group suffered a severe hemorrhage, an ICH. No severe hemorrhages were observed in the non-immunized group and the HPA-1a positive control group. Five cases (1%) of perinatal mortality were observed in the HPA-1a positive control group. One was a case of severe perinatal asphyxia, with no other abnormalities after MRI and pathologic examination. The other four were IUFDs of which one had fetal anemia due to a massive fetomaternal hemorrhage, one declined post-mortem examination and in two the cause was unknown after examination.

HPA-1a positive (n = 779)	*p*-value	Risk ratio (95% CI)**
39^{+6} (38^{+5} – 40^{+4})	0.407	-
42 (5)	0.017	3.9 (1.7 – 8.9)
8 (1)	0.038	8.1 (1.8 – 36.2)
396 (51)	0.750	1.1 (0.8 – 1.6)
3510 (3130 – 3845)	0.006	-
88 (11)	0.451	1.0 (0.9 – 1.2)
11 (1)	0.290	3.1 (0.4 – 22.7)
10 (1)	0.001	13.0 (4.4 – 38.5)
0	0.025	-
0	0.025	-
5 (1)	0.637	-

Immunized cases, non-immunized HPA-1a negative cases and HPA-1a positive cases had similar proportions of first pregnancies and first-born children (Table 9.3). Hypertensive disorders, either pregnancy-induced hypertension (PIH) or pre-eclampsia, were present in five (21%) immunized pregnancies and 53 (7%) pregnancies from HPA-1a positive controls (risk ratio, 3.1; 95% CI, 1.4 to 7.0). Obstetric history, if applicable, revealed a miscarriage in ten (63%) immunized cases and 196 (39%) HPA-1a positive controls (risk ratio, 1.6; 95% CI, 1.1 to 2.4).

Immunized cases

Of the 24 anti-HPA-1a immunized pregnancies, four resulted in clinically relevant FNAIT, one severe FNAIT and three cases with mild FNAIT (Table 9.4). The case of severe FNAIT was a severe bilateral intracranial hemorrhage detected at ultrasound that was performed due to reduced fetal movements at 29 weeks' gestation. In follow-up MRI assessment in the following weeks, extensive damage to the fetal brain tissue with large cysts was observed and after extensive counseling a late termination of pregnancy at 34 weeks' gestation was performed. The three cases of mild FNAIT consisted of petechiae in a male infant born after an emergency cesarean section due to fetal distress, a girl with a cephalic hematoma after an uncomplicated pregnancy and delivery and a case of hematomas in a female infant that was admitted to the neonatal intensive care unit (NICU) because of prematurity.

Table 9.3 – Obstetric characteristics

	HPA-1a negative (*n* = 238)		**HPA-1a positive**	***p*-value**	**Risk ratio**
	Immunized (*n* = 24)	Non-immunized (*n* = 214)	**(*n* = 779)**		**(95% CI)**
Primigravidae	8 (33)	69 (32)	271 (35)	0.764	0.9 (0.4 – 2.2)
Nulliparae	10 (42)	85 (40)	342 (44)	0.525	0.9 (0.4 – 2.1)
Hypertensive disorder this pregnancy	5 (21)	18 (9)	53 (7)	0.039	3.1 (1.4 – 7.0)
Miscarriage in obstetric history*	10 (63)	47 (32)	196 (39)	0.028	1.6 (1.1 – 2.4)
IUFD in obstetric history**	1 (7)	1 (1)	6 (1)	0.258	5.2 (0.7 – 40.2)

Data presented as n (%) or median (interquartile range); CI, confidence interval; HPA, human platelet antigen; IUFD, intrauterine fetal demise.

* risk ratio of immunized cases versus HPA-1a positive cases.

** primigravidae excluded; immunized *n* = 16, non-immunized *n* = 145, HPA-1a positive *n* = 508.

*** nulliparae excluded; immunized *n* = 14, non-immunized *n* = 128, HPA-1a positive *n* = 435.

Discussion

Implementing population-based screening in order to timely detect and prevent disease burden caused by HPA-1a mediated FNAIT is a long debated topic. Lacking knowledge on natural history and incidence of the disease and, more importantly, the absence of reliable diagnostic tools to select alloimmunized pregnancies at high risk for bleeding, complicate implementation. We present the preliminary results of a large, nationwide, non-interventional, prospective screening study.

The incidence of HPA-1a negativity and subsequent alloimmunization in our study can be best compared to the three largest prospective antenatal screening studies performed in Norway, Scotland and England (screening 100,448, 26,506 and 24,417 pregnant women, respectively).[15,27,28] In our cohort 2.4% of the pregnant women were HPA-1a negative, similar to the reported 2.1% – 2.5%. Although the material used for HPA-1a typing varied (platelet-rich plasma or whole blood in the previous studies and plasma in ours), all three studies performed serological HPA-1a typing (either ELISA or flow-cytometry), followed by supportive genotyping. Further, the studies describe an antenatal anti-HPA-1a detection of 7.2% – 9.6%, comparable to the 9.2% alloimmunizations detected in our HPA-1a negative samples. In contrast to our single measurement at 27 weeks' gestation, previously mentioned studied screened for antibodies 2 to 5 times during pregnancy, between 8 and 36 weeks' gestation. For anti-HPA-1a detection none of the studies used PAKLx bead-based assay, like was performed in our cohort. Both Kjeldsen-Kragh *et al.*[15] and Williamson *et al.*[27] used the monoclonal antibody immobilization of platelet antigens (MAIPA) assay and Turner *et al.*[28] used a somewhat comparable ELISA technique. A study that compared MAIPA to PAKLx in 100 cases with suspected FNAIT, detected 26 anti-HPA-1a immunizations were by both MAIPA and

PAKLx and one case with anti-HPA-1a that was only detected by PAKLx.[29] Despite we performed antibody screening only once during pregnancy, the use of a probably more sensitive technique might explain the relatively high immunization rate of 9.2%.

Within the 24 immunizations that we detected, three cases with only minor bleeding and one case with severe bleeding were identified. The comparison to reported incidences in literature is troubled by the absence of completely observational studies. The Norwegian screening study reported two cases of severe bleeding in 144 antenatally detected HPA-1a immunizations, the occurrence of minor bleeding complications was not described.[15] The Scottish study detected three minor and no severe bleeding complications in 25 immunizations and the English study found seven minor hemorrhages and one case of severe bleeding within 37 antenatally identified alloimmunizations.[27,28]

Obviously, the small numbers from our study make it difficult to adequately extrapolate. With a birth rate of approximately 170,000, this would result into the identification of 4,080 (2.4%) HPA-1a negative pregnant women and 375 anti-HPA-1a immunizations (9.2%) each year in the Netherlands.[30] Further extrapolation would mean that these immunizations lead to 63 cases of clinically relevant FNAIT (375/24*4), of which 47 with only minor hemorrhage and 16 with severe bleeding complications.

It should be noted that, because antibody screening was performed with the sample collected at 27 weeks' gestation, the detected alloimmunizations in our study might still be an underestimation. Besides potentially missing immunizations that occur in third trimester and after delivery, immunizations that resulted in severe bleeding and pregnancy termination or IUFD before 27 weeks' gestation will also not be included. Further underestimation might occur due to the absence of routine neonatal brain ultrasound. Intracranial hemorrhages that will not lead to clinical problems during pregnancy or in the first week of life can be missed. To estimate the extent of this problem, a prospective study with routine ultrasound examination and structured long-term follow-up will be necessary. The Norwegian screening study did perform routine cranial ultrasound of all newborn's brains.[15] They detected two ICHs. One was already detected on ultrasound at 34 weeks' gestation and both had petechiae at birth. The other was a small grade I bleed, also with petechiae but no neurological symptoms. Within our study design, the first and large ICH would have been detected, but we would not have identified the latter. There were two other prospective screening studies that detected an ICHs. The first, performed by Williamson *et al.*[27], performed routine cerebral ultrasound in all HPA-1a alloimmunizations, and detected a severe and symptomatic ICH, leading to the formation of porencephalic cyst and hydrocephalus. Blanchette *et al.*[11] detected the second, a symptomatic ICH amongst three anti-HPA-1a immunizations.

Table 9.4 – HPA-1a immunizations

Classification	G/P	Obstetric history	Pregnancy	Delivery	Sex
Severe FNAIT	G3P1	1 miscarriage	Bilateral ICH on US at 29 weeks	Late pregnancy termination at 34+0	Male
Mild FNAIT	G3P2		Gestational diabetes	Primary CS at 33+5	Female
	G1P0	NA		Spontaneous delivery at 40+6	Female
	G3P1	1 miscarriage	PIH	Secondary CS at 39+6 due to fetal distress	Male
No clinical bleeding problems	G2P0	1 miscarriage	PIH	Induction of labor at 34+1	Female
	G2P0	1 miscarriage		Spontaneous delivery at 39+6	Male
	G4P2	1 miscarriage		Spontaneous delivery at 39+3	Female
	G1P0	NA		Spontaneous delivery at 40+1	Female
	G1P0	NA	PPROM at 31 weeks	Forcipal extraction at 31+4, after PPROM	Male
	G3P2			Spontaneous delivery at 40+2	Female
	G1P0	NA		Spontaneous delivery at 38+5	Female
	G1P0	NA	Oligohydramnios	Induction of labor due to oligohyramnios at 41+2	Male
	G7P4	2 miscarriages, 1mola pregnancy		Induction of labor at 37+0	Male
	G1P0	NA		Spontaneous delivery at 39+1	Male
	G3P2		Anemia	Primary CS at 37+3	Male
	G1P0	NA		Spontaneous delivery at 39+6	Male
	G4P2	1 miscarriage		Spontaneous delivery at 41+0	Male
	G2P1			Spontaneous delivery at 40+5	Male
	G1P0	NA	PIH	Vacuum extraction at 40+5	Female
	G2P1		PIH	Induction and spontaneous delivery at 37+6	Female
	G2P1			Spontaneous delivery at 40+2	Male
	G3P1			Spontaneous delivery at 36+3	Male

	Birth weight (g)	Signs of bleeding	Other	Other antibody	MFI anti-HPA-1a
	2482	Massive ICH, hematomas (PLT 12)	Severe bilateral ICH, severe damage to brain tissue, large cysts	None	13485
	2016	Hematomas (PLT 102)	Admission to NICU for prematurity, respiratory distress, hyperglycemia	None	11068
	3140	Cephalic hematoma		None	6420
	2960	Petechiae, conjunctival bleeding (PLT 80)	SGA (<p10)	None	9117
	1715	None	SGA (<p2.3)	HLA, HPA-5b	1385
	3655	None		None	3285
	2970	None		HLA	2933
	3410	None		None	163
	1750	None	Admission to NICU because of prematurity	None	598
	3245	None		None	1006
	2790	None	Bilateral clubfeet	None	595
	3405	None	Hypospadias	None	390
	2950	None		HLA	18526
	3045	None	Admission to NICU due to hyperbilirubinemia	None	1899
	2975	None		None	1163
	3320	None		None	5649
	4410	None		None	443
	4360	None		None	5694
	3215	None	Pediatric consultation due to vacuum	None	365
	3195	None	Apgar score 5/6	None	341
	3715	None		None	145
	2700	None	Admission to NICU and hypothermic treatment due to asphyxia	None	487

Table 9.4 – Continued

Classification	G/P	Obstetric history	Pregnancy	Delivery	Sex
	G7P1	4 miscarriages, 1 abortus provocatus		Spontaneous delivery at 39+2	Female
	G4P2	1 miscarriage		Spontaneous delivery at 40+6	Male

AS, Apgar score; CS, cesarean section; HLA, human leukocyte antigen; G/P, gravidity/parity; HPA, human platelet antigen; ICH, intracranial hemorrhage; IUFD, intrauterine fetal demise; NA, not applicable; NICU, neonatal intensive care unit; PIH, pregnancy-induced hypertension; PLT, platelet count; PPROM, premature prelabor rupture of membranes; SGA, small for gestational age; US, ultrasound.

The lack of routinely collected neonatal platelet counts in our study might be considered a limitation as well. Besides hampering comparison with the previously performed prospective studies, this complicates estimation of the consequences of our detected anti-HPA-1a alloimmunizations. Previous studies suggest that there will be more cases of severe thrombocytopenia than cases with minor hemorrhage.[12,14,27,28] However, we do feel that the complete neonatal follow-up data, including the occurrence of minor bleeding, compensates this lack of information. After all, within health care and screening the goal is to prevent clinical disease instead of laboratory values.

At the moment of performing and writing this interim-analysis, the collection of clinical data is still ongoing. The rate of cases with currently incomplete clinical data is larger in the control group compared to the immunized group (26% versus 10%, respectively). After completion of the collection we expect these proportions to be similar. Also, whereas data is collected blinded for outcome of serological HPA-1a typing or antibody screening and data is collected per health care institution (either hospital or midwifery practice), we do not expect this to introduce a possible selection bias. Further, because we used left-over material from the Dutch population-based screening program for RBC immunization, we only included RhD negative and Rhc negative pregnant women. Additionally, we missed pregnant women with anti-RhD or anti-Rhc alloantibodies in the first trimester of their pregnancy, because no additional RBC antibody screening at 27 weeks' gestation will be performed in these women. However, RhD negative or Rhc negative blood type has never been associated with HPA-type, HPA-alloimmunization or FNAIT disease severity. Neither has an association RBC alloimmunization and HPA-1a immunization or disease severity been described before. Therefore, we do not expect the use of this specific study population to introduce a selection bias.

	Birth weight (g)	Signs of bleeding	Other	Other antibody	MFI anti-HPA-1a
	3435	None		None	18726
	3460	None	Admission to NICU because of hyperbilirubinemia	None	18676

In conclusion, our prospective and more importantly non-interventional study reports incidence numbers of HPA-1a negative and subsequent HPA-1a alloimmunization in the Netherlands, that are comparable to the literature. Further, estimates on incidence of clinical relevant FNAIT are in line with expected numbers as well and do not seem to possibly hamper implementation of a population-based screening program. For exact extrapolation and firm conclusions, completion of the HIP-study needs to be awaited.

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