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

Vos, T.W. de

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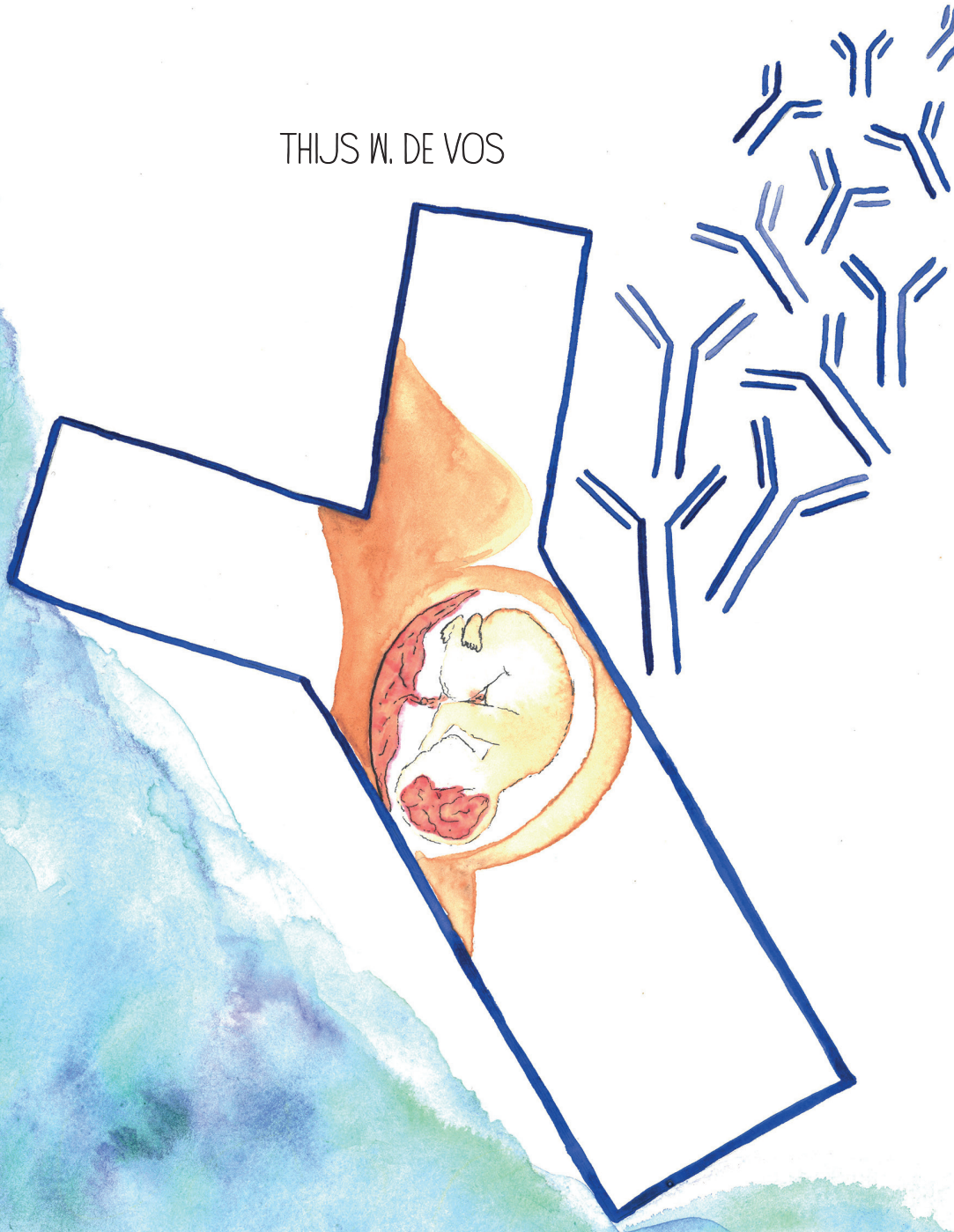
THIJS W. DE VOS



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The research described in this thesis was performed at the Willem-Alexander Children's Hospital, the Department of Obstetrics at the Leiden University Medical Center (Leiden), the Department of Experimental Immunohematology, the Department of Translational Immunohematology at Sanquin Research (Amsterdam) and Sanquin Diagnostic Services (Amsterdam).

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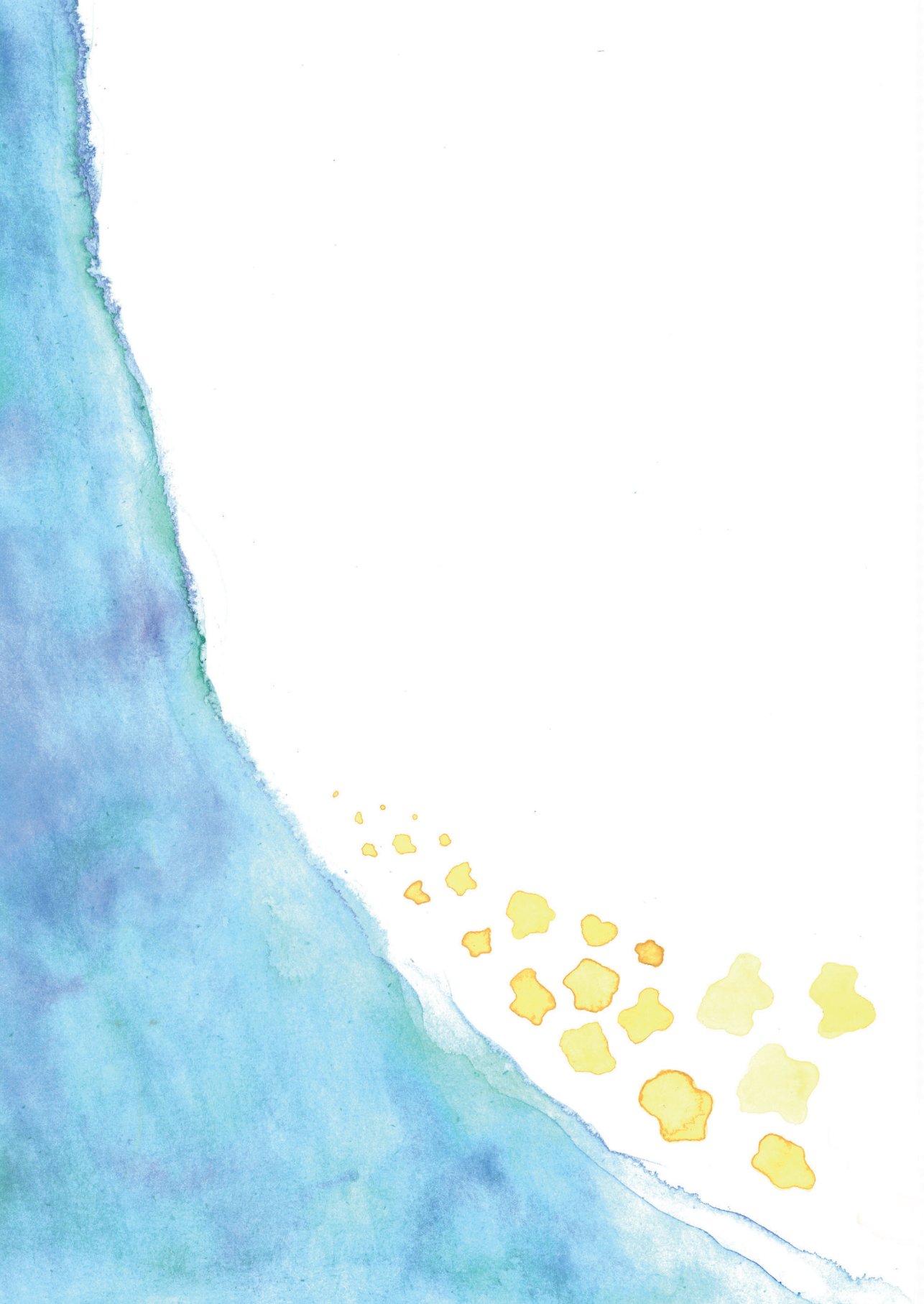
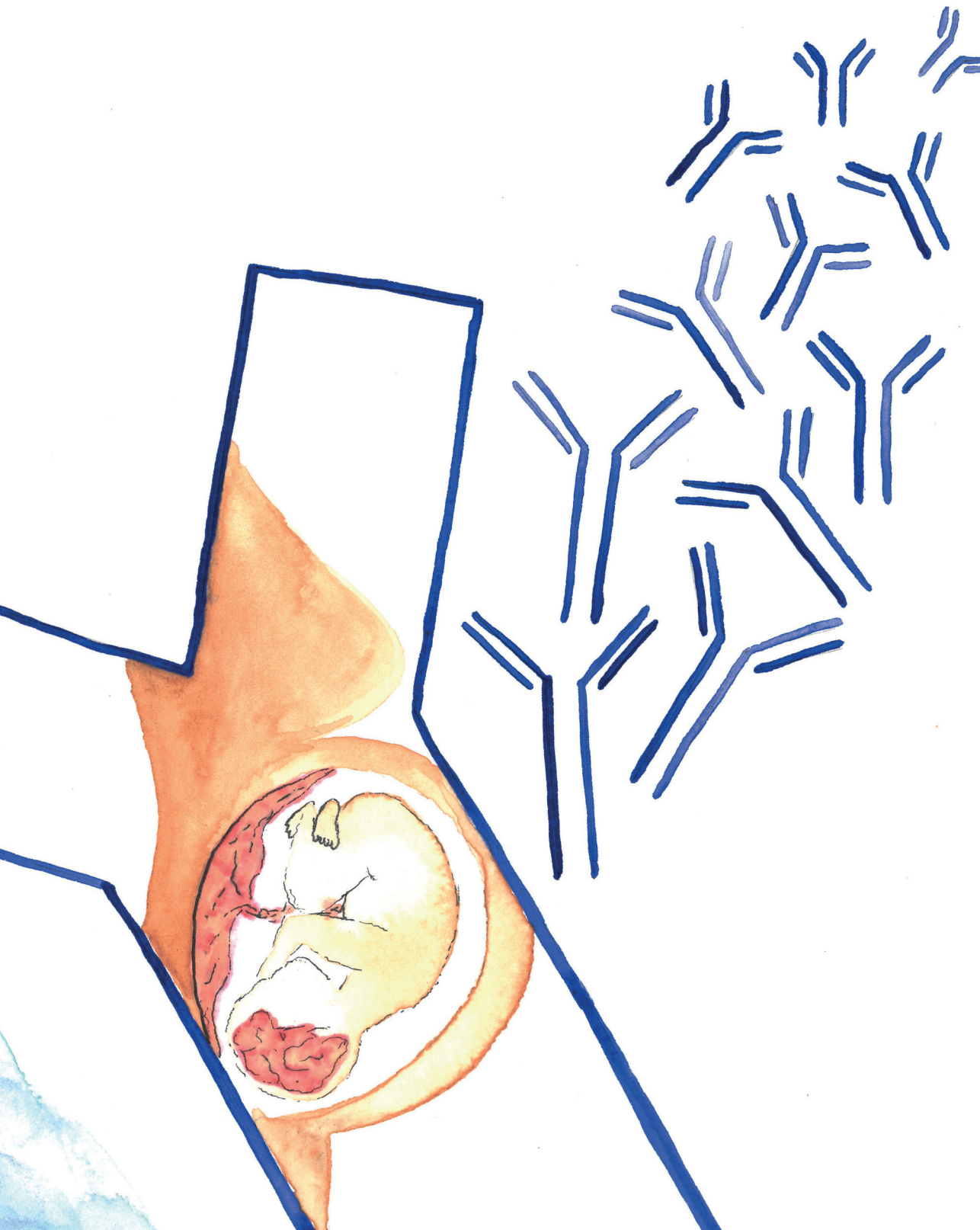


PART ONE

Overview



General introduction and scope of the thesis

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PATHOPHYSIOLOGY OF FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but potentially severe life-threatening disease that can lead to intracranial hemorrhage (ICH) and other severe bleeding symptoms[1] in babies during fetal development and the neonatal period. ICH is associated with perinatal morbidity and mortality and can lead to long-term neurodevelopmental impairment.[2]

In uncomplicated pregnancies, women tolerate their semi-allogenic fetus while maintaining their own immune system to protect themselves from pathogens. In pregnancies affected by FNAIT this miraculous interplay is disrupted. Essential for the development of FNAIT is that there is an incompatibility between the fetal and maternal human platelet antigens (HPA).[3] Exposure of these fetal foreign HPA to the maternal immune system can lead to the formation of HPA directed antibodies of the IgG class. During pregnancy maternal IgG antibodies are actively transported across the placenta to the fetus, therefore HPA antibodies end up in the fetal circulation. In the fetus, these antibodies bind to fetal cells expressing the involved HPA antigen. Binding to platelets and endothelial cells results in platelet phagocytosis,[4] impaired formation of platelets,[5] and for some type of HPA antibodies possibly with interference of the endothelial cell function and even impaired angiogensis.[6, 7] In the placenta, certain subtypes of HPA antibodies may bind to the syncytiotrophoblast cells and involvement of placenta pathology in FNAIT has been suggested.[8-11]

Although the pathophysiology of ICH is not fully understood. It is proposed to result from the combination of thrombocytopenia and antibody interaction with endothelial cells resulting in this severe outcome of the increased bleeding tendency in the fetus and neonate. The clinical outcome of infants from HPA immunized pregnancies can differ from being asymptomatic with normal platelet counts to thrombocytopenia with or without skin bleeding or being severely affected by ICH or organ bleeding (Figure 1).

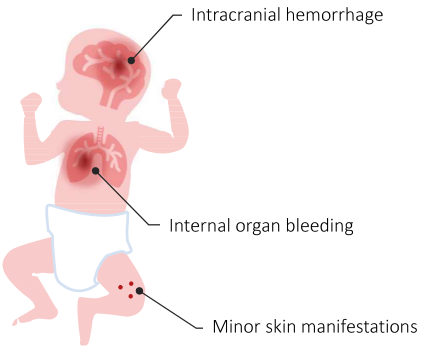

FIGURE 1. Neonatal outcome in FNAIT

HISTORY OF HPA-1a-MEDIATED FNAIT

In 1953 Harrington *et al.*[12] reported on two thrombocytopenic infants from healthy mothers with normal platelet counts. In both cases there was no evidence of sepsis, perinatal asphyxia, metabolic disease or congenital abnormalities. HPAs had not been discovered at that time and serological examinations to prove the presence of maternal alloantibodies were not available back then. However, in their[12] study and other reports that followed,[13, 14] it was suggested that maternal antibodies might play a role in the development of neonatal thrombocytopenia in otherwise healthy born infants. In 1962 Shulman *et al*.[15] implicated that neonatal thrombocytopenia could be explained by maternal alloantibodies directed against a paternally inherited platelet antigen named PL^{A1}. Already in 1959, in the Netherlands, at the Central Laboratory of the Blood Transfusion Service in Amsterdam, this platelet antigen was described as Zw^a by Van Loghem *et al*.[16]. These antigens, PL^{A1} or Zw^a are nowadays better known as HPA-1a.

Currently 41 different HPA have been described.[17] HPAs are small protein-based changes or variations at the glycoproteins expressed on the cell membrane of platelets (Figure 2). These changes, result from single nucleotide polymorphisms (SNPs). HPAs are located on different glycoprotein complexes: GPIb-V-IX (von Willebrand receptor), GPIIb/IIIa (αIIb/β3 integrin, fibrinogen receptor) GPIa/IIa (collagen receptor) and CD109. In addition, women of African or Asian ancestry can have inherited mutations that prevent glycoprotein IV (CD36, Nak) from being expressed on platelets which can result in the formation of so called isoantibodies.[18] Platelets express high levels of the fibrinogen receptor αIIbβ3 carrying HPA-1a which is the most frequently involved antigen in FNAIT in the white population.[19] As with other blood groups, allele frequencies for HPAs involved in FNAIT differ between ethnic populations.

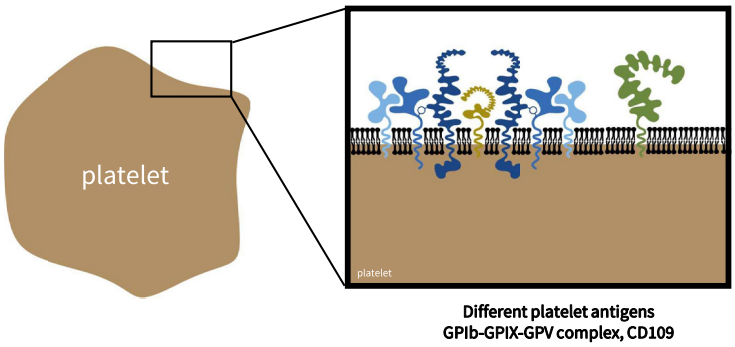

FIGURE 2. Human platelet antigens

Initially, only assays were available with indirect evidence for the presence of platelet antibodies (e.g. platelet aggregation test, complement fixation techniques) in FNAIT suspected cases.[20] Later in the 1970s, more sensitive and specific methods became available with the advantage of fluorescent labelled tools to detect platelet antibodies (e.g. platelet immune fluorescence tests).[20] In the years that followed, many efforts were done to improve assays particularly aimed at preventing non-specific reactions. For years, the monoclonal antibody immobilization of platelet antigens (MAIPA) as published in 1987 by Kiefel *et al*.[21], has been further developed and is now widely used and established as the golden standard for HPA antibody detection. Disadvantages of the MAIPA are that the assay is technically challenging with the necessity of availability of HPA typed platelets, time consuming, and uses relatively large amounts of patient material. More recent assays, such as an assay with platelet GP-coated beads and Luminex technology for antibody detection (e.g. PAK Lx assay from Immucor) can be much more easily implemented and has the advantage to use only low amounts of patient material.[22]

Because the risk of fetal ICH during pregnancy is substantial, if not greatest,[23] during pregnancy, fetal therapy is essential in the treatment of FNAIT. In 1984, Daffos *et al*.[24] were the first to perform an intrauterine platelet transfusion (IUPT) in a pregnancy from a woman of which her previous child was diagnosed with ICH due to FNAIT. IUPTs were successful in raising platelet counts and an ICH did not occur in this child, suggesting that this treatment could prevent the occurrence of severe bleeding in FNAIT. However, in comparison to sequential intrauterine transfusions for fetal anemia in hemolytic disease of the fetus and neonate (HDFN), cordocentesis in thrombocytopenic fetuses is associated with a high risk of fetal bleeding and the short life span of platelets requires weekly transfusions. Therefore, in 1988 Bussel *et al*.[25], studied the effect of administration of intravenous immune globulins (IVIg) to HPA immunized pregnant women. Although IVIg was used regularly after this initial study, the efficacy of this treatment has never been proven in a randomized controlled trial. However, a systematic review[26] concluded that IVIg was effective in preventing ICH in the vast majority (99%) of the cases.

AIM AND OUTLINE OF THE THESIS

FNAIT is currently diagnosed in cases suspected for FNAIT, because of bleeding symptoms or if thrombocytopenia is detected as a finding by chance. Therefore, the larger part of the FNAIT cases is only diagnosed postnatally. In these children, treatment mainly consists of preventing further complications with postnatal platelet transfusions. In subsequent pregnancies, antenatal treatment regimens are used to prevent the occurrence of severe ICH.[27] The successful prevention programs of HDFN, the red cell counterpart of FNAIT, made people postulate that a severe outcome of FNAIT may also be prevented by timely

identification of pregnancies at risk. This would be possible if existing screening programs during pregnancy were supplemented with a screening for platelet antibodies.

To guide the decision on the introduction of a population-based screening program, ten principles (or criteria) of screening on early disease detection formulated by Wilson and Jungner (W&J) were published by the World Health Organization in 1968 (Figure 3). Although these criteria were adapted and supplemented,[28] it was stated that the value of the principles as postulated in 1968 remain preserved.[29] Nationwide population-based screening on platelet antibodies during pregnancy has not been implemented thus far. Missing knowledge on principle 1 important health problem, 2 accepted treatment, 5 suitable test, 7 natural history, 8 whom to treat and 9 costs of case finding hamper the addition of anti-HPA screening to the existing screening programs. The research in this thesis aims to gain new knowledge about FNAIT so that HPA screening during pregnancy can be considered.

Principles of screening on early disease detection by Wilson and Jungner (1968)

1. The condition should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic phase.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including a diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuous process and not a “once and for all” project.

FIGURE 3. Wilson and Jungner Criteria, World Health Organization, 1968

In the first part we provide an overview of the current knowledge on FNAIT. The narrative review in chapter 1 describes the knowledge available on the epidemiology and management (W&J 1 and 2) of FNAIT.

The second part of this thesis focusses on the natural history of HPA-1a mediated FNAIT. Despite several antenatal screening studies were performed by others,[30-39] knowledge on the natural history of FNAIT (W&J 7) is still uncertain. In the majority of screening studies to date the result of HPA-1a testing and antibody screening was reported to pregnant women and caregivers allowing perinatal medicine, which might have reduced the risk of bleeding in these studies. Chapter 2 describes the study-protocol of the HIP study (HPA-screening in pregnancy study), a large observational prospective study in which caregivers were not

informed about the results of HPA screening tests. In chapter 3 the results of this screening study are reported. Main outcomes in this study are the incidence of severe bleeding, pregnancy outcome and neonatal outcome in HPA-1a immunized pregnancies. (W&J 7) In addition, we explore whether we can select pregnancies at risk for a severe neonatal outcome with characteristics described in the literature (antibody levels[40] and maternal HLA DRB3*01:01 carrier status[41]). (W&J 5 and 8) Evidence emerges that in addition to bleeding symptoms, HPA-1a immunization may be associated with placental damage.[9-11, 42] In chapter 4 we explore this broadening of the clinical spectrum of FNAIT and assess the signs of antibody-mediated placental damage in FNAIT.

The second most frequent involved antibody in FNAIT is directed against HPA-5b.[19] Given the high prevalence of these antibodies in pregnant women (1.8%),[43] thrombocytopenia and/or bleeding may be incidental in HPA-5b immunized pregnancies. In the third part of the thesis (chapter 5) the clinical outcome of anti-HPA-5b cases is described, and the association between HPA-5b and clinical FNAIT is assessed. (W&J 7 and 8)

Evidence on supporting the postnatal treatment in FNAIT is limited. Clinical guidelines on the postnatal management of FNAIT are based on expert opinion and small observational studies.[44] Hence, the fourth part of this thesis (chapter 6) focusses on the postnatal treatment and outcome of FNAIT cases. (W&J 2) We performed an international multicentre study to describe contemporary postnatal treatment and outcome of liveborn FNAIT cases.

To estimate the benefits of a possible screening program, knowledge about the long-term outcome of FNAIT cases is indispensable. The fifth part of this thesis focusses on the long-term outcome of children that were affected by FNAIT. It is known that FNAIT cases with ICH have a high risk of severe neurodevelopmental problems on the long-term.[2, 23] However, knowledge on the long-term outcome of cases newly diagnosed with FNAIT without ICH is limited. In chapter 7 we assess the neurodevelopment at school age, in a cohort of children that were newly diagnosed with FNAIT. (W&J 1) In chapter 8, we describe the long-term outcome of a cohort of children whose mothers were treated with IVIg during pregnancy because a previous child in their family was affected by FNAIT. (W&J 2)

The knowledge of the earlier parts of this dissertation eventually comes together in part six. Chapter 9 we compare the costs of a situation with platelet antibody screening to the situation without antibody screening (W&J 9). Finally, in part seven of this thesis (chapter 10) we discuss the results of the chapters from this thesis and the other available literature guided by the Wilson and Jungner principles.

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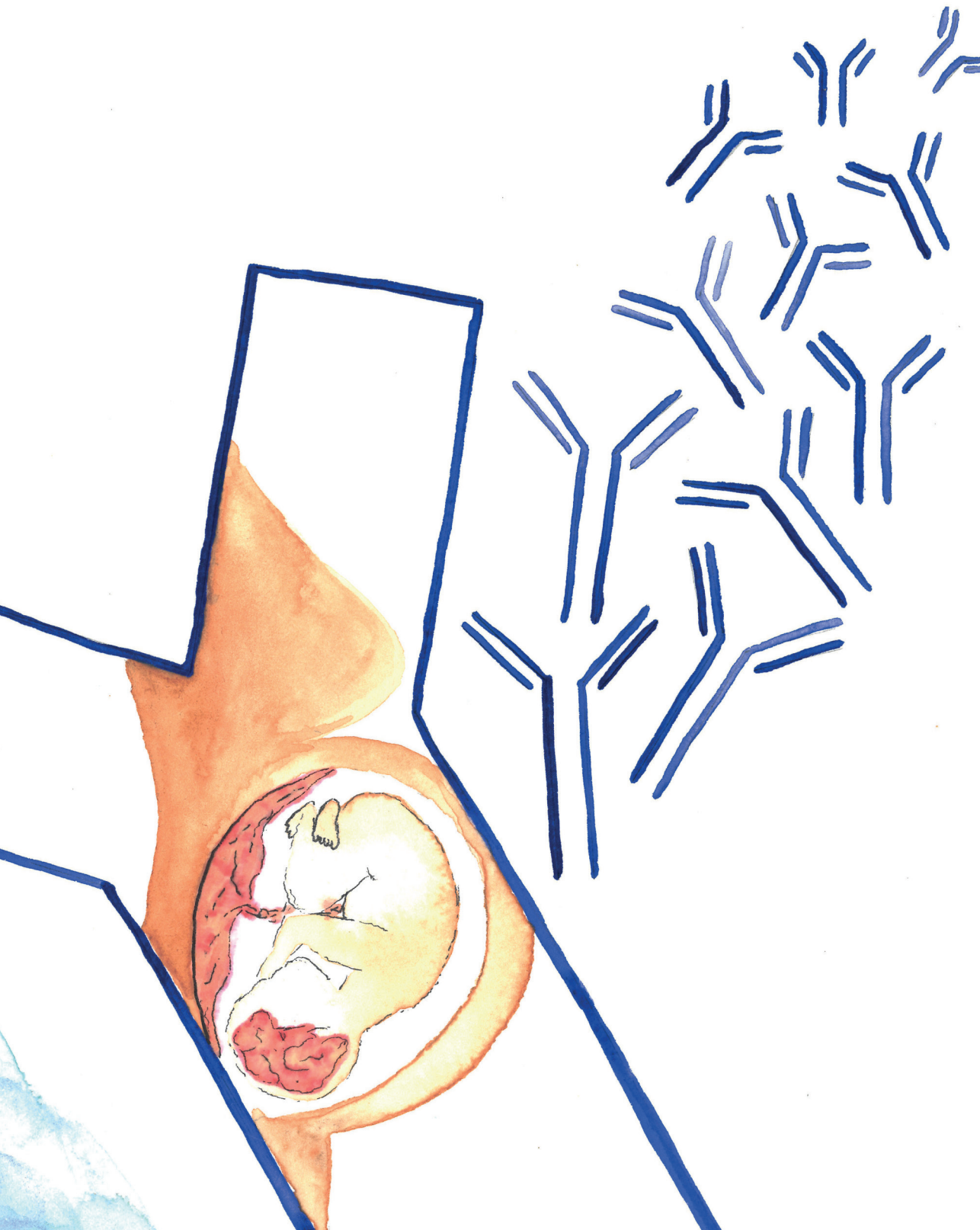

CHAPTER 1

Epidemiology and management of fetal and neonatal alloimmune thrombocytopenia

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ABSTRACT

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a disease in pregnancy characterized by maternal alloantibodies directed against the human platelet antigen (HPA). These antibodies can cause intracranial hemorrhage (ICH) or other major bleeding resulting in lifelong handicaps or death. Optimal fetal care can be provided by timely identification of pregnancies at risk. However, this can only be done by routinely antenatal screening. Whether nationwide screening is cost-effective is still being debated. HPA-1a alloantibodies are estimated to be found in 1 in 400 pregnancies resulting in severe burden and fetal ICH in 1 in 10.000 pregnancies. Antenatal treatment is focused on the prevention of fetal ICH and consists of weekly maternal IVIg administration. In high-risk FNAIT treatment should be initiated at 12-18 weeks gestational age using high dosage and in standard-risk FNAIT at 20-28 weeks gestational age using a lower dosage. Postnatal prophylactic platelet transfusions are often given in case of severe thrombocytopenia to prevent bleedings. The optimal threshold and product for postnatal transfusion is not known and international consensus is lacking. In this review practical guidelines for antenatal and postnatal management are offered to clinicians that face the challenge of reducing the risk of bleeding in fetuses and infants affected by FNAIT.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but severe disease in pregnancy. FNAIT develops in pregnancy due to maternal alloimmunization and results in thrombocytopenia and a risk of bleeding in the fetus and neonate. Clinical presentation may vary from an asymptomatic thrombocytopenia as a finding by chance to skin bleeding or severe organ bleeding.[1] The most severe complication being intracranial hemorrhage (ICH), that can lead to perinatal death or lifelong neurologic sequelae.[2] FNAIT is the most frequent cause of thrombocytopenia in otherwise healthy born neonates with an incidence of approximately 1 in 1,500 pregnancies.[3] As FNAIT is a rare condition and often not recognized by clinicians, this entails difficulties to determine the burden of this disease on population level.[4] In addition, strong evidence for the optimal antenatal and postnatal treatment is lacking due to the limited number and size of randomized trials or observational studies.[5, 6] In this review we aim to summarize the current knowledge about the epidemiology of FNAIT and provide an overview on antenatal and postnatal management strategies based on the most recent literature.

PATHOGENESIS

In FNAIT, maternal exposure to incompatible, paternally derived, fetal HPA can lead to immunization and formation of alloantibodies. During pregnancy, active transport of IgG takes place form the mother to the unborn child over the placenta by binding to the neonatal Fc-receptor (FcRn).[7, 8] Hence, IgG class HPA-alloantibodies bind to fetal platelets, leading to phagocytosis of the fetal platelets causing thrombocytopenia and a risk of bleeding in these infants.[9, 10] Thrombocytopenia in FNAIT does not only result from the destruction of platelets, but may also worsen due to the suppression of platelet production by megakaryocytes. Suppression of megakaryocytopoesis has recently been shown in *in vitro* experiments in the presence of HPA-1a alloantibodies.[11]

HUMAN PLATELET ANTIGENS

Maternal alloantibody formation is triggered by exposure to incompatible HPA. Nowadays 41 HPAs are known and described. HPAs are epitopes present on five cell surface platelet glycoproteins (GP).[12, 13] These glycoproteins play an important role in platelet function for instance adhesion, aggregation and hemostatic plug formation.[14] Approximately 80% of the FNAIT cases in the white population are caused by alloantibodies directed the HPA-1a epitope. Therefore, we will focus on anti-HPA-1a-mediated FNAIT in this review unless stated otherwise. The HPA-1a is carried by the β3 integrin, which is part of the fibrinogen receptor.[15] The β3 integrin is also expressed in complex with the αV integrin as the vitronectin receptor, by endothelial cells, the placenta syncytiotrophoblast and by other tissue during

development of the human embryo.[16-18] This finding has led to interesting ideas about early immunization and functional effects of alloantibodies in FNAIT.

IMMUNIZATION

Immunization in FNAIT can occur early in pregnancy and lead to clinically relevant disease already in first affected pregnancies. The relevance of these immunizations was questioned by Skogen *et al.*[19] mentioning that the rate of immunization in primigravidae was 8-24%. However, in two case series describing intracranial hemorrhages (ICH) due to FNAIT, ICH occurred in first pregnancies in 24-27% of the cases, underlining that immunization and clinically relevant disease can occur already in early stages of first pregnancies.[2, 20] Previous research highlighted the contribution of several risk factors that play a role in HPA immunization during pregnancy. For some HPA antigens, such as HPA-1a, the exposure of the HPA-1a epitope on the placenta may play an important a role.[21] Furthermore, if HPA-1a negative women carry the MHC class II allele HLA-DRB3*01:01, this may promote the anti-HPA-1a immune response, due to optimal HPA-1a peptide presentation.[22, 23]

FUNCTIONAL EFFECTS OF ANTIBODIES

Approximately 10-25% of children with a severe thrombocytopenia caused by FNAIT develop an ICH.[4] However, the relationship between ICH and thrombocytopenia is not linear and the majority of FNAIT children do not develop an ICH. It may very well be that cranial bleedings are not solely caused by thrombocytopenia. The observation in a murine model with thrombocytopenic fetuses that were born without ICH supports this idea.[24] The expression of the β3 chain on both platelets and endothelial cells lead to the idea that bleedings in FNAIT can occur by the binding of antibodies to endothelial cells. Animal studies and *ex vivo* studies with human cord blood derived endothelial cells showed that HPA-antibodies directed against the β3-chain reduced endothelial cell proliferation, angiogenesis and endothelial integrity.[25, 26] Santoso *et al.*[27] recently found a larger proportion of vitronectin-receptor specific antibodies in maternal sera of FNAIT cases with ICH, compared to the sera from pregnancies without the occurrence of an ICH in the infant. The occurrence of ICH in fetuses and neonates with FNAIT may result from the combination of severe thrombocytopenia and endothelial damage, leading respectively to insufficiency of the hemostatic system and vessel wall injury.

TABLE 1. Prospective cohort studies assessing the incidence of FNAIT

Author, year	HPA-1a negative	Antenatal anti-HPA-1a	PLT < 50 × 10^9/L	Mild bleeding	Severe bleeding	Intervention
Mueller-Eckhardt, 1985 [28]	26/1,211 (2.1)	2/26 (7.7)	0	0	0	None
Reznikoff-Etievant, 1988 [29]	27/860 (3.1)	0/27 (0)	0	0	0	None
Blanchette 1990 [30]	81/5,000 (1.6)	3/50 (6.0)	1	0	1	NTCS, PP
Doughty, 1995 [31]	74/3,473 (3.2)	1/71 (1.4)	0*	0*	0	FBS/IUPT, IVIg, PP
Durand-Zaleski, 1996 [32]	52/2,066 (2.5)	4/45 (8.9)	1	0	0	FBS, IVIg, CTS
Williamson, 1998 [33]	618/24,417 (2.5)	37/385 (9.6)**	8	7	1	PP
Davoren, 2003 [31]	54/4,090 (1.3)	2/34 (5.9)	1	1	0	FBS, IUPT, PP
Maslanka, 2003 [34]	144/8,013 (1.8)	12/122 (9.8)	3	1	0	IUPT, IVIg
Turner, 2005 [35]	546/26,506 (2.1)	25/318 (7.9)	5	3	0	PP
Kjeldsen-Kragh, 2007 [36]	2,111/100,448 (2.1)	144/1,990 (7.2)	48	NR	2	NTSC, PP
Debska, 2018 [37]	373/15,204 (2.5)	22/373 (5.9)	3	NR	NR	IUPT, IVIg

*One HPA-1a negative women delivered two severely affected twin children, anti-HPA-1a antibodies detected after birth, not detected by prenatal screening.

** Two pregnancies ended in loss of the baby, one at 15 weeks, another as neonatal death from immaturity after CS at 25 weeks for severe pre-eclampsia.

Numbers are n/N (%)

Abbreviations: CST, antenatal corticosteroids; FBS, fetal blood sampling; FNAIT, fetal and neonatal alloimmune thrombocytopenia; HPA, human platelet antigen; IUPT, intrauterine platelet transfusion; IVIg, antenatal intravenous immunoglobulins; NR, not reported; NT, not tested; NTCS, near-term cesarean section; PLT, platelet count; PP, postnatal platelets available for transfusion. Severe FNAIT is defined as neonatal platelet count at birth <50 x 10^9/L. Mild bleeding is defined as only skin or mucosal bleeding. Severe bleeding is defined as internal organ hemorrhage or ICH.

EPIDEMIOLOGY

Knowledge about the epidemiology of FNAIT is obtained from retrospective data of cases sent to reference laboratories and prospective screening studies. Studies with a retrospective study design are often prone for bias. Not the least because FNAIT cases are often missed and underdiagnosed in the absence of a nationwide screening programme.[38, 39] FNAIT as a cause of intrauterine fetal demise (IUFD) is often not considered and the burden of FNAIT might be even larger when miscarriages and IUFD are taken into account. Large prospective screening studies that address the incidence of FNAIT have been performed in several countries (Table 1). In general, studies with a prospective study design are less prone for bias. However, interventions in these studies might influence the outcome of the disease, which makes it difficult to assess the natural course of the disease. In addition, it should be noted that two types of screening studies have been performed; postnatal screening studies that screen for alloantibodies in thrombocytopenic neonates[40-45] and antenatal screening studies that assess neonatal outcome after screening for antibodies, mainly performed in HPA-1a-negative women.[28-37, 46]

INCIDENCE OF HPA-ALLOANTIBODIES

Incidence of HPA-alloantibodies varies due to genetic variation between populations.[13] In 2004 Davoren *et al.*[47], described the HPA specificity of 1162 different FNAIT cases in a white population.

Based on this study, around 80% of the FNAIT cases are caused by HPA-1a alloantibodies. HPA-5b alloantibodies are responsible for ~10% of the FNAIT cases. When cases with HPA-2, -3 and -15 alloantibodies are added to this list approximately 95% of the FNAIT cases are covered.[47] In the Asian population most FNAIT cases are caused by antibodies directed against HPA-5b, followed by anti-HPA-4b; the HPA-4 antigens are present on the β3-integrin like HPA-1.[48] Since most studies are based on patient series that were collected retrospectively it is difficult to estimate natural occurrence and pathogenicity of these alloantibodies. Based on the available studies, antibodies against low frequent antigens are rare, and their contribution to the morbidity of FNAIT in the white population is negligible.[49]

Kamphuis *et al.*[3] performed a systematic review on the incidence of FNAIT in 2010. In total 176,084 antenatal screened pregnancies were included. Based on these studies the authors estimated that 2.1% of the pregnant women are HPA-1a negative in the white population and therefore at risk for FNAIT. The risk of antibody formation in HPA-1a negative women during reproductive age was calculated to be 9.7% (294 of the 3,028 cases). In 2018 Dębska *et al.*[37] found that the risk of antibody formation was 8.6% in the study population of Polish screening program. In a recent Norwegian study, the risk of postpartum immunization for an HLA-DRB3*01:01 positive women delivering an HPA-1a positive child was calculated to 12.7%.[50]

INCIDENCE OF SEVERE THROMBOCYTOPENIA

Clinical symptoms in FNAIT vary from asymptomatic thrombocytopenia to severe (intracranial) hemorrhages. In literature, FNAIT is often defined as neonates with alloantibodies and a platelet count < 50×10^9/L with or without ICH. Differences in definitions on severe FNAIT makes it difficult to compare different studies and assess the natural history of FNAIT. Kamphuis *et al.*[4] assessed the incidence of severe FNAIT in a review in 2014 and compared antenatal and postnatal screening studies. The incidence of severe FNAIT, defined as platelet count < 50×10^9/L, was estimated to be 0.04% in both antenatal and postnatal screening studies.[4]

INCIDENCE OF INTRACRANIAL HEMORRHAGES (ICH)

The development of ICH is not directly associated with the severity of thrombocytopenia, given that only a small proportion of severely thrombocytopenic infants suffer from bleeding complications.[4] In addition to this, severe bleedings have been described in cases with moderate thrombocytopenia.[51] Shortage of platelets is therefore unlikely to be the only factor that causes ICH. Over the last years more evidence emerged showing that the maternal alloantibodies can also cause damage to the endothelium that might result in ICH.[26, 27] It is not known at which moment in pregnancy the developing brain is most vulnerable for damage induced by these kinds of alloantibodies. It may also be that these types of alloantibodies do not only lead to ICH but also to other type of cerebral damage or small bleedings. These lesions

can remain subclinical directly after birth but lead to developmental delay on the long term.

Two cohort studies by Tiller *et al.*[20] and Winkelhorst *et al*[2] described the localization and extensiveness of FNAIT-related ICH. In these studies, 43 and 21 cases, respectively were described. Approximately 90% of these cases were caused by HPA-1a alloantibodies. Tiller *et al.*[20] described that most of the bleedings were intraparenchymal. This finding was confirmed by Winkelhorst *et al.*[2] showing that 19 bleedings were intraparenchymal complicated by hydrocephalus in 11 cases. The survival rate of children with ICH due to FNAIT varied from 65% to 52%. The risk on neurological sequelae in survivors was high, ranging from 70% to 53%.

The incidence of ICH varies from 9.9% to 25% of the severe FNAIT cases based on antenatal and postnatal screening studies, respectively (Figure 1).[4] Antenatal screening studies may underestimate the incidence of ICH as imaging was not performed routinely in all screening studies. However, Refsum *et al.*[52] performed a retrospective cohort study and assessed the presence of HPA alloantibodies in a cohort of neonates born with an ICH. In this study, HPA alloantibodies were only found in 3 of the 105 maternal serum samples. The authors acknowledge that this study might be affected by bias, although the study implicates that the true incidence on FNAIT-related ICH might be less than previously thought.

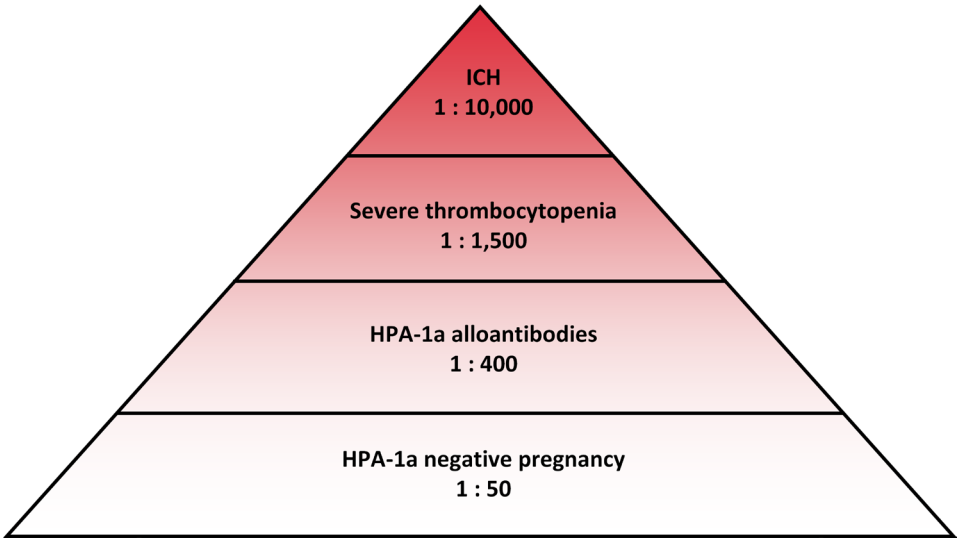

FIGURE 1. Epidemiology of anti-HPA-1a induced FNAIT
Severe thrombocytopenia is defined as a platelet count below 50×10^9/L.
Abbreviations: HPA, Human platelet antigen; FNAIT, fetal and neonatal alloimmune thrombocytopenia; ICH, intracranial haemorrhage; L, liter.

The recurrence rate of ICH in FNAIT is difficult to assess since studies are retrospective and biased by different treatment interventions. In addition, as severe cases might be more likely to be published, studies on this subject are also more prone for publication bias. In 2003 Radder *et al.*[53] performed a retrospective cohort study to assess the recurrence risk of ICH after a pregnancy complicated by an ICH due to FNAIT. The recurrence rate of an ICH due to FNAIT in subsequent pregnancies was estimated on 79%. Besides ICH, other life-threatening organ bleedings, like pulmonary, ocular and gastrointestinal bleedings, are described in FNAIT.[54, 55] Since these bleedings are less well known and probably underreported, it is impossible to estimate the incidence of these bleedings.[1]

ANTENATAL MANAGEMENT

The majority of ICH in FNAIT develop during pregnancy.[20] These hemorrhages have an important contribution to the burden of FNAIT. To prevent these ICH, treatment during pregnancy must be initiated early enough in pregnancy. Alloantibodies develop without clinical symptoms during pregnancy. Details about diagnostics of these antibodies are published by Porcelijn *et al.*[56]. The only way to prevent severe complications in first pregnancies is by screening and timely antenatal treatment. In the absence of routine screening, antenatal treatment is mostly started in subsequent pregnancies after the first affected child was born. When FNAIT is suspected, diagnostic tests should confirm or rule out FNAIT, in this way clinical management can be adapted adequately in the future. Clinicians are challenged to reduce the risk of bleedings in FNAIT, this can be done by treatment during pregnancy. In the past, the mainstay of treatment was based on invasive intrauterine platelet transfusions, similar to blood transfusions in red cell immunization. Nowadays, non-invasive approaches using maternal IVIg administration have been shown to be equally or even more effective, and preferable due to a reduced risk of complications.

RISK STRATIFICATION

If FNAIT is confirmed antenatally, pregnancies should be closely monitored by a specialized obstetrician. Currently we use the clinical outcome of a previous FNAIT pregnancies for risk stratification. Some studies show that alloantibody levels could predict disease severity[57] an approach which has been used in Norway for two decades.[58] However severe FNAIT cases have been described with low antibody levels implicating that sensitivity is low.[59] It may be that pregnancies with HPA-5b or HPA-15b antibodies could be considered as low risk pregnancies and alloantibodies against HPA-1a and HPA-3a as high risk, however, the risk for the individual pregnancy on an HPA-alloantibody mediated bleeding in the infant can yet not be predicted. Data set from large prospective studies comparing clinical outcome of different

HPA antibodies will be the first step to adjust clinical management for different HPA types. Despite efforts to find a reliable prognostic marker until know, no single marker has been found to predict disease severity accurately. Maternal HLA DRB3*01:01 status is suggested as a prognostic marker for immunization and formation of potent HPA-1a antibodies during pregnancy. More research is needed to confirm these findings.

INTRAUTERINE TRANSFUSIONS

Fetal blood sampling (FBS) and intrauterine platelet transfusions (IUPTs) were traditionally the only treatment options to reduce the risk of ICH and severe thrombocytopenia. Intrauterine intravascular, ultrasound-guided red blood cell transfusions to treat fetal anemia were introduced in the 1980s. Daffos and colleagues[60] were the first that applied this technique on FNAIT. After their successful first intra-uterine platelet transfusion others followed. However, there are important differences between intra-uterine red blood cell transfusions for fetal anemia compared to platelet transfusions for fetal thrombocytopenia. Since the fetus is at risk for bleeding due to FNAIT, puncture of the umbilical cord is a dangerous procedure. In addition, the half-life of the transfused platelets is short, making it necessary to perform weekly transfusions. The high risk of complications related to fetal blood sampling or intra uterine transfusions was confirmed in a recent systematic review by Winkelhorst *et al*. [5]. In total, 26 studies were included, of which 4 were randomized controlled trails, 5 prospective studies and 17 retrospective studies. The most frequently reported complication due to FBS or IUPT was an emergency cesarean section. The authors describe that complications occurred in 11% of the pregnancies treated with invasive therapy (54 of 497 treated pregnancies in 24 studies). Complications resulted in fetal death or neonatal loss in 26% of cases. The overall mortality rate was 4%, with more than half of the mortality related to FBS/IUTP.[5]

MATERNAL INTRAVENOUS IMMUNOGLOBULINS (IVIG) ADMINISTRATION

In the past, IVIg administration was successfully used in pregnancies complicated by maternal idiopathic thrombocytopenic purpura (ITP). Later, in 1988, Bussel and colleagues[61] were the first to report a positive effect of antenatal maternal IVIg treatment in pregnancies with FNAIT. IVIg are made from human IgG antibodies extracted from pooled human donor blood. The exact therapeutic mechanism of maternal IVIg treatment is unknown. The various proposed mechanism that could be responsible for the effect are discussed by Wabnitza *et al.*[62]. Evidence about optimal treatment options is obtained mostly from cases series and was recently summarized in a systematic review as part of an international guideline on FNAIT.[5, 63] In the absence of a reliable clinical or biochemical marker to predict platelet count or clinical outcome on FNAIT, treatment is based on the obstetric medical history. FNAIT pregnancies are usually classified as 'high-risk' in case of a previous pregnancy with a severely affected child with ICH or severe hemorrhage, while all other pregnancies with sibling with FNAIT without ICH are considered as 'standard risk'. First line treatment in high-risk pregnancies is weekly maternal IVIg administration from 12-16 weeks of gestational age with a standard

dosage regime of 1 g/kg/week. In some centers, dosage is increased to 2 g/kg/week around 20 weeks gestational age and/or corticosteroids are added. Since side effects of IVIg treatment are dose-dependent and the beneficial effect of increment in dosage is based on low evidence, therapy is not intensified in high-risk pregnancies in the Netherlands. In a randomized trial to assess the optimal dosage in FNAIT the risk on adverse outcomes was not different between low-dose (0.5 g/kg/week) or high dose (1.0 g/kg/week).[64] As this trial was stopped early due to poor recruitment a definitive conclusion regarding dose could not be drawn from this trial. Yet the standard dosage regimen in standard risk pregnancies at our center has been lowered to 0.5 g/kg/week. Treatment in standard risk pregnancies is usually started from 20 to 28 weeks gestational age, antenatal management strategies in the Netherlands are summarized in figure 2.

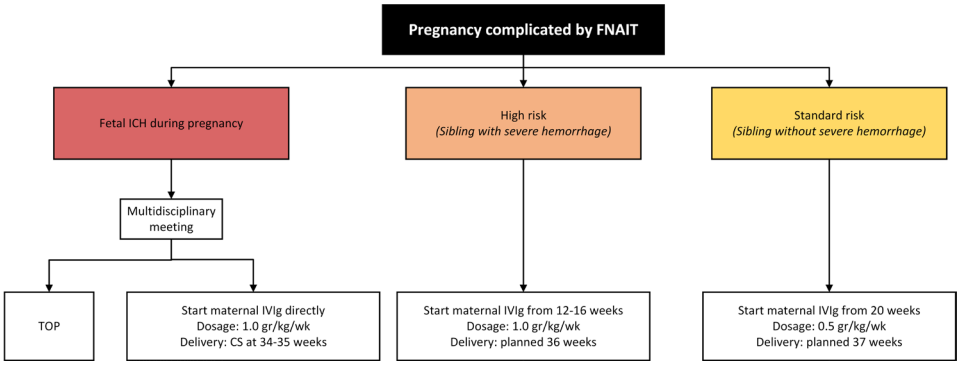

FIGURE 2. Flowchart antenatal treatment
Abbreviations: FNAIT, Fetal and neonatal alloimmune thrombocytopenia; ICH, intracranial haemorrhage; TOP, termination of pregnancy; IVIg, Intravenous immune globulins; gr/kg/week: grams per kilogram (bodyweight) per week.

IVIg administration is performed blindly, without FBS or IUPT, and this makes it impossible for clinicians to monitor the therapeutic effect of this treatment. Side effects can be classified in maternal and fetal side effects. The most important maternal side effects are headache and flu-like symptoms. One case of maternal pancytopenia was reported, in this case full blood count was normalized spontaneously 6 weeks after pregnancy.[65, 66] Possible long-term effects on the development the immune system of children are not well known. In one study, complete blood count and leukocyte differentiation were assessed in 20 cord blood samples, and in this study no abnormal maturation of the immune system was seen in children with FNAIT who received antenatal IVIg treatment or IUPT.[67] In the same cohort, a questionnaire study was performed and showed no increase of pediatric infections in IVIg treated cases. However, treatment regime in this cohort was heterogeneous and groups were small.[68] Since side effects of maternal IVIg treatment during pregnancies on the development of the fetus were not assessed adequately and no randomized placebo-controlled trial was performed

to show clinical efficacy, IVIg treatment for this indication is still 'off label'. This, even though IVIg treatment is recommended in virtually all guidelines. Additional studies on maternal and neonatal effects of IVIg are required, including the effects on long-term neurodevelopment outcome and immune system should be performed.

In conclusion, invasive antenatal management are equally effective but invasive interventions have a higher risk of complications. Therefore, maternal IVIg administration is first line treatment as antenatal management in FNAIT. This recommendation was integrated in the most recent international guideline on clinical management strategies of FNAIT.[63]

CORTICOSTEROIDS

In addition to maternal IVIg treatment, corticosteroids are sometimes added as antenatal treatment in some centers and mentioned in guidelines. However, the evidence for the use of corticosteroids is very weak, and the fetal and maternal side effects should not be underestimated.[63] Winkelhorst *et al.* [5] assessed 11 studies that compared IVIg treatment with and without the addition of corticosteroids. Only one study showed an increase in platelet count,[69] while all other studies failed to report beneficial effects. Given the important side effects and the lack of evidence for a beneficial effect, we think that this treatment should not be applied as first line treatment in FNAIT.

MODE AND TIMING OF DELIVERY

Peripartum management should be focused on reduction of the risk of bleeding by minimizing the factors contributing to bleeding complications. Strong evidence for best peripartum strategies is lacking. There is consensus about reticence in the use scalp electrodes, scalp blood samplings or assisted vaginal delivery. Elective cesarean delivery is the preferred mode of delivery in some centers. This is based on the assumption that delivery could trigger ICH in FNAIT cases, based on a retrospective cohort study. In this study, 200 FNAIT cases were described, all 17 FNAIT related ICH occurred before or within 24 hours after birth.[51] However, in this study no routine ultrasound prior to and after delivery was performed. Time-point of development of ICH is therefore not certain or by definition induced by delivery. Van den Akker *et al.*[70] performed a cohort study on standard risk FNAIT pregnancies. In this study 23 vaginal deliveries and 9 cesarean sections (CS) were included, and they showed that vaginal delivery was not associated with an increased risk on ICH. Another argument to reconsider routine near term elective cesareans is the risk on neonatal complications. Neonatal complications related to pulmonary maladaptation or prematurity were observed in 37 (21.5%) of the neonates in the large prospective Norwegian screening study that performed near team CS to prevent ICH. [36] In our opinion, given the lack of evidence and the potential increased risks of neonatal morbidity, CS should not routinely be performed in FNAIT pregnancies.

FUTURE TREATMENT OPTIONS

In analogy to the prevention of RhD using immunoprophylaxis, prevention of immunization and development of FNAIT has been shown to be effective in a murine model.[71] However, no clinical trials have yet confirmed this effect in humans. Kjær and Skogen[72] discuss the challenges of developing a hyperimmune anti-HPA-1a IgG for the prevention of HPA-1a-immunization. Another future therapeutic option might be an FcRn receptor blocker that can inhibit the transportation of alloantibodies over the placenta. Preclinical studies showed promising results,[73] and a study in red cell alloimmunized pregnancies is currently ongoing.[74]

POSTNATAL MANAGEMENT: OPTIMAL TRANSFUSION THRESHOLDS AND PLATELET PRODUCT

TRANSFUSION THRESHOLD

The optimal postnatal management strategy for neonates with FNAIT is unknown and is currently mostly based on expert opinions and single center observational data. Due to the rarity of disease and its heterogeneity, randomized trials will need a high number of inclusions and a large consortium contributing to the study. A recent systematic review on the postnatal treatment concludes that there is no sufficient evidence on the optimal postnatal treatment to prevent bleeding in infants that suffer from FNAIT.[6] After birth, prophylactic platelet transfusions are advised in case of severe thrombocytopenia in virtually all guidelines, but transfusion thresholds vary. Prophylactic platelet transfusions are given to prevent bleedings, but the evidence for the preventive effect of platelet transfusions is controversial and has recently been questioned[75-77] In a recent systematic review, the opposite was found to be more plausible, as transfusions in thrombocytopenic preterm neonates were associated with increased risk of bleeding.[78] A recently published randomized trial on prophylactic platelet transfusions thresholds in preterm infants confirmed the results of the systematic review.[79] This study showed that a higher platelet transfusion threshold (50×10^9/L) was associated with a higher mortality rate and bleeding than a more restrictive transfusion threshold (25×10^9/L). Although this study included only preterm neonates and excluded neonates with FNAIT, one could argue that prophylactic transfusions in FNAIT neonates, with potentially fragile vessel walls due to endothelial damage, could be harmful too. A randomized trial in FNAIT neonates would be required to determine the optimal transfusion threshold. Until then, we suggest to follow national guidelines and transfer knowledge obtained from randomized trails performed in preterm infants. Figure 3 provides an overview of a Dutch transfusion guideline in FNAIT. A transfusion threshold of 50×10^9/L in neonates suffering from FNAIT with active bleeding is suggested and a threshold of 25×10^9/L in neonates without active bleeding.

PLATELET PRODUCT

HPA-typed platelets, negative for the implicated HPA antigen, have traditionally been regarded as the optimal platelet product in FNAIT neonates, as these products would not be susceptible to the pathogenic antibodies present in these children. In a recent study in a national cohort in 102 firstly diagnosed FNAIT cases, we found a similar platelet increment in both HPA-matched and random platelet transfusions.[80] In our opinion, random platelet transfusions could be viewed as first-line treatment in case acute bleeding or severe thrombocytopenia. In most countries, HPA-matched platelets are not readily available and reducing delay by using random platelets may be preferable. No large benefits of neonatal IVIg treatment were observed in our small cohort.[80] Also the beneficial effect of IVIg treatment postnatal is questioned in a recent systematic review on postnatal treatment in FNAIT.[6] Pragmatically we advise to perform a platelet transfusion without delay using random platelets, at a platelet count < 25 × 10^9/L or in case of a severe bleeding, unless an HPA-matched product is readily available. IVIg treatment is not advised as first line postnatal treatment.

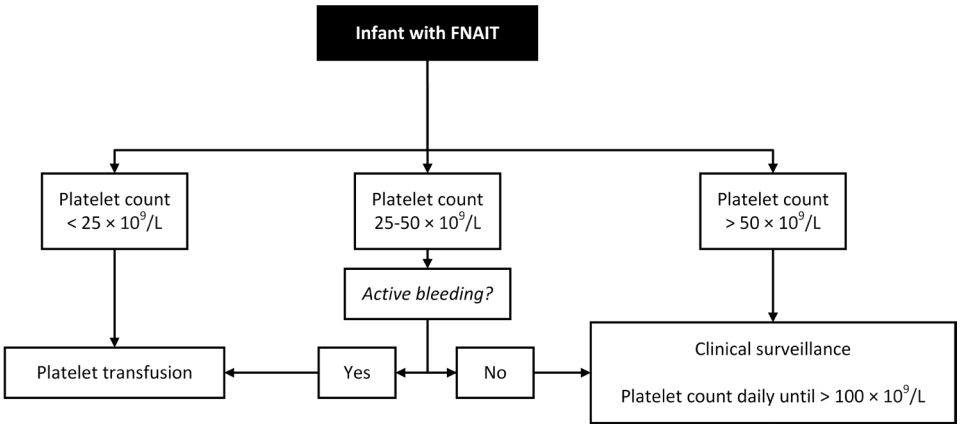

FIGURE 3. Flowchart postnatal treatment
This flowchart is based on the Dutch transfusion guideline for infants.
Abbreviation: L, liter.

LONG-TERM OUTCOME

Literature about the long-term outcome on FNAIT without ICH is scarce.[2,20] The first follow-up study on FNAIT cases was performed by Ward *et al.*[81] and published in 2006. They concluded that development of children treated for FNAIT was better compared to their non-treated siblings. Their conclusions were based on questionnaires taken by telephone, assessing the behavioral outcome of the children and were limited by a 40% lost-to-follow-up rate. A second follow-up study including 39 children was published by a research group from our center in 2004. This study showed that the outcome in children with FNAIT and exposed to maternal IVIg treatment was similar to the normal population.[68] However, this study included a heterogenic group of children with different treatment strategies including IUT, hampering definitive conclusions. More research is needed to provide insight in the long-term development of children that suffered from FNAIT.

FUTURE PERSPECTIVES

The introduction of an antenatal screening on FNAIT has been a topic of scientific debate in the past decades and has been discussed by the health authorities in several countries, for instance Norway, Denmark, the United Kingdom and the Netherlands. The World Health Organization stimulated the use of the criteria from Wilson and Jungner to decide whether a population screening program is of benefit to a population and the overall health care system. These criteria can be used to guide the debate on antenatal screening on FNAIT.[82] Two of these criteria should be priority in research on FNAIT. First, the natural course and incidence on FNAIT should be addressed. Second, attention should be paid to the development of diagnostic tools to identify the pregnancies at risk for severe neonatal outcome. As discussed earlier, most studies that address the incidence of FNAIT are either performed retrospectively or performed prospectively in combination with interventions. Therefore, results on the natural course of the disease could be biased. Importantly, most studies used platelet count as a primary outcome marker instead of clinically more relevant outcomes such as major bleeding and/or perinatal death.[4] Conducting a study that addresses these outcomes will be extremely challenging since the incidence of these major bleeding is estimated on 1 in 10.000 pregnancies and thousands of pregnancies would have to be screened. An important research priority on this subject is the development of diagnostic tools to predict clinical outcome, hence, to identify the cases who need antenatal therapy and interventions. Future international collaboration involving both clinicians and scientists might lead to this diagnostic assay to identify pregnancies at risk and prevent burden of FNAIT in future.

TOWARDS ROUTINE HPA-SCREENING IN PREGNANCY TO PREVENT FNAIT – THE HIP STUDY

In 2017, a large prospective screening study started in the Netherlands to assess the incidence on clinically relevant FNAIT cases.[83, 84] Maternal serum samples of HPA-1a negative and HPA-1a positive controls and clinical data are collected. We expect to end our study in spring 2020. In contrast to other screening studies, this study is completely observational without any perinatal management applied, which allows the assessment of the natural history of this disease. More importantly, this study will provide a uniform serum sample collection (alloimmunized pregnancies without clinical disease) that can be used to develop and test diagnostic assays to identify pregnancies at risk. In this way the HIP study will contribute important knowledge and provide arguments in the debate on antenatal screening on FNAIT.

CONCLUSION

FNAIT causes severe burden and fetal ICH in 1 in 10.000 pregnancies. Antenatal, non-invasive treatment strategies are nowadays viewed as preferred management since they bear a lower risk of intrauterine complications. In high-risk FNAIT, antenatal IVIg treatment should be initiated at 12-18 weeks gestational age, using a high dosage. In standard-risk FNAIT antenatal IVIg should be started between 20-28 weeks gestational age using a lower dosage. Postnatal treatment strategies consist of platelet transfusions in case of severe thrombocytopenia, using either HPA-selected or unselected, depending on which one is more readily available. The optimal threshold for prophylactic transfusion is not known and varies between countries. A more restrictive transfusion strategy using a lower platelet threshold was recently shown to be superior to a liberal strategy using a higher threshold in preterm neonates. Whether this may also apply to term neonates with FNAIT is not known. Further studies are needed to evaluate the natural history and long-term neurodevelopmental outcome in FNAIT to optimize risk assessment and identify pregnancies at risk for ICH. Prevention of FNAIT is key and can be achieved by antenatal screening. Implementation of national screening programs will probably strongly reduce the burden of this severe disease.

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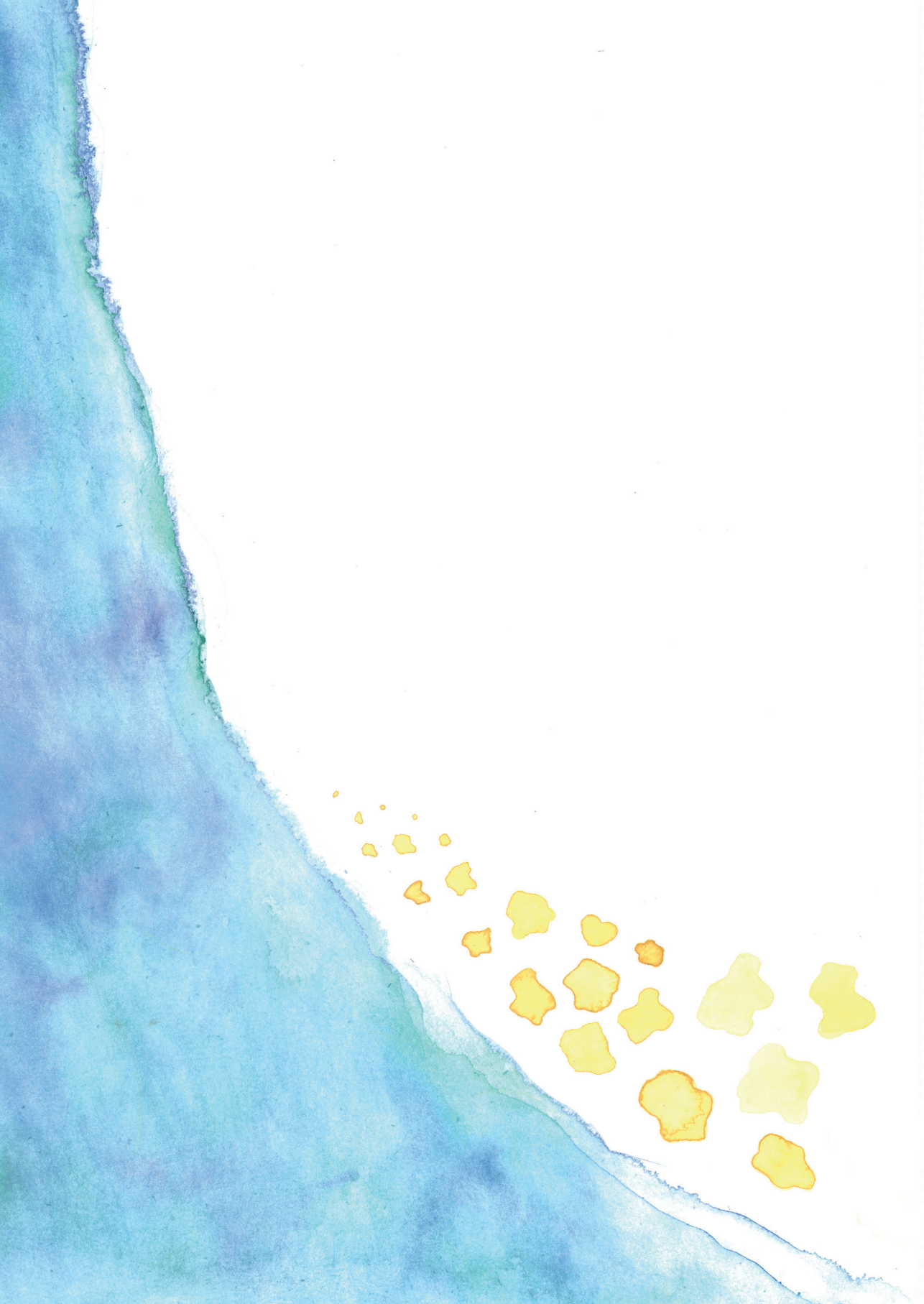
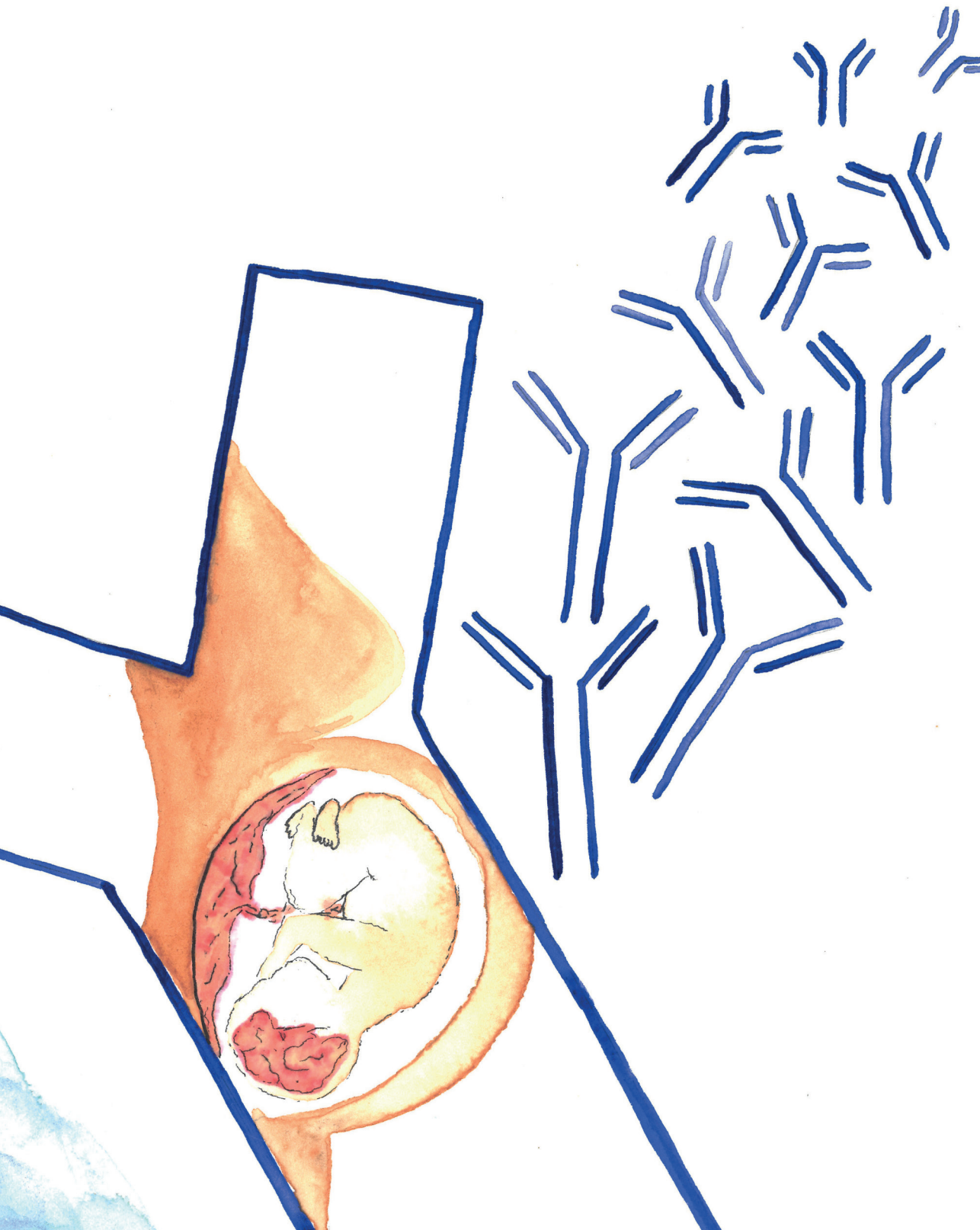


PART TWO

Natural history of HPA-1a mediated FNAIT



CHAPTER 2

HIP study (HPA-screening In Pregnancy): Protocol of a nationwide, prospective and observational study to assess incidence and natural history of fetal/neonatal alloimmune thrombocytopenia and identifying pregnancies at risk

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ABSTRACT

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) may lead to severe fetal or neonatal bleeding and/or perinatal death. Maternal alloantibodies, targeted against fetal human platelet antigens (HPAs), can result thrombocytopenia and bleeding complications. In pregnancies with known immunisation, fetal bleeding can be prevented by weekly maternal intravenous immunoglobulin (IVIg) infusions. Without population-based screening, immunisation is only detected after birth of an affected infant. Affected cases that might have been prevented, when timely identified through population-based screening. Implementation is hampered by the lack of knowledge on incidence, natural history and identification of pregnancies at high-risk of bleeding. We designed a study aimed to obtain this missing knowledge.

METHODS AND ANALYSIS

The HIP study (HPA-screening In Pregnancy) is a nationwide, prospective and observational cohort study, aimed to assess incidence and natural history of FNAIT, as well as identifying pregnancies at high-risk for developing bleeding complications. For logistic reasons we invite RhD or Rhc-negative pregnant women, that take part in the Dutch population-based prenatal screening program for erythrocyte immunisation, to participate in our study. Serological HPA-1a typing is performed and a Luminex-based multiplex assay will be performed for the detection of anti-HPA-1a antibodies. Results will not be communicated to patients or caregivers. Clinical data of HPA-1a negative women and an HPA-1a positive control group will be collected after birth. Samples of HPA-1a immunised pregnancies with and without signs of bleeding will be compared to identify parameters for identification of pregnancies at high-risk for bleeding complications.

ETHICS AND DISSEMINATION

Ethical approval for this study has been obtained from the Medical Ethical Committee Leiden-The Hague-Delft (METC-LDD) (P16.002). Study enrolment began in March 2017. All pregnant women have to give informed consent for testing according to the protocol. Results of the study will be disseminated through congresses and publication in relevant peer-reviewed journals.

TRIAL REGISTRATION NUMBER

NCT04067375

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The HPA-screening In Pregnancy (HIP) study is a unique prospective and completely non-interventional screening study with a large cohort that enables assessing the true natural history of fetal and neonatal alloimmune thrombocytopenia (FNAIT).
- The unique infrastructure in the Netherlands with one national referral laboratory for FNAIT (Sanquin, Amsterdam) collaborating with the national fetal therapy centre (LUMC, Leiden) will result in complete data and focus on both laboratory and clinical parameters.
- A limitation of the study is that we rely on the clinical judgement of bleeding tendency after birth, and do not obtain cord blood platelet counts or perform routine neonatal cerebral ultrasounds. Therefore, we may still underestimate disease prevalence due to subclinical cases.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most frequent cause of severe thrombocytopenia in term born infants.[1, 2] FNAIT is caused by the production of maternal alloantibodies against the paternally derived, fetal human platelet antigens (HPAs). Clinical consequences can vary from an asymptomatic thrombocytopenia to minor skin haemorrhage, such as haematoma or petechiae, or ultimately severe internal organ and intracranial haemorrhage (ICH).[3, 4] Bleeding complications that, in subsequent pregnancies can be effectively prevented by weekly administration of intravenous immunoglobulin (IVIg) to the mother.[5] The vast majority of cases with (severe) clinical consequences are caused by maternal alloantibodies targeted against fetal HPA-1a.[6-8] FNAIT is considered to be the platelet counterpart of haemolytic disease of the fetus and the newborn (HDFN) because of their similar pathophysiologic fundaments. In this comparison, HPA-1a, that causes 90% of the ICH caused by FNAIT, is regarded to be the equivalent of RhD of the red blood cell (RBC) in HDFN.[8] Important differences, however, exist as well. First, whereas RhD is only expressed on red blood cells, the HPA-1a epitope expressed on platelets is also present on the membrane of endothelial cells and syncytiotrophoblast cells.[9, 10] Second, whereas RhD is mainly a problem of second or subsequent incompatible pregnancies, more than half of the severe cases of HPA-1a-mediated FNAIT already occur in firstborn children.[4, 11] For decades, the possibility of prevention of FNAIT by population-based screening for HPA-1a is discussed, in analogy to the RhD prophylaxis and erythrocyte immunisation screening.[12-14]

Careful evaluation of the feasibility, benefits, harms and cost effectiveness of a possible FNAIT screening program showed that knowledge is missing on different aspects of the disease. First, despite a couple of large prospective cohort studies, no data exist on the natural history of the disease. Most of the large prospective, screening studies performed, were not only observational, but included some kind of intervention, thereby making it impossible to draw any firm conclusion on the natural history of FNAIT.[15-19] Further, more accurate estimates of incidence and prevalence of the disease in the Dutch population need to be known. One of the most important differences, making it hard to implement a program similar to the antenatal screening program for erythrocyte immunisation, is the lack of tools to identify pregnancies at high risk for developing bleeding complications. Detecting HPA-1a negative women and further HPA-1a alloimmunised pregnancies can be done easily. When alloimmunisation is detected in HDFN several parameters, laboratory as well as clinical, are available to assess disease severity and to predict which cases would benefit from treatment. For example, RBC alloantibody titre and functional assays such as an antibody-dependent cellular cytotoxicity assay can be performed, followed in pre-selected cases by estimation of fetal anaemia by Doppler-based assessment of flow velocity in the middle cerebral artery of the fetus. In this way, high risk cases are identified that most likely benefit from fetal blood sampling (FBS), followed by an intrauterine transfusion.[20] Treating all HPA-alloimmunised

pregnancies with IVIg would lead to a considerable and undesirable overtreatment. So, identification of HPA-alloimmunised pregnancies at high risk for disease, like in HDFN, would be preferable as well. FBS to determine fetal platelet count and if necessary, administer intrauterine platelet transfusion, can be performed in these pregnancies as well. However, in potentially thrombocytopenic fetuses this is a risky procedure with a high rate of associated complications.[5] Unfortunately, no non-invasive laboratory or clinical diagnostic tests to select HPA-alloimmunised pregnancies that would benefit from treatment are applicable in a clinical setting.

To obtain information necessary to judge the effectiveness and feasibility of a potential population-based screening, we designed the HIP (HPA-screening In Pregnancy) study. With the HIP study we aim to collect data on the incidence of HPA-1a alloimmunisation and clinically relevant FNAIT in the Netherlands. The study will be completely observational. This way we will be able to conclude on the natural history of FNAIT. Ultimately, by comparing test characteristics of blood samples from pregnancies with and without clinical manifestations of bleeding we aim to develop one or more diagnostic tools, allowing more effective and personalised management by selecting pregnancies at high risk for bleeding complications that have the highest chance to benefit from antenatal preventive treatment with IVIg. This would not only be desirable in current management of FNAIT but especially in potential future screening setting.

METHODS AND ANALYSIS

STUDY OBJECTIVES

The primary objective of this study is to determine incidence of HPA-1a alloimmunisation and the incidence of clinically relevant HPA-1a-induced FNAIT in the Netherlands. Clinically relevant FNAIT will be defined as minor bleeding (haematoma, bruising, petechiae or small visceral bleeding) and severe bleeding (ICH or internal organ haemorrhage) with the presence of an anti-HPA-1a alloantibody. Additionally, as secondary objective, we aim to collect a set of blood samples that can contribute to the development of a risk assessment model to be used as a diagnostic tool enabling the identification of alloimmunised pregnancies that are at high risk of developing bleeding complications.

STUDY DESIGN

The HIP study is a nationwide prospective and observational cohort study, conducted in all settings of obstetric care in the Netherlands, for a period of two and a half years.

PATIENT AND PUBLIC INVOLVEMENT

In 2008, the ministry of Health, Welfare and Sport (in Dutch: Ministerie van VWS) gave instructions to investigate preventive interventions for 27 significant health problems that could be cost-effective. As a result the National Institute for Public Health and the Environment (in Dutch: RIVM) published a report stating that antenatal screening for FNAIT would be cost saving, but they advised that more knowledge on natural history of the disease and treatment of detected cases should be obtained to support possible implementation of screening.[21] Also, the RIVM was involved in the design of the study. There was no further involvement of patients or public in the recruitment or the conduct of the study.

STUDY POPULATION

For logistic purposes, RhD or Rhc negative pregnant women were selected for enrolment in the HIP study. As part of the Dutch prenatal screening program for infectious disease and erythrocyte immunisation (in Dutch: PSIE), these women are offered a free of charge red cell antibody screening and/or fetal RHD typing at 27 weeks' gestation. For this, nine ml ethylenediamine tetra-acetic acid (EDTA) anticoagulated blood is drawn by their midwife or at certified, local laboratories all over the Netherlands (n = ±90) and transported to the Sanquin laboratory in Amsterdam by regular surface mail or private courier service. The program has a voluntary participation grade of 99%.[22, 23] With approval of the RIVM, that organises this population screening program, left-over material can be used for the HIP study for HPA-1a typing and stored for further antibody testing after informed consent.

Inclusion criteria

Prior to enrolment, participants have to fulfil these following criteria:

- Pregnant women participating in the currently implemented prenatal screening program for erythrocyte immunisation and who are typed RhD or Rhc negative.
- Ability to make an informed decision on participating in the population screening program as well as in the HIP study.

Exclusion criteria

- Cases with insufficient material to perform HPA-1a typing by enzyme-linked immunosorbent assay (ELISA).
- Cases with known HPA-1a alloimmunisation.

PARTICIPATING CENTRES

All obstetric care centres, hospitals, midwifery practices as well as general practices that provide obstetric care, in the Netherlands are able to enrol pregnant women to participate in the HIP study. In order to ensure that obstetric caregivers were equipped to inform and counsel pregnant women, communicatory symposia were organised at six locations all over the Netherlands. Additionally, an informational leaflet was produced in different languages

(Dutch and English on paper; Spanish, Arabic, Turkish and Polish digitally available; supplemental material). Two informational videos were made informing on FNAIT as well as the HIP study. Lastly, a website was created containing news and information about the HIP study (www.HIPstudie.nl).

STUDY OUTCOMES

The main study parameters / primary endpoints are:

- Incidence of HPA-1a negativity in the RhD or Rhc-negative pregnant population in the Netherlands at 27 weeks of pregnancy
- Incidence of HPA-1a alloantibodies in the tested population at 27 weeks of pregnancy
- Incidence of clinically relevant HPA-1a-mediated FNAIT; classified as mild or severe FNAIT
- Severe FNAIT
  - ICH
  - Internal organ haemorrhage
- Mild FNAIT
  - Neonatal bleeding signs other than ICH or internal organ haemorrhage: haematoma, bruising, petechiae, purpura, mucosal or visceral bleeding
  - Thrombocytopenia for which treatment was administered (platelet transfusion or IVIg) or for which clinical observation was performed
- Our secondary study parameters / endpoints are:
  - Neonatal treatment for thrombocytopenia: platelet transfusion (with random-donor platelets versus compatible platelets), IVIg, red blood cell transfusion
  - Neonatal morbidity: small for gestational age, infection, hours/days in hospital (NICU versus Medium Care), need for additional treatment, congenital abnormalities, other causes causing increased bleeding tendency
  - Neonatal laboratory findings: platelet count, haemoglobin, CRP

HIP STUDY PROCEDURE

As part of the prenatal screening program for erythrocyte immunisation, an EDTA tube of blood of RhD and Rhc negative pregnant women will be sent to Sanquin at 27 weeks' gestation. These women are eligible for enrolment in the HIP study and will be informed about the study and asked for consent by their obstetric caregivers. This consent or decline of participation is added to the regular laboratory request form for the 27th week assessment, that is already sent to Sanquin with each tube of blood. No additional blood will be drawn for the HIP study. Once the tubes of blood are sent to Sanquin the consent is either received digital or on paper, depending on the route and location (various hospitals, midwifery practices and local laboratories).

The procedures that are performed after consent and enrolment in the HIP study can be divided into four separate phases, depending on the time in and after pregnancy (Figure 1).

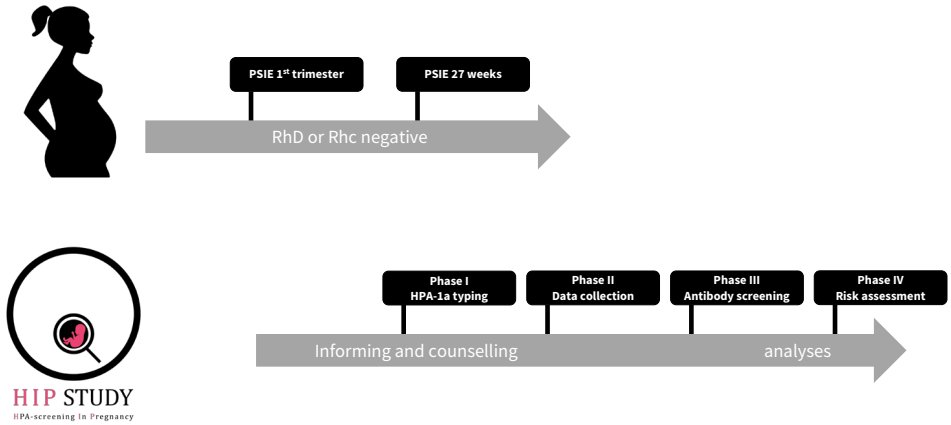

FIGURE 1. Schedule of selection, enrolment and tests in the HIP study
Abbreviations: HPA, human platelet antigen; PSIE, prenatal screening of infectious diseases and erythrocyte immunisation; RhD, rhesus D; Rhc, rhesus c.

Phase I

After regular screening, authorisation and correspondence of the results for the prenatal screening program for erythrocyte immunisation, the tubes are made available for the HIP study. For the HIP study the platelet containing plasma of the stored blood tubes is serologically typed for HPA-1a, using a sandwich ELISA. In short, 20 µL of plasma containing platelets will automatically be pipetted into microtiter plates that have been coated with a monoclonal antibody CLBthromb/1 (C17) directed against glycoprotein IIIa, at a concentration of 3 µg/mL to capture all platelets from the plasma. Then HRP-conjugated B2G1, an antibody targeting HPA-1a, will be added and plates will be centrifuged and incubated for 45 minutes. Lastly, after washing of the plates, HRP-substrate solution will be added for 15 minutes and after stopping of this reaction the reactions will be quantified using an ELISA reader (Biochrom Anthos, Cambridge, United Kingdom). This HPA-1a ELISA was specifically designed for the HIP study, thus for quick and high-throughput screening. All samples with an ELISA value below a defined optic density (OD) are called HPA-1a negative. The HPA-1a typing result is supported with an allelic discrimination polymerase chain reaction (PCR) assay. Plasma and buffy-coat of samples that are typed HPA-1a negative will be stored at -20°C, using only a study number. Additionally, for each HPA-1a negative case, material of one HPA-1a positive control will be stored simultaneously.

Because this first phase comprises serological HPA-1a typing, which is performed with fresh material, and a delay in the arrival of consent forms might exist, this phase is performed with all samples from pregnant women who did not decline participation for the HIP study. All consecutive phases, such as antibody screening, risk-assessment development and clinical data retrieval, are solely performed in case of informed consent for the HIP study.

Phase II

Of all samples stored with consent, obstetric caregivers will be contacted to obtain clinical information. An overview of these clinical parameters is provided in Figure 2. The clinical data will be stored in a secured digital database, designed by the LUMC, called ProMISe. First, study numbers of HPA-1a negative cases and HPA-1a positive controls with corresponding obstetric caregivers are entered into the database. Then, for each case, ProMISe randomly generates a code. Thereafter obstetric caregivers will receive a secured digital invitation to add clinical data to a digital case report form (CRF) for the cases from their practice. This secured invitation contains the initial personal data for the sample sent for the erythrocyte immunisation screening program together with the code generated by ProMISe. In the digital CRF they fill in this code and the clinical data. Clinical data is stored in ProMISe, only by anonymous study codes. This way, no personal information is being transferred or entered in our database, nor is the obstetric caregiver in possession of a key that links the anonymous study number to personal information, nor does the caregiver know whether their patients or clients are HPA-1a negative or positive.

Medical history:	known with immune thrombocytopenic purpura (ITP).
Obstetric history:	previous pregnancies, deliveries, miscarriage (spontaneous as well as pregnancy terminations) or intra-uterine fetal demises.
Pregnancy:	gestational diabetes, hypertensive disorders (pregnancy induced-hypertension, pre-eclampsia), intrauterine growth restriction (IUGR).
Perinatal:	gestational age at delivery, mode of delivery, Apgar score, birth weight.
Neonatal:	gender, chromosomal disorder, laboratory assessment (CRP or platelet count), consultation of paediatrician, admission to the neonatal care unit, mortality.
FNAIT related:	haematomas, petechiae, visceral bleeding, internal organ haemorrhage, intracranial haemorrhage, platelet count (if tested), treatment for thrombocytopenia.

FIGURE 2. Clinical parameters
These clinical parameters will be collected in the HIP study.

Phase III

The next step is to evaluate the incidence of alloimmunisation. Of all HPA-1a negative women that gave consent for the HIP study, we will use the stored left-over plasma to screen for HPA-1a alloantibodies. For antibody screening the Pak Lx assay, a qualitative immunoassay, will be used, according to the manufacturer's' recommendations (LIFECODES Pak Lx Assay, Immucor GTI Diagnostics, Norcross, United States of America). In short, plasma samples are incubated with reconstituted beads and for the removal of unbound antibodies, the beads are washed. Next a conjugate (anti-human immunoglobulin G antibody conjugated to phycoerythrin) is added and incubated with the sample for 30 minutes at room temperature. Lastly, the Luminex 200 instrument is used to analyse the data. The advantage of this assay is that it is quick and uses only a small amount of plasma so there will be enough left-over for further testing in phase IV.

Phase IV

Combining the results from phase II and phase III will enable us to select cases of alloimmunisation with and without clinical manifestations of FNAIT to identify possible parameters to predict the development of (severe) bleeding complications. For this we will be testing different laboratory parameters as well as clinical parameters (Figure 2). Laboratory parameters that will be tested to assess risk at bleeding complications are: HLA-DRB3*0101 status, antibody level, Fc-core glycosylation and FcγRIII-binding index, endothelial cell binding, endothelial cell function.[24-28]

SAMPLE SIZE CALCULATION

The HIP study was designed to assess the incidence of clinically relevant FNAIT in pregnant women in the Netherlands. Therefore, the incidence of ICH in HPA-1a immunised cases was compared with HPA-1a positive women. The estimated risk of ICH in immunised cases was 3%. For our power calculation we took a marge of 1% on the estimated incidence of ICH in FNAIT.[19,29] In our control group we assumed a risk on symptomatic ICH of 4.9 in 10.000 (0.05%).[30] To achieve a power of 80% at an alpha level of 5%, we calculated that a total study population of 2,400 pregnant women is needed. Within this calculation, we took into account the unequal distribution between HPA-1a positive controls and immunised cases. We considered 5% of our total study population to consist of immunised cases, which means that we needed to include 120 immunised cases. Calculations were performed using logistic regression model making use of PASS 11.

Each year, approximately 60,000 RhD or Rhc negative pregnant women participate in the prenatal screening program for erythrocyte immunisation and are therefore eligible for enrolment in the HIP study. To include 120 immunised cases, we need to include 1,200 HPA-1a negative women (immunisation rate of approximately 10%).[29] Because 2.1% of the white population is HPA-1a negative, the total study population should exist of 60,000 pregnant women (Table 1). Based on previous experience with the OPZI-study and the highly positive attitude toward potential HPA-screening in pregnancy women expressed in our previous study, the expected enrolment was 50%.[31, 32] This would correspond with a study period of two years.

TABLE 1. Estimated cases in HIP study

	%	Incidence	Cases in the Netherlands Total pregnancies n = 170,000	Cases during study period Total included n = 60,000*
HPA-1a negative	2.1	1 : 50	3,570	1,260
HPA-1a antibodies	10	1 : 400	428	126
Severe FNAIT	30	1 : 1,300	129	36
ICH	10 – 30	1 : 12,500	13	3-4

* Assuming 50% enrolment of the 60,000 RhD/Rhc negative women each year, for two years

Abbreviations: FNAIT, fetal and neonatal alloimmune thrombocytopenia; HIP, HPA-screening in pregnancy; HPA, human platelet antigen; ICH, intracranial haemorrhage.

STATISTICAL ANALYSIS

Clinical data will be entered into a validated data capture system, 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 (version 23.0) and GraphPad (version 8.0). An interim analysis after one year will be performed.

ETHICS AND DISSEMINATION

The introduction of an antenatal screening program requires a careful balance between benefit and potential harm. To investigate the true natural history of FNAIT we aimed to collect data from pregnancies without additional interventions based on screening test results. This observational non-intervention design is ethically challenging. It would be unethical to share the antibody screening results with pregnant women but withhold them from therapy, therefore antibody screening will be performed far after due date. There will be no direct beneficial effect for pregnant women participating in the HIP study, pregnant women will be informed by their caregivers about this before they give consent to our study. Ethical approval for this study has been obtained from the Medical Ethical Committee Leiden-The Hague-Delft (METC-LDD) (P16.002).

Patient recruitment started in March 2017 and the study is planned to close to recruitment on the spring/summer of 2019. However, to ensure the inclusion of 1,000 – 1,500 HPA-1a negative women the inclusions period might take longer. Accurate predictions on the duration of the study will be made after interim-analysis at one year. Results will be published in relevant scientific journals and be disseminated in international conferences when inclusion and clinical data collection is finished.

DISCUSSION

FNAIT can cause severe bleeding complications in fetuses and neonates, with a high risk of associated morbidity and mortality.[33] A preventive antenatal treatment, that effectively prevents these bleeding complications from occurring, is available.[5] In current practice, this prevention is only available in pregnancies with known alloimmunisation, usually after a previously affected child. To prevent these first cases as well, timely detection by prenatal and population-based screening is necessary.

Current lack of prospective non-interventional studies providing data on natural history of the disease as well as a reliable risk assessment tool to identify alloimmunised pregnancies

that are at high risk for developing bleeding, complicates the implementation of such population-based screening. The aim of the HIP study is to gather this missing knowledge necessary to adequately evaluate the potential efficacy and feasibility of prenatal population-based screening in order to timely detect and prevent FNAIT-related complications. With the current study design and logistics, making use of the current national screening program for red blood cell immunisation with a participation grade of 99.1%. We do not think that selection of RhD and Rhc negative women would influence the outcome of our study (i.e. immunisation rate or bleeding symptoms). RhD and Rhc status has never been associated with platelet immunization during pregnancy and inheritance is unrelated since the RhD and Rhc genes are located on chromosome 1 and the HPA-1 allele on chromosome 17. Therefore we expect our results to give an adequate representation of the Dutch population of pregnant women.

A potential limitation of this study protocol is the lack of routine determination of neonatal platelet counts. However, the goals of potential screening and prevention of FNAIT is not to prevent a low platelet count as reflected as a laboratory result, but to prevent symptomatic disease, mainly ICH, with associated morbidity caused by FNAIT. However, routine neonatal cerebral ultrasound is not performed either. Therefore, cases of subclinical ICH without symptoms (such as convulsions or reduced consciousness) or additional bleeding manifestations might be missed, although in theory these might lead to developmental problems later in life. However, major ICHs detected in prospective studies that did perform routine cerebral ultrasound, were cases that were symptomatic as well.[19, 34]

Further underestimation might occur due to the fact that we will perform only a single screening for anti-HPA-1a alloantibodies, that is at 27 weeks' gestation. Immunisations that occur later in pregnancy or after delivery will not be detected. Also, immunisations that will result in complications and termination of pregnancy or IUFD before 27 weeks' gestation will not be identified. However, in terms of assessing feasibility and cost-effectiveness of population-based screening, a slight underestimation is unquestionably preferred to an overestimation. On the contrary we use another antibody screening method compared to earlier screening studies which is possibly more sensitive compared to the MAIPA technique (Monoclonal Antibody Immobilization of Platelet Antigens [35]) The PAK Lx assay was tested on a series with 100 cases with suspected FNAIT by our research group in 2014. In 26 of these cases anti-HPA-1a was detected by MAIPA, all cases were detected and one more by PAKLx. Overall, to our knowledge, the HIP study will be a unique study to prospectively and observationally collect data on incidence and natural history of FNAIT by including this large number of pregnant women without performing any kind of intervention. Additionally, it will be the first study to be able to identify a unique and unbiased study group, that is, immunised pregnant women without disease and without intervention. This is the pre-eminent group to be used for the development of a risk-assessment platform in order to select immunised

pregnancies that are at high risk to develop bleeding complications and would therefore benefit from antenatal preventive measures, such as IVIg treatment.

AUTHOR CONTRIBUTION STATEMENT

DW, DO, CEvdS, MdH designed the study, MK, LP, EL commented on the design of the HIP study; DW, TWdV, LP, DO, CEvdS and MdH wrote study materials and coordinated the study design, wrote and reviewed this paper.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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DATA AVAILABILITY STATEMENT

A Data Availability Statement was not applicable, this article does not contain any data.

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HIP-study

In the Netherlands all pregnant women are screened for antibodies against **red blood cells** as these antibodies can lead to anemia or jaundice in the newborn. In the blood of pregnant women, antibodies against **platelets** may also be present, these platelet antibodies can lead to bleeding problems in the newborn.

Can we prevent bleeding problems caused by antibodies against platelets? To answer this question we first need to know the following:

- How often are **antibodies** against platelets present in the blood of pregnant women?
- If these antibodies are present how often do they cause bleeding problems in newborn babies?
- Which newborns are at high risk of suffering from bleeding problems?
- Can we predict bleeding problems by testing the blood of pregnant women?
- How can we prevent these **bleeding problems** in newborn babies?

The HIP-study can provide answers to these questions. To answer all these questions , we need the help of midwives, gynaecologists and pregnant women. By agreeing to participate, you can make an important contribution to the HIP-study.

More information

For further information, please check
www.HIPstudie.nl
Or scan this QR-code to watch our informative video.

Please do not hesitate to consult your obstetric caregiver with any further questions you may have.
Or send an email to: **info@HIPstudie.nl**

Principal investigator:
drs. Dian Winkelhorst, medical doctor and researcher
Independent medical doctor:
Dr. M. Sueters, gynecologist, LUMC

Research study on platelet antibodies in pregnancy

HIP STUDY
HPA-screening In Pregnancy

Sanquin

LUMC
Leids Universitair Medisch Centrum

Why do you receive this flyer?

In the first trimester of your pregnancy, your red blood cell type was tested. The results showed that your red blood cells are RhD or Rhc negative. All RhD and Rhc negative pregnant women in the Netherlands are tested again at 27 weeks gestation by Sanquin in Amsterdam. We would like to perform an extra test, for the HIP-study, with the remainder of this blood sample. Here we ask your consent to perform this extra test.

What does this mean for you?

Participation is completely voluntary and will have no further consequences for the care you receive during your pregnancy. If you participate, no additional actions are necessary. No extra blood is drawn nor will you be personally contacted. You and your baby will be unaware of participation.

It is important to know that there is no personal gain in participating. However, your participation will contribute to improving knowledge on diseases caused by antibodies against platelets and will help to improve treatment during pregnancy in the future.

Which tests and actions are part of the HIP-study?

If you consent to participate in the HIP study, we will perform an extra test on the blood sample that has been already drawn. With this test we will determine the blood type of your platelets. This blood type is called **HPA-1a**. If you don't have this blood type, you are HPA-1a negative (1 in 50 women). If you are HPA-1a negative, we will store the tested blood sample. We will also store the blood sample from a small number of HPA-1a positive women as part of the so called 'control-group'.
After the expected delivery date all stored blood samples will be tested for antibodies against platelets . A member of the study team will contact your obstetric care giver to enquire on the health of your child during the first hours-days of life. All results and data will be anonymized and saved in a secured database. Results will not be reported to you or your obstetric care giver. The study results cannot be requested by third parties.

Participate?

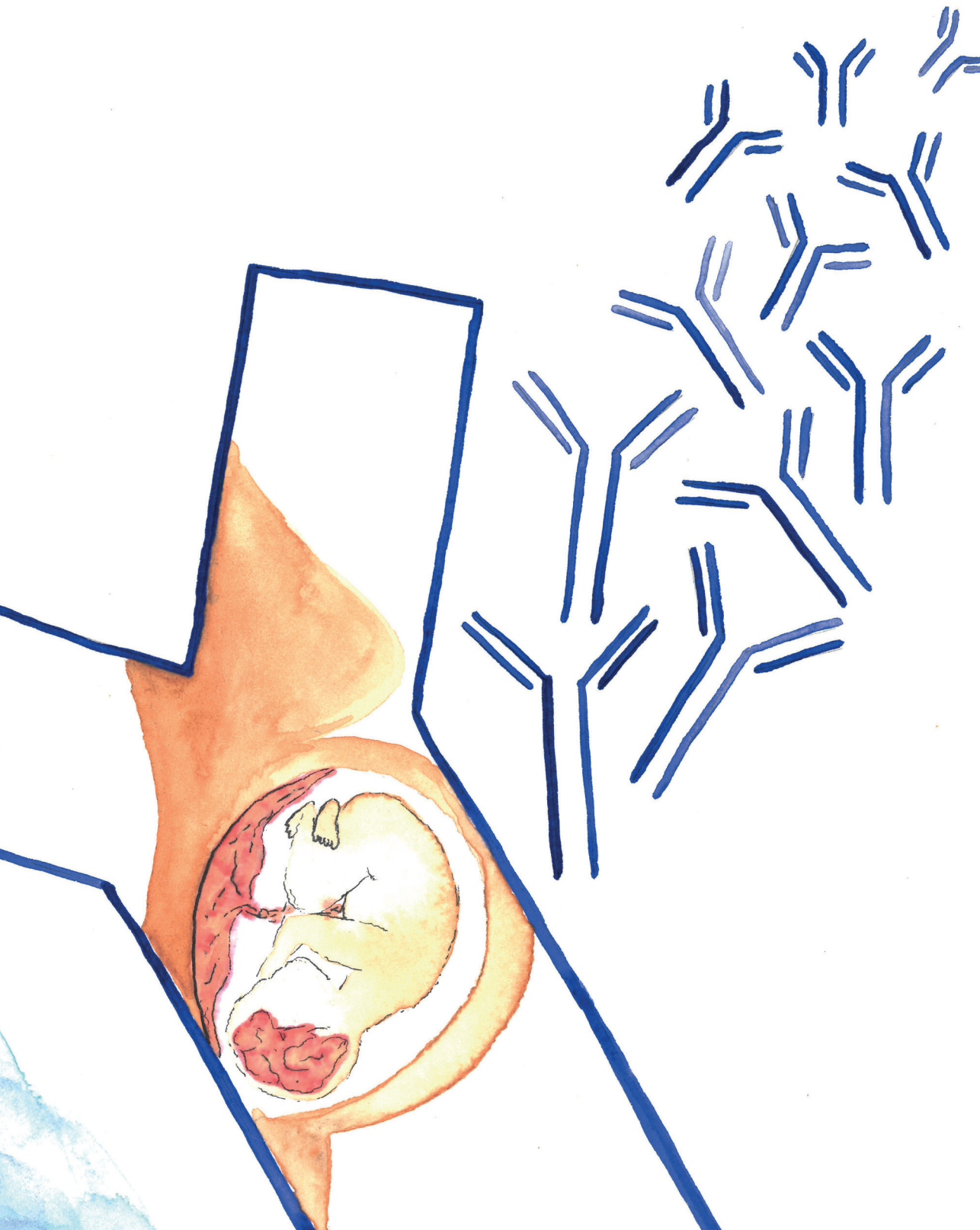
Your obstetric care giver will ask you if you are willing to participate in the HIP-study.
Your answer will be indicated on the HIP-study special check box on the blood collection form.

Background information

Our blood contains billions of cells. For example: red blood cells, white blood cells and **platelets**. All these cells express characteristics that are called blood types. Our body can produce **antibodies** against these blood types. During pregnancy a pregnant women can produce antibodies against the blood type of her child. Sometimes these antibodies are able to destroy the blood cells of the child. This can lead to disease and the need to start timely treatment.

*'**HIP**' in **HIP**-study stands for:*
HPA-screening In Pregnancy

***HPA** is an abbreviation for a blood type on platelets and means: Human Platelet Antigen.*



CHAPTER 3

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

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Submitted

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.

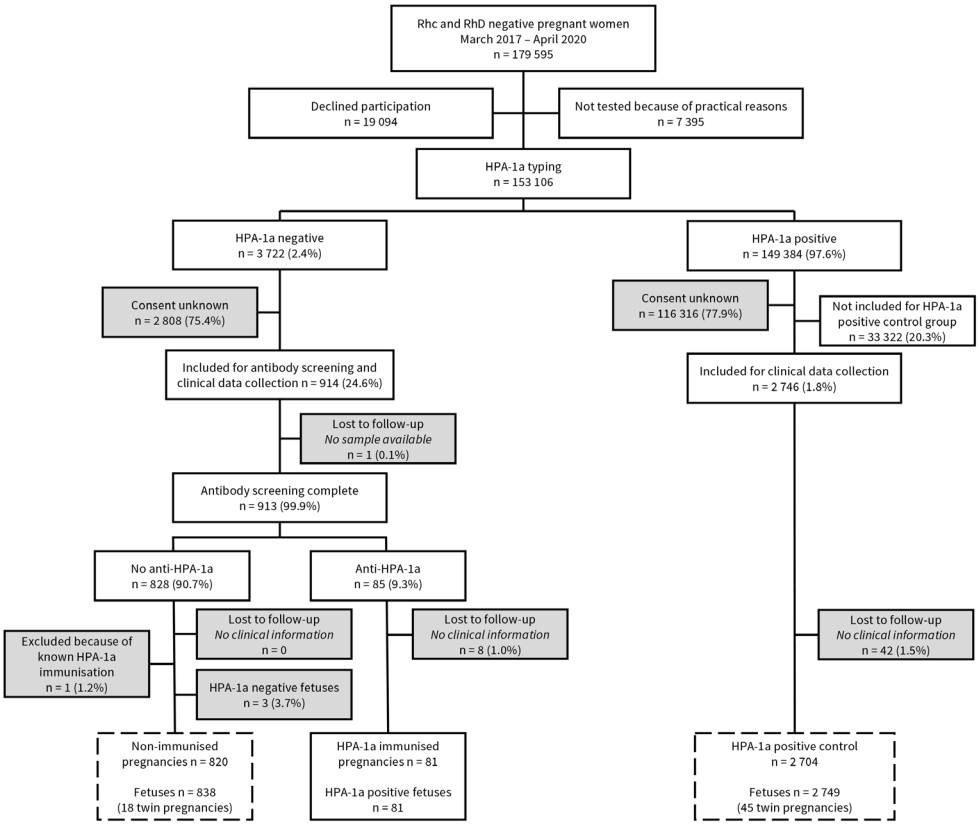

FIGURE 1. Study population
Flowchart of the study population.
Abbreviations: RhC, rhesus c; RhD, rhesus D; HPA-1a, human platelet antigen-1a.

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.

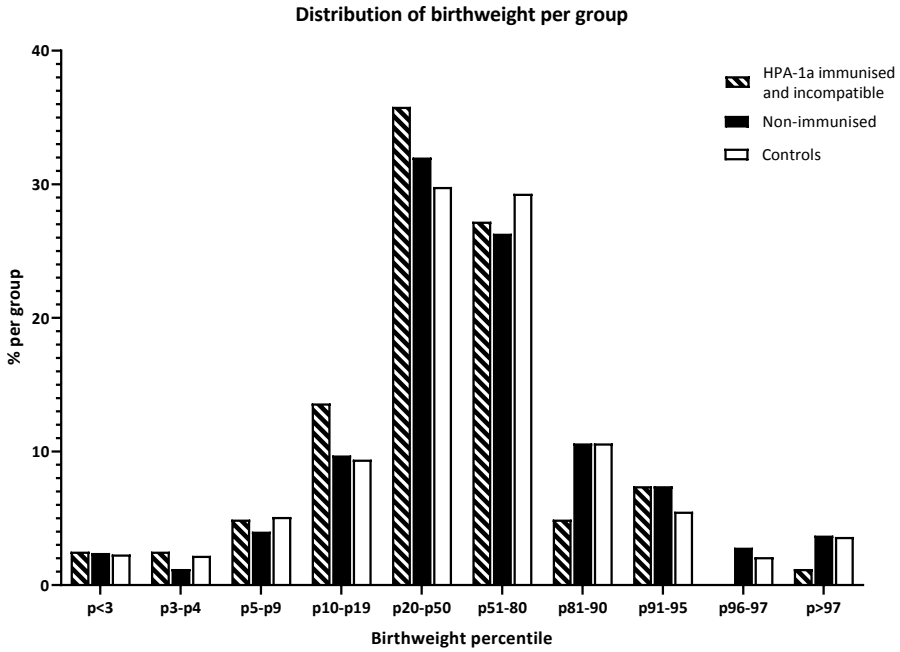

FIGURE 2. Birthweight distribution
Distribution of the birthweight percentile per study group according to the birthweight charts for the Dutch population.
Abbreviations: HPA, human platelet antigen; p, percentile

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.

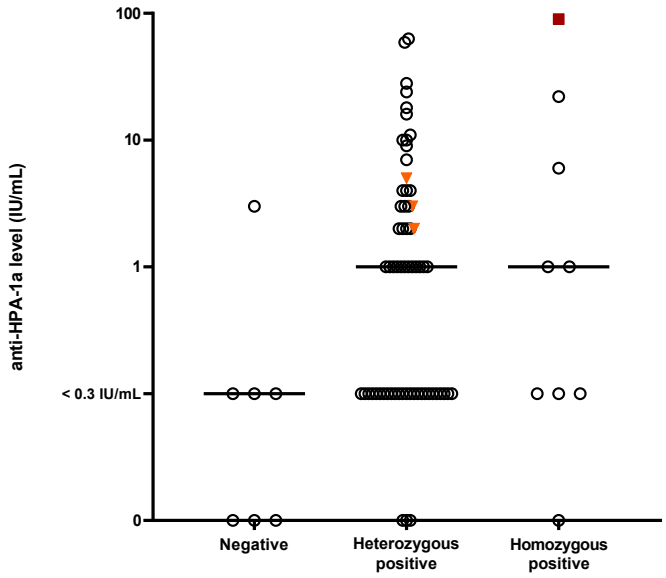

FIGURE 3. Maternal HLA DRB3*01:01 status and antibody level
Alloantibody levels as determined by the modified MAIPA stratified by maternal HLA DRB3*01:01 status. The red square dot represents the case with major bleeding. The orange triangles represent the cases with minor bleeding.
Abbreviations: HLA, 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

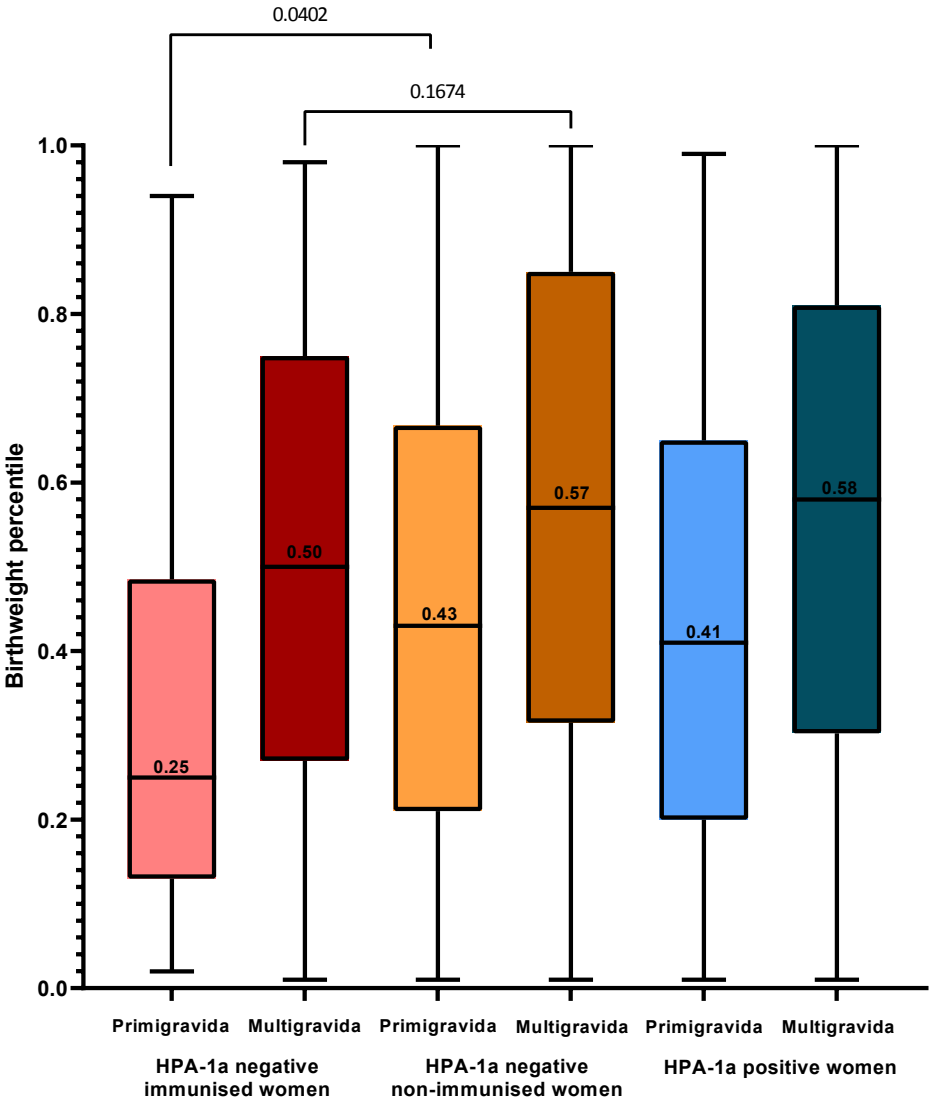
HIP	FNAIT	G/P	Obstetric history	Current pregnancy	GA at delivery	Deliver mode	Sex	BW (p)	Signs of bleeding	Clinical course	Other antibody	MFI	Quant. (IU/mL)	HLA DRB3*01:01 alleles
145974	No	G1P0		PIH	40+4	SVD	Female	3280 (25)	None			2816	1	1
148643	No	G3P1	Miscarriage (1)		41+5	SVD	Female	3718 (53)	None			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

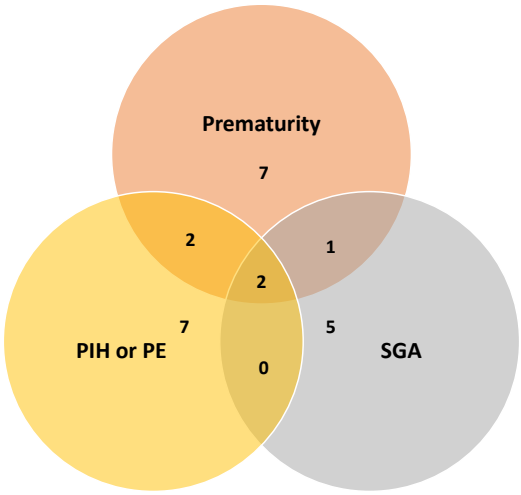
SUPPLEMENTAL TABLE 4. Obstetric history

	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)

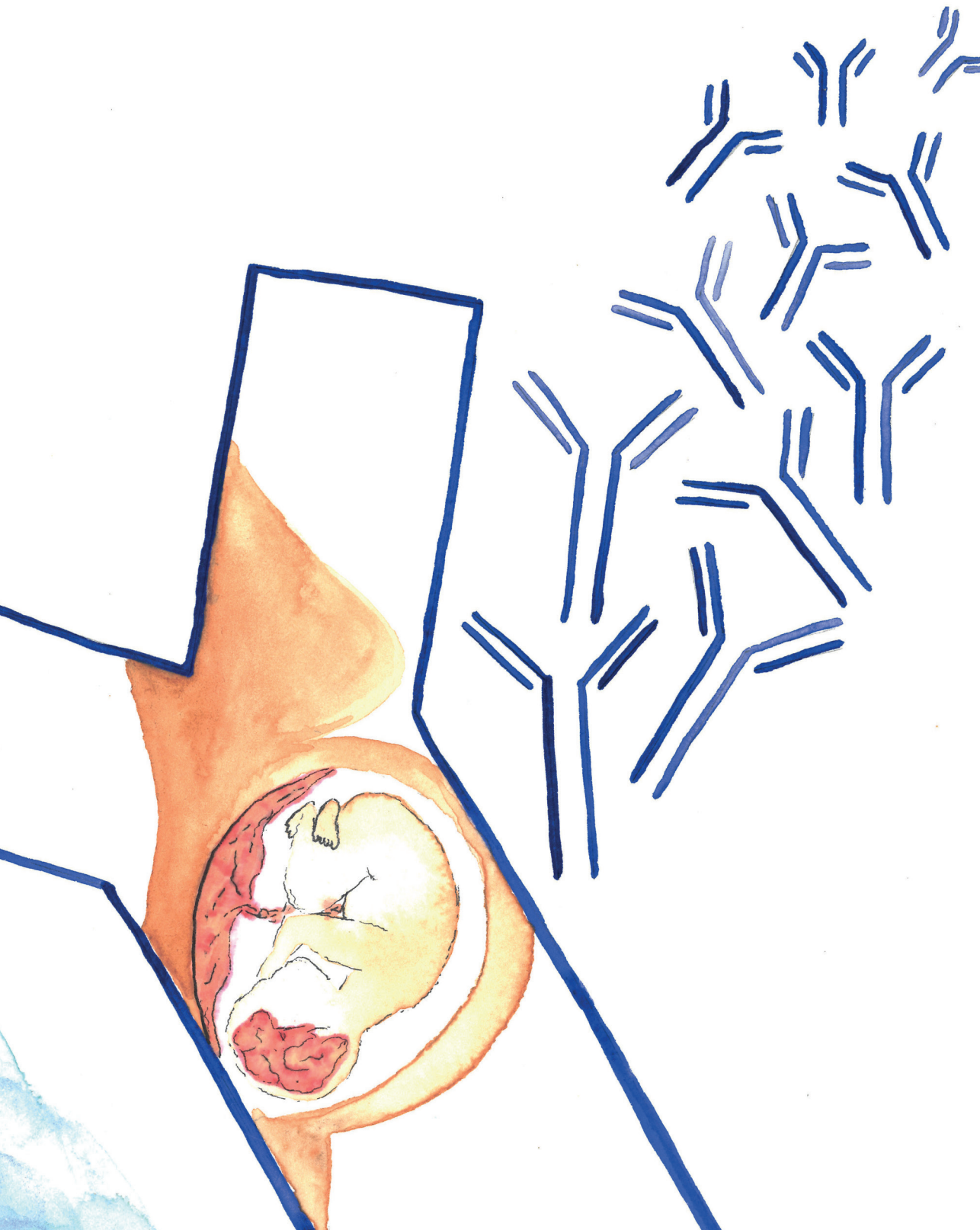
† HPA-1a immunised cases were compared with HPA-1a positive cases
‡ Primigravidae excluded
§ Nulliparae excluded
Abbreviations: HPA, human platelet antigen; IUFD, intrauterine fetal demise.



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.



CHAPTER 4

Placental complement activation in fetal and neonatal alloimmune thrombocytopenia: an observational study

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ABSTRACT

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a disease that causes thrombocytopenia and a risk of bleeding in the (unborn) child that result from maternal alloantibodies directed against fetal, paternally inherited, human platelet antigens (HPA). It is hypothesized that these alloantibodies can also bind to the placenta, causing placental damage. This study aims to explore signs of antibody-mediated placental damage in FNAIT. We performed a retrospective study that included pregnant women, their newborns, and placentas. It comprised 23 FNAIT cases, of which nine were newly diagnosed (14 samples) and 14 were antenatally treated with intravenous immunoglobulin (IVIg) (21 samples), and 20 controls, of which 10 with anti-HLA-class I antibodies. Clinical information was collected from medical records. Placental samples were stained for complement activation (C1q, C4d, SC5b-9 and mannose-binding lectin) using immunohistochemistry. Histopathology was examined according to the Amsterdam criteria. A higher degree of C4d deposition was present in the newly diagnosed FNAIT cases (10/14 samples), as compared to the IVIg treated FNAIT cases (2/21 samples, $P = 0.002$) and anti-HLA-negative controls (3/20 samples, $P = 0.006$). A histopathological examination showed delayed maturation in four (44%) placentas in the newly diagnosed FNAIT cases, five (36%) in the IVIg treated FNAIT cases and one of the controls (NS). C4d deposition at the syncytiotrophoblast was present in combination with low-grade villitis of unknown etiology in three newly diagnosed FNAIT cases that were born SGA. We conclude that anti-HPA-1a antibodies bind to the placenta and that a higher degree of classical pathway-induced complement activation is present in placentas from pregnancies with untreated FNAIT. This may affect placental function and fetal growth.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the leading cause of thrombocytopenia and bleeding tendency in otherwise healthy and term-born infants. FNAIT is caused by maternal alloantibodies directed against paternally inherited human platelet antigens (HPA).[1] Immunoglobulin G (IgG) class alloantibodies cross the placenta and bind to fetal platelets, resulting in thrombocytopenia and risk of bleeding. Bleeding complications in FNAIT are often caused by HPA-1a specific alloantibodies and vary from minor skin bleedings to severe intracranial hemorrhages (ICH) leading to lifelong neurological impairment or death.[2] Administration of intravenous immune globulins (IVIg) to the mother during pregnancy can prevent the hemorrhagic complications due to FNAIT.[3] However, since FNAIT is predominantly diagnosed after birth, prenatal treatment can usually only be administered in subsequent pregnancies.

The HPA-1a/HPA-1b alloantigenic epitopes are formed due to a single nucleotide substitution (C29523T), resulting in a Leu33Pro amino acid polymorphism within the integrin β3 subunit. Platelets express high levels of HPA-1a at the integrin β3 on the fibrinogen receptor (α2β3, glycoprotein IIbIIIa, CD41/CD61) and at a much lower level on the vitronectin receptor (αvβ3, CD51/CD61). Platelet-directed antibodies may lead to antibody-mediated destruction of senzitised cells, e.g. leading to thrombocytopenia, and also impair the function of these integrins.[4-6] Trophoblast cells and endothelial cells express high levels of the vitronectin receptor, and interference of HPA-1a antibodies with their cellular function is likely involved in the bleeding tendency.[7] In a Norwegian cohort study,[8] the presence of anti-HPA-1a in maternal serum was associated with a reduced birthweight in infants diagnosed with FNAIT, which was also reported in other cohorts.[9, 10] Furthermore, immune-induced placental dysfunction was observed in murine FNAIT models, in which the mice had high levels of anti-β3 antibodies and showed fetal growth restriction (FGR) and miscarriages.[11] Yougbaré *et al*.[12] showed abnormal placental vascularisation and poor placental perfusion resulting in FGR in these mice.

Antibodies in pregnancies complicated by antiphospholipid syndrome or systemic lupus erythematosus (SLE) can also bind to trophoblast cells, which results in classical pathway complement activation.[13] In these pregnancies, complement activation is associated with adverse pregnancy outcomes, such as fetal loss and children born small for gestational age.[14, 15] On the basis of these observations, we hypothesized that placental classical route complement activation might also occur in FNAIT and could lead to placental dysfunction. The aim of this study was to explore the presence of classical route complement activation and histopathological abnormalities in placentas from FNAIT cases.

MATERIALS AND METHODS

STUDY COHORT AND PLACENTA COLLECTION

A total of 43 placentas were included in this study; they were categorized into four groups: newly diagnosed FNAIT cases (n = 9, Group 1), antenatally IVIg-treated FNAIT cases (n = 14, Group 2), and controls with and without anti-HLA class I antibodies (n = 10, Group 3 and n = 10 Group 4, respectively). Only FNAIT cases caused by anti-HPA-1a antibodies were included. Newly diagnosed FNAIT cases (Group 1) were identified upon diagnostic testing at Sanquin Diagnostics, Amsterdam, The Netherlands. Cases were selected from a cohort of 77 cases diagnosed between January 2006 and January 2017; 22% of the new-borns with newly diagnosed anti-HPA-1a mediated FNAIT were small for gestational age.[9] Pathology departments were contacted to request placenta material. All cases with available material were included. In total, nine placentas of newly diagnosed FNAIT cases were available and could be included in Group 1. These placentas had initially been evaluated for other clinical reasons: small for gestational age (SGA) (n = 3), fetal distress during delivery (n = 2), a previous mola pregnancy (n = 1), suspected abruptio placentae (n = 1), premature delivery (n = 1) and a suspected congenital infection (n = 1). Group 2 consisted of 14 placentas from pregnancies complicated by FNAIT of which mothers received IVIg treatment at the Leiden University Medical Center (LUMC). Cases were identified because they were included in a randomized trial comparing low dose IVIg (0.5 g/kg/week, n = 7, 10 samples) versus standard dose IVIg (1 g/kg/week, n = 7, 11 samples).[16]

Twenty controls were selected from a cohort of cases of uncomplicated pregnancies that resulted in the delivery of a healthy child at the obstetric department of the LUMC or in the affiliated hospitals in the region. Placentas were stored after informed consent for research purposes: furthermore storage of the placenta material, HLA typing, and HLA antibody screening took place for all these cases. Although the role of anti-HLA class I antibodies in FNAIT is the subject of scientific debate, anti-HLA class I reactive antibodies can bind to platelets, immune cells and endothelial cells in the placenta.[17] Ten controls were selected based on the presence of anti-HLA class I type antibodies and a new-born positive for the targeted antigens, these cases were categorized as Group 3. Ten controls were selected based on the absence of anti-HLA class I or II antibodies and placed in Group 4. All mothers in Group 3 and 4 were genotyped and were HPA-1a positive.

CLINICAL DATA COLLECTION AND DEFINITIONS

Clinical data concerning obstetric and neonatal treatment were collected from the medical records. A low placental weight was defined as a weight below the 10th percentile.[18] Small for gestational age (SGA) was defined as a birthweight below the 10th percentile according to the Dutch reference curves for birthweight.[19] Clinical data collection was performed separately from laboratory experiments; data were de-identified and linked to a study number by an independent research nurse.

ETHICS

Ethical approval was provided by the medical ethical committee Leiden-Delft-The Hague for cases by study protocol B18.033 and for the controls by protocol P13.084. Placentas and serum samples of control cases were collected after informed consent.

HPA ANTIBODY DETECTION

FNAIT was diagnosed by the presence of fetal or neonatal thrombocytopenia and/or bleeding symptoms in presence of fetal-maternal HPA incompatibility and HPA alloantibodies. HPA incompatibility was confirmed by maternal, paternal and/or neonatal genotyping. HPA alloantibody screening was performed using PIFT (platelet immunofluorescence test) and MAIPA (monoclonal antibody immobilization of platelet antigens assay) as described by Porcelijn *et al*. [20].

HLA ANTIBODY DETECTION AND HLA TYPING

Maternal anti-HLA antibodies were detected and typed using the Luminex Single bead Antigen assay (Lifecodes, Immucor, Norcross, United States of America) for HLA class I and II at either the Department of Immunogenetics, Sanquin, Amsterdam (Group 1 and 2) or the Department of Immunology at the LUMC (Group 3 and 4). If HLA antibodies were present in the maternal serum, low-resolution genotyping was performed using PCR-SSP and sequence-based typing to determine whether or not the maternal HLA alloantibodies were child-specific.

HISTOPATHOLOGY

Formalin-fixed paraffin-embedded (FFPE) tissues (one slide of the umbilical cord, one of the membranes, and at least two sections of normal placental parenchyma) were H&E stained according to standardized protocol.[21] Histopathology was reviewed according to the Amsterdam criteria by two experienced perinatal pathologists (PGKN and LEvdM). The pathologists were blinded to clinical information, except for gestational age.

IMMUNOHISTOCHEMISTRY

The presence of complement proteins was investigated using immunohistochemistry for C1q, C4d, membrane attack complex (MAC, SC5b-9) and mannose-binding lectin (MBL). Detailed protocols are reported in Supplemental Table 2. In brief, sections were deparaffinized and antigen retrieval was performed. After blocking for endogenous peroxidase, the sections were incubated with the primary antibody. Binding of the primary antibody was visualized with the appropriate secondary antibody (Dako Envision+) and diaminobenzidine as a chromogen. Isotype specific controls were used as negative controls (Supplemental Table 2). Sections were counterstained with hematoxylin.

QUANTIFICATION OF IMMUNOHISTOCHEMICAL STAINING

The immunohistochemical staining was scored semi-quantitatively as either absent (<10%), focal (10% - 50%), or diffuse (>50%) by two blinded observers (TWdV and RDMvB). In case of discrepancy between the scores, consensus was reached in the presence of an independent blinded third researcher (MB). Examples for the semi-quantitative score can be found in Supplemental Figure 3.

STATISTICAL ANALYSIS

Data analysis was performed using IBM SPSS Statistics 25.0 (Chicago, IL, USA). Descriptive statistics were used to report clinical characteristics by proportions and medians with interquartile ranges. Quantitative clinical characteristics were compared by the Kruskal–Wallis analysis. Categorical parameters were compared by the Chi-square analysis or Fisher Exact Test as appropriate. An ordinal logistic regression model was used for the comparison of complement deposits in the placenta. This statistical model was chosen because in some FNAIT cases only one sample was available instead of two.

TABLE 1. Clinical characteristics

	Group 1 FNAIT cases Newly diagnosed n = 9	Group 2 FNAIT cases IVIg-treated n = 14	Group 3 Controls anti-HLA I positive n = 10	Group 4 Controls anti-HLA I negative n = 10
Maternal characteristics				
Maternal age (years) – median (IQR)	31 (29 – 34)	33 (31 – 36)	33 (31 – 35)	33 (30 – 39)
Gravidity – median (IQR)	2 (1 – 2)	3 (2 – 4)	3 (2 – 3)	2 (1 – 4)
Parity – median (IQR)	0 (0 – 1)	1 (1 – 2)	1 (0 – 1)	1 (0 – 1)
Multiparous women – n (%)	6 (67%)	14 (100%)	9 (90%)	7 (70%)
Pre-eclampsia – n (%)	0	0	0	0
Delivery				
Spontaneous vaginal delivery – n (%)	1 (11%)	8 (57%)	4 (40%)	4 (40%)
CS fetal distress – n (%)	3 (33%)	1 (7%)	0	0
GA at delivery (weeks^{+days})** – median (IQR)	37^{+1} (33^{+5} – 40^{+5})	38^{+3} (37^{+2} – 38^{+6})	39^{+4} (39^{+1} –40^{+4})	37^{+1} (38^{+4} – 40^{+2})
Neonatal data				
Sex (male) – n (%)	5 (56%)	7 (50%)	5 (50%)	5 (50%)
Birthweight (grams)** – median (IQR)	2405 (2099 – 3535)	3133 (2674 – 3493)	3700 (3319 – 3805)	3323 (3214 – 3814)
Small for gestational age* – n (%)	4 (44%)	1 (7%)	0	0
Skin bleeding only* – n (%)	4 (44%)	0	0	0
Intracranial hemorrhage* – n (%)	2 (22%)	1 (7%)ϕ	0	
Perinatal asphyxia – n (%)	1 (11%)	0	0	0
Platelet count nadir (x 10^9/l)** – median (IQR)	17 (9 – 43)	64 (21 – 170)	NT	0
HPA alloantibodies				
Mother HPA-1a negative – n (%)	9 (100%)	14 (100%)	0	0
Fetus HPA-1a positive – n (%)	9 (100%)	14 (100%)	NT	NT
Antibodies directed against;			NT	NT
HPA-1a – n (%)	6 (67%)	14 (100%)		
HPA-1a and 3a – n (%)	1 (11%)			
HPA-1a and 5b – n (%)	2 (22%)			
Anti-HLA class I present fetus-specific † – n (%)	4 (57%), 2 missing	4 (44%), 5 missing	10 (100%)	0
Placenta characteristics¶	n = 9	n = 9	n = 7	n = 8
Placenta weight (grams) – median (IQR)	460 (268 – 636)	500 (440 – 577)	572 (420 – 790)	610 (533 – 730)
Placenta weight < p10* – n (%)	4 (44%)	1 (7%)	0	0

*Data show a statistically significant difference ($P < 0.05$) when compared with the pooled controls by the Chi-Square test.

** Data show a statically significant difference ($P < 0.05$) when compared with the controls by Kruskal–Wallis analysis.

Φ Intracranial hemorrhage developed at 27 weeks' gestational age before administration of intravenous immune globulins to the mother was started.

† Assessed in 7/9/10/10 cases, missing values for 7 (16%) cases due to lack of serum/DNA.

¶ Assessed in 9/9/7/8 cases, missing values for 10 (23%) cases.

Abbreviations: FNAIT, fetal neonatal alloimmune thrombocytopenia; IVIg, intravenous immune globulins; HLA, human leukocyte antigen; CS, caesarean section; GA, gestational age; l, liter; HPA, human platelet antigen p, percentile.

RESULTS

CLINICAL CHARACTERISTICS

Clinical characteristics are shown in Table 1. Newly detected FNAIT cases (Group 1) and IVIg treated FNAIT cases (Group 2) were comparable to controls in terms of maternal age, gravidity, parity, and presence of pre-eclampsia. Gestational age at delivery was lower in both the newly detected FNAIT cases (Group 1) and IVIg-treated FNAIT cases (Group 2) compared to controls. ICH was observed in one of the IVIg-treated FNAIT cases (Group 2); this ICH was detected by ultrasound at 27 weeks' gestational while the IVIg-treatment was planned to start at 28 weeks. Placental weight was below the 10th percentile in four (44%) newly diagnosed FNAIT cases, one (7%) FNAIT IVIg-treated case and none of the controls ($P = 0.022$). Three of these four cases were SGA.

C4D DEPOSITION ON SYNCYTIOTROPHOBLAST IS INCREASED IN NEWLY DETECTED FNAIT CASES

Placental complement deposition was scored semi-quantitatively; representative examples are shown in Supplemental Figure 1. More C4d deposition was present at the syncytiotrophoblast in newly diagnosed FNAIT cases (Group 1; 10/14 samples), as compared to IVIg-treated FNAIT cases (Group 2; 2/21 samples; $P = 0.002$) and controls without anti-HLA class I antibodies (Group 4; 3/20 samples; $P = 0.006$; Figure 1A). C4d can be regarded as the footprint of complement activation, which may be either induced trough the classical route (e.g. antibody-mediated) or lectin pathway activation. C1q deposition, a marker for the classical pathway of complement activation, was equally present in all study groups in either a focal (20-45%) or diffuse (20-57%) staining pattern (Figure 1B). In 11 (92%) of the samples from FNAIT cases (Group 1 and 2) with C4d deposition, C1q deposition was also present. Deposition of MBL, which indicates complement system activation via the lectin route, was observed only once (Figure 1C). The staining pattern for ongoing complement activation resulting in the formation of a membrane attack complex (MAC) did not differ between the study groups (Figure 1D). In 41% (5/12) of the samples from FNAIT cases (Group 1 and Group 2) with C4d deposition at the syncytiotrophoblast, MAC was also present. C4d deposition was present in all four placentas of newly detected FNAIT children born SGA and absent in the IVIg-treated FNAIT case born SGA. In the samples from FNAIT cases (Group 1 and 2), C4d deposition was equally present in placentas from term (6/6) and preterm (2/4) pregnancies (GA < 37 weeks). No relationship was observed between the mode of delivery and C4d deposition at the syncytiotrophoblast.

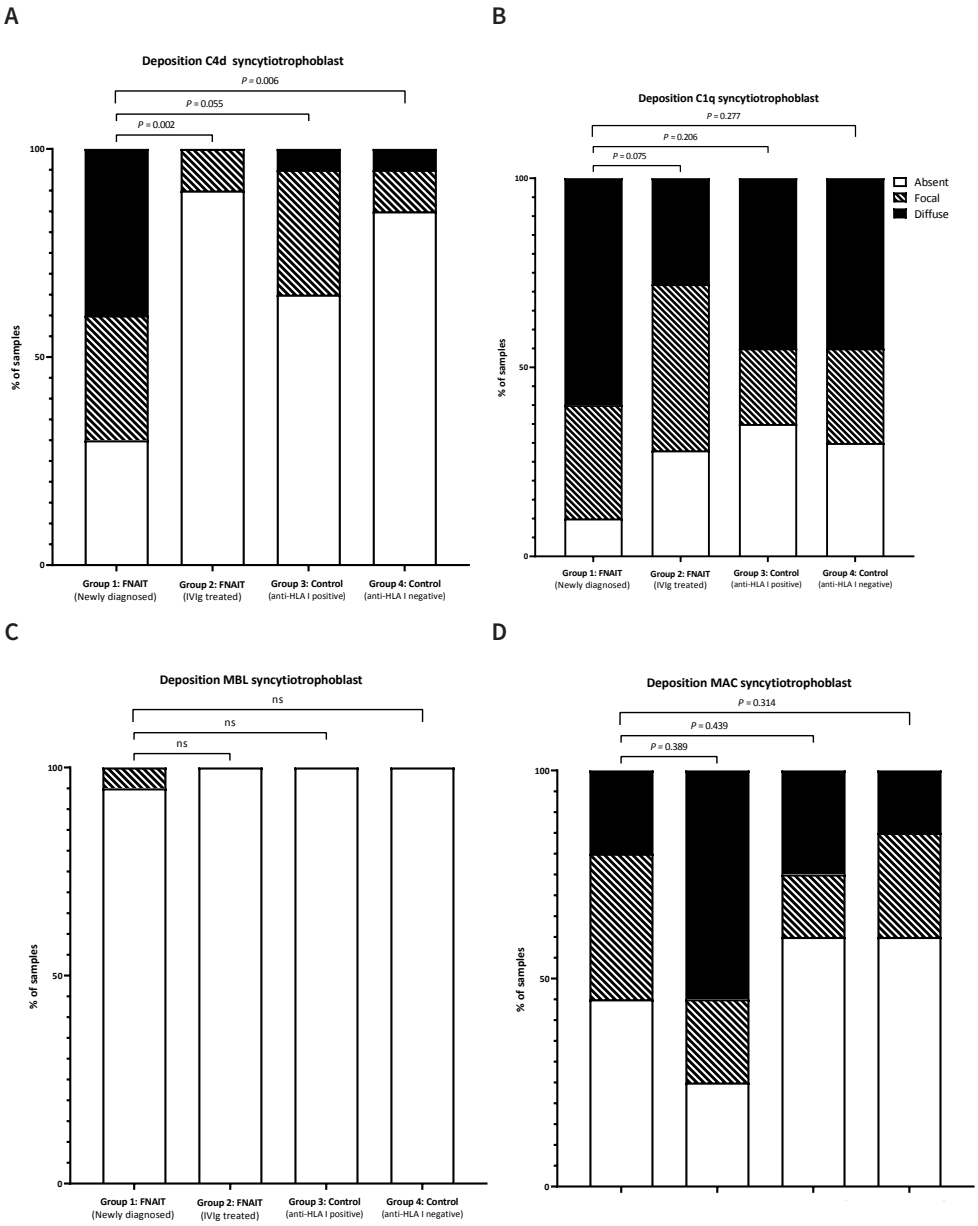

FIGURE 1. Semi-quantitative scoring of complement deposition syncytiotrophoblast

All complement depositions shown by immunohistochemistry at the syncytiotrophoblast were scored semi-quantitatively as absent (<10%), focal (10-50%) or diffuse (>50%). Figure **A** summarizes the scoring of C4d deposition at the syncytiotrophoblast. In Figure **B** scoring of C1q deposition, in Figure **C** scoring of MBL deposition and in Figure **D** scoring of MAC deposition are summarised. Ordinal logistic regression was used to compare complement deposition score between the groups.

Abbreviations: FNAIT, fetal neonatal alloimmune thrombocytopenia; IVIg, intravenous immune globulins; HLA, human leukocyte antigen; MBL, mannose-binding lectin; MAC, membrane attack complex.

The presence of complement depositions in the fetal vessels is shown in Supplemental Figure 3. The pattern of C4d deposition in the fetal vessels was only different between the newly diagnosed FNAIT cases (Group 1; 50% focal, 21% diffuse) as compared to the IVIg-treated FNAIT cases (Group 2; 62% focal, 10% diffuse; *P* = 0.012). C4d deposition was observed to a similar extent, though with different distribution, in controls. C1q deposition in the fetal vessels was more often diffuse (25% focal, 75% diffuse) in the control group with anti-HLA class I antibodies (Group 3) as compared to the newly detected FNAIT cases (Group 1; 71% focal, 21% diffuse; *P* = 0.003). We did not observe other significant differences.

PLACENTA MATURATION WAS DELAYED IN NEWLY DIAGNOSED FNAIT CASES

Histopathological findings in the placental tissue are described in Table 2. In four placentas of newly diagnosed FNAIT cases (Group 1; 4/9 cases; 44%) and five of the IVIg-treated FNAIT cases (Group 2; 5/14 cases; 36%), a delayed placental maturation was observed whereas this was not observed in any of the controls (*P* = 0.118). In three of the nine cases (Group 1 and 2; 3/9 cases; 33%) with delayed maturation, the presence of C4d at the syncytiotrophoblast was detected. In one placenta of the newly diagnosed FNAIT cases with delayed maturation the infant was born SGA. In both Group 1 and Group 2, three cases showed maternal vascular malperfusion (*P* = 0.156). Low-grade villitis of unknown etiology was observed in all groups. In all three cases with low-grade villitis of unknown etiology in the group of newly diagnosed FNAIT cases (Group 1), C4d deposition at the syncytiotrophoblast was observed and these three infants were all born SGA. Mild signs of fetal hypoxia were seen in two of the newly diagnosed FNAIT cases (Group 1; 2/9 cases; 22%) and in one of the IVIg-treated FNAIT cases (Group 2, 1/14 cases; 7%; *P* = 0.475). Decidual arteriopathy, chronic intervillositis and vascular necrosis were not seen in any of the cases nor in the controls. An overview of clinical characteristics, complement deposition and histopathological findings for all individual FNAIT cases is listed in Supplemental Table 2.

TABLE 2. Placenta histopathology

Pathology	Group 1 FNAIT cases Newly diagnosed n=9	Group 2 FNAIT cases IVIg-treated n=14	Group 3 Controls Anti-HLA I positive n=10	Group 4 Control Anti-HLA I negative n=10
Maturation				
Delayed	4 (44%)	5 (36%)	1 (10%)	0
Corresponding with GA	3 (33%)	9 (64%)	9 (90%)	10 (100%)
Accelerated	2 (22%)	0	0	0
Maternal vascular malperfusion	**3 (33%)**	**3 (21%)**	**0**	**0**
Retroplacental hematoma	1	0	0	0
Infarction	3	0	0	0
Ischemia	0	2	0	0
Distal villous hypoplasia	1	3	0	0
Fetal vascular malperfusion	**1 (11%)**	0	0	**3 (30%)**
Avascular villi	1	0	0	3

TABLE 2. Continued

Pathology	Group 1 FNAIT cases Newly diagnosed n=9	Group 2 FNAIT cases IVIg-treated n=14	Group 3 Controls Anti-HLA I positive n=10	Group 4 Control Anti-HLA I negative n=10
Ascending intrauterine infection	**2 (22%)**	**1 (7%)**	**1 (10%)**	**2 (20%)**
Stage 1	2	1	0	2
Fetal response	0	0	1	0
Villitis of unknown etiology	**3 (33%)**	**3 (21%)**	**2 (20%)**	**1 (10%)**
Low grade focal	3	3	1	1
Low grade multifocal	0	0	1	0
Massive perivillous fibrin depositions	**0**	**1 (7%)**	**0**	**0**
Signs of fetal hypoxia	**2 (22%)**	**1 (7%)**	**0**	**0**
Mild hypoxia	2	1	0	0
Chorangiosis	**0**	**0**	**2 (20%)**	**0**
Meconium	**1 (11%)**	0	0	0

Abbreviations: FNAIT, Fetal Neonatal Alloimmune Thrombocytopenia; IVIg, intravenous immune globulin; HLA, human leukocyte antigen; GA, gestational age.

DISCUSSION

MAIN FINDINGS

The aim of this study was to explore complement activation and histopathological anomalies in the placenta of FNAIT cases. We observed an increase in C4d deposition at the syncytiotrophoblast in newly diagnosed FNAIT cases (10/14 samples), as compared to antenatally IVIg treated FNAIT cases (2/21 samples) and healthy controls (P = 0.006). C4d deposition was present at the syncytiotrophoblast in all infants born SGA with newly diagnosed FNAIT, but not in the SGA new-born from a pregnancy treated with IVIg. Histopathological examination revealed a delayed maturation of the placenta parenchyma in 44% of the newly diagnosed FNAIT cases, 36% of the IVIg-treated FNAIT cases, and only in one of the controls (10%) (NS). Both low-grade villitis of unknown etiology and C4d deposition at the syncytiotrophoblast were observed in three out of four newly diagnosed FNAIT cases that were born SGA.

STRENGTHS AND LIMITATIONS

As a result of the retrospective nature of our study, we unfortunately had limited availability of placental tissue from newly diagnosed FNAIT cases. FNAIT is often diagnosed after delivery. The need for a clinical indication to send the placenta to pathology may have led to selection bias. This was not the case for the IVIg-treated FNAIT cases and our control cohort as these placentas were routinely stored for examination. Differences in the inclusion of our groups made it challenging to decipher whether alterations in complement deposits were due to less severe FNAIT or due to the effect of IVIg treatment. The inclusion of newly diagnosed FNAIT cases was based on the nationwide Dutch retrospective cohort study (2006-2017).[9] A strength of our study is that the pathology departments of all referring hospitals were

contacted to retrieve the maximum amount of placenta material available. We previously reported that 22% of the infants in the nationwide Dutch retrospective cohort were SGA;[9] therefore the number of SGA in this newly diagnosed FNAIT cohort (44%) does not appear to be an overrepresentation. Furthermore, Buurma *et al*.[22] found a comparable amount of C4d depositions in IUGR and control placentas. Thus, SGA was suggested to not be associated with C4d deposition in the placenta. A limitation of our study is that the baseline characteristics between groups were slightly different. FNAIT cases had a lower gestational age at birth, likely due to the routine near-term induction in this group. When comparing term and preterm cases, however, our main outcome measure of C4d deposition was found to be equally present in both groups. Similar to our study, Buurma *et al*.,[22] also did not report an association between placental complement activation and the mode of delivery.

INTERPRETATION

In line with other antibody-mediated disorders, such as the antiphospholipid syndrome and SLE, we hypothesized that anti-HPA-1a binding could lead to classical pathway complement activation in the placenta during FNAIT pregnancies.[13, 15] The complement system is tightly regulated in the placenta. C1q is reported to be an important factor in physiological trophoblast migration.[23] The balance between an active role of C1q and prevention of ongoing complement activation is controlled by complement regulatory proteins, which inhibit the formation of MAC on the membrane surface.[24]

In the newly diagnosed FNAIT cases, a significantly higher degree of C4d deposition was observed at the maternal interface of the placenta of newly diagnosed FNAIT cases compared to IVIg treated FNAIT pregnancies (P = 0.002) and anti-HLA antibody-negative controls (P = 0.006). This may be related to the high IgG levels in the maternal circulation of the IVIg-treated women, which could have reduced anti-HPA-1a antibody levels.[25] We did not observe increased staining for MAC at the syncytiotrophoblast in the newly diagnosed FNAIT cases. This may be explained by the notion that the complement system is tightly regulated in the placenta by complement inhibitory proteins.[24] For instance, membrane cofactor protein (CD46), decay-accelerating protein (CD55), and MAC-inhibitory protein (CD59) inhibit the continuation of the complement cascade beyond C4b formation.[26] C4d is seen as a footprint of complement activation, whereas MAC deposition can be transient.[13] Moreover, C4d is an accepted biomarker in antibody-mediated transplant rejection and is also recognized to have a role in antibody-mediated pregnancy complications,[13] to which HPA-1a alloimmunization may be added based on our current results.

It is known that subtypes of HPA-1a-directed antibodies can bind to endothelial cells.[4, 6] In contrast to the differences in complement depositions between the groups at the syncytiotrophoblast no differences were observed in complement depositions between the fetal vessels of the FNAIT cases and the controls. Several factors may explain these

differences between the syncytiotrophoblast and fetal vessels, such as lower antibody levels in the fetus, lower availability of complement proteins on the fetal vessel side, or differences in the expression levels of complement regulatory proteins at the surface of endothelial cells and the syncytiotrophoblast.

Delayed placental maturation was found in 44% of the newly diagnosed cases, 36% of the IVIg-treated FNAIT cases and only once in the control group (10%). Delayed maturation may be caused by villitis due to the binding of alloantibodies to trophoblasts, which may lead to altered growth and maturation of the terminal villi. Delayed maturation is associated with fetal hypoxia[27] and was also found in the placentas of β3-immunised mice.[12] C4d deposition and low-grade villitis of unknown etiology were present in three out of four newly diagnosed FNAIT cases born SGA. This finding suggests a possible correlation between complement deposition and villitis with fetal growth restriction in FNAIT.[28] Our sample size, however, was relatively small and low grade villitis of unknown etiology was also present in some of the control cases, indicating that this finding should be interpreted with caution. In other cohort studies that assessed placental histopathology in FNAIT, chronic villitis, chronic chorioamnionitis and chronic intervillositis were observed.[29, 30] Aberrant histopathology was less pronounced in our FNAIT cases as compared to the findings of these studies, however, median gestational age at delivery was 34 weeks in the French study as compared to 37 weeks in our study which may explain the histopathological differences.[30]

FUTURE PERSPECTIVES

In earlier studies, the expression of the β3-integrin on the syncytiotrophoblast and extravillous trophoblasts was shown.[12, 31] In these studies an antibody directed against the β3-integrin was used. Eksteen *et al.*[32] showed binding of the HPA-1a antibody to the αvβ3 receptor on isolated trophoblasts. To further support our hypothesis that binding of anti-HPA-1a can lead to placenta damage, future research should focus on demonstrating binding of anti-HPA-1a to the syncytiotrophoblast. On the basis of our observations of increased classical route complement activation in FNAIT placentas, in combination with the histopathological findings, it is imperative to perform additional investigations in the placentas of HPA-1a negative women with and without alloantibodies and in infants born SGA.

CONCLUSION

Our findings support the hypothesis that HPA-1a antibodies affect placental function and may impair fetal growth. We demonstrated that increased classical pathway-mediated complement activation was present in placentas of pregnancies complicated by FNAIT. To what extent binding of HPA-1a antibodies to trophoblast lead to placenta dysfunction should be further explored.

FOOTNOTES

AUTHOR CONTRIBUTIONS

MdH, MB, HB and DW conceptualised and designed the research study; TV, KD, RB and MB performed experiments; TV, DW, LP, CK and MB performed clinical data collection; LM and PN performed histopathological examination, ME, CK and MvdH collected control samples; TV and MB analysed the data; TV drafted the manuscript; DW, HB, HB, LM, PG, LP, CS, GV, ME, RK, CK, LA, DO, EL, MvdH, MB and MdH critically revised the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Medical Ethical Committee Leiden-Delft-The Hague for cases by study protocol B18.033 (July 11th, 2018) and for the controls by protocol P13.084 (2013).

INFORMED CONSENT STATEMENT

Patient consent was waived for cases with FNAIT, these cases and clinical data were anonymised by a thrusted third party before inclusion. This was in complies with the Code of Proper Use by the Federation of Medical Scientific Societies (Code Goed Gebruik door de Federatie Medisch Wetenschappelijke Verenigingen in Dutch) and approved by the Ethical Committee. Placentas and serum samples of control cases were collected after informed consent.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions. Outcomes of immunohistochemistry and histopathology assessment per case can be found in Supplemental Material Table 2.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results

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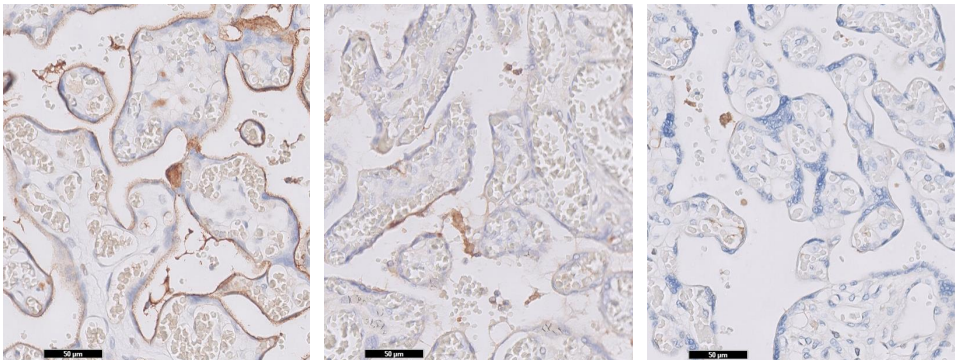
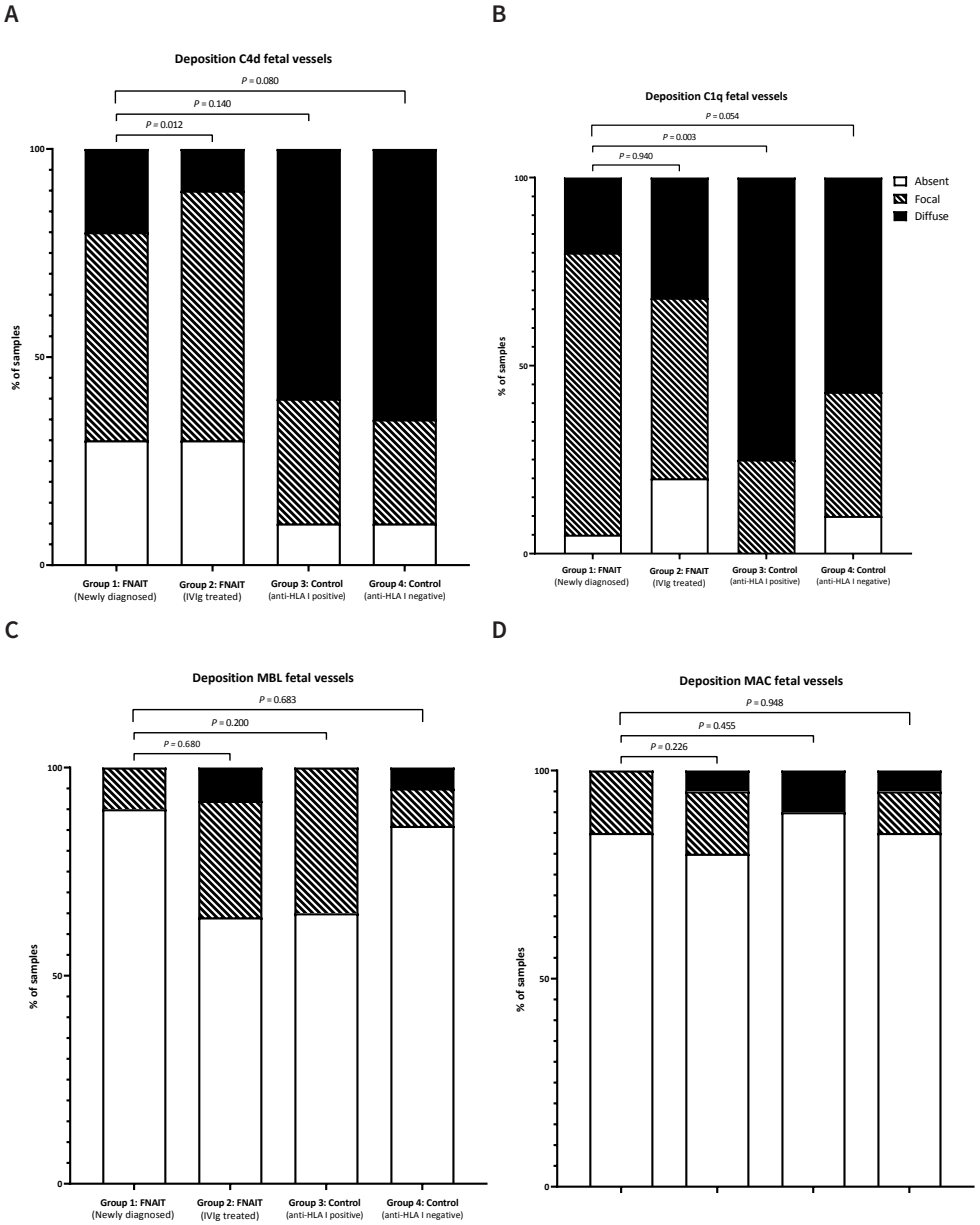
SUPPLEMENTAL MATERIALS



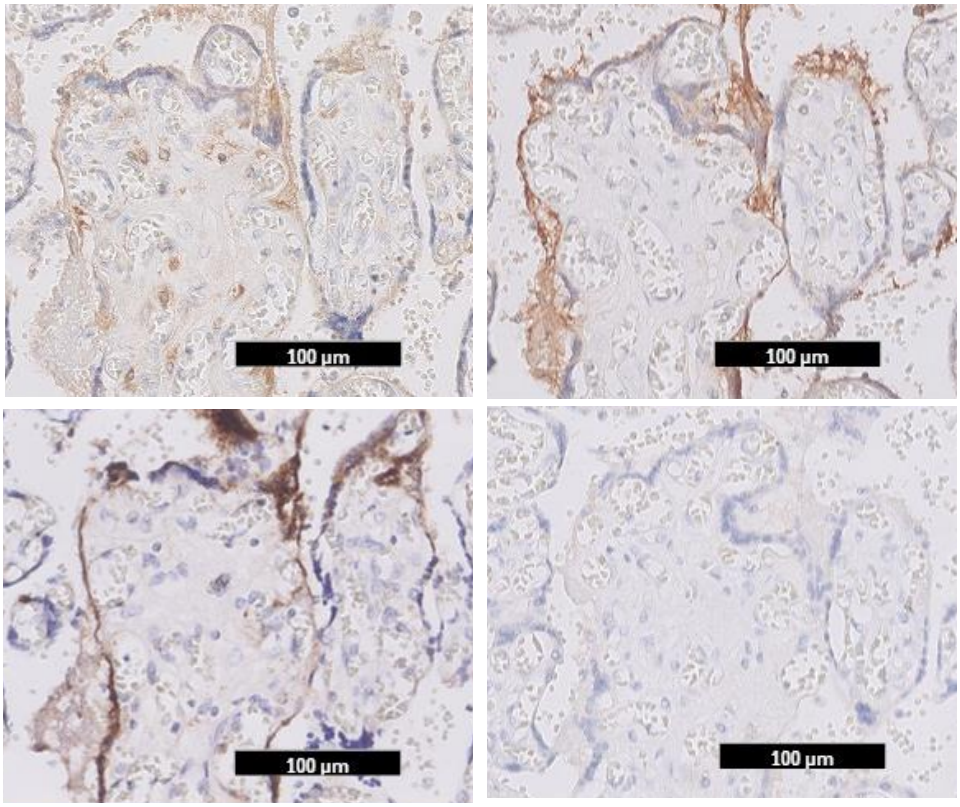
SUPPLEMENTAL FIGURE 1. Semi quantitative scoring complement depositions
Sections of placentas stained immunohistochemically for C4d, **A** shows diffuse deposition of C4d to the syncytiotrophoblast, in **B** focal binding to syncytiotrophoblast of C4d appreciated and **C** shows an example where C4d binding to syncytiotrophoblast was absent.



SUPPLEMENTAL FIGURE 2. Semi quantitative scoring of complement deposition fetal vessels

All complement depositions shown by immunohistochemistry in the fetal vessels were scored semi-quantitatively as absent (<10%), focal (10-50%) or diffuse (>50%). **Figure 2A** summarizes the scoring of C4d deposition at the fetal vessels, in **Figure 2B** scoring of C1q deposition, in **Figure 2C** scoring of MBL deposition and **Figure 2D** scoring of MAC deposition are summarized. Ordinal logistic regression was used to compare complement deposition score between the groups.

Abbreviations: FNAIT, fetal neonatal alloimmune thrombocytopenia; IVIg, intravenous immune globulins; HLA, human leukocyte antigen; MBL, mannose-binding lectin; MAC, membrane attack complex.



SUPPLEMENTAL FIGURE 3. Complement deposition in the placenta

Sequential sections of placentas stained immunohistochemically for components of the complement system. **A** shows diffuse deposition of C1q to the syncytiotrophoblast, in **B** diffuse binding of C4d to syncytiotrophoblast can be seen and **C** shows an example of binding of (MAC) SC5b-9. Figure **D** shows that the mannose-binding lectin (MBL) is absent.

SUPPLEMENTAL TABLE 1. Immunohistochemistry protocols

	C1q	C4d	MAC (SC5b-9)	MBL
Material type	Sections of paraffin embedded placenta			
Preparation/fixation	Deparaffinize			
Antigen retrieval	Heat antigen retrieval (Tris/EDTA, 10 mM, pH 9.0)	Heat antigen retrieval (Tris/EDTA, 10 mM, pH 9.0)	Enzym antigen retrieval, Prot 24 (XXIV) (Sigma Aldrich, Saint Louis, Missouri, USA, P8038-50MG)	Heat antigen retrieval (Citric acid, 10mM, pH 6.0)
Block endogenous peroxidase	H2O2 (30%) was used 1:250 in distillated water (Merck, Kenilworth, New Jersey, United States of America, 1.07209.0250)			
Block	NGS 1:20 in PBS/1% BSA (Normal goat serum, DakoCytomation, Glostrup. Denmark, X0907)			
Primary antibody	anti-C1q, Rabbit, HRP conjugated, 1:2000 (DakoCytomation, Glostrup. Denmark, A0138)	anti-C4d, Rabbit, HRP conjugated, 1:75 (Biomedica, Vienna, Austria, BI-RCD4D)	anti-SC5b-9 (neoantigen), Mouse, HRP conjugated, 1:1000 (Quidel Corporation, San Diego, California, USA, A239)	anti-MBL, Rabbit, HRP conjugated 1:500 (Sigma Aldrich, Saint Louis, Missouri, USA HPA002027)
Incubation primary antibody	60 minutes	60 minutes	Overnight	60 minutes
Isotype specific control (negative control)	Rabbit negative control fraction, (DakoCytomation, Glostrup. Denmark, X0936)	Rabbit negative control fraction, (DakoCytomation, Glostrup. Denmark, X0936)	Mouse negative control, IgG2b (DakoCytomation, Glostrup. Denmark, X0944)	Rabbit negative control fraction, (DakoCytomation, Glostrup. Denmark, X0936)
Secondary antibody	HRP anti-rabbit envision+ system (DakoCytomation, Glostrup. Denmark, K4003)	HRP anti-rabbit envision+ system (DakoCytomation, Glostrup. Denmark, K4003)	HRP anti-mouse envision+ system (DakoCytomation, Glostrup. Denmark, K4001)	HRP anti-rabbit envision+ system (DakoCytomation, Glostrup. Denmark, K4003)
Tertiary antibody	Not applicable			
Visualisation staining	DAB, diaminobenzidine (DakoCytomation, Glostrup. Denmark, K3468)			
Counterstaining	Hematoxylin			

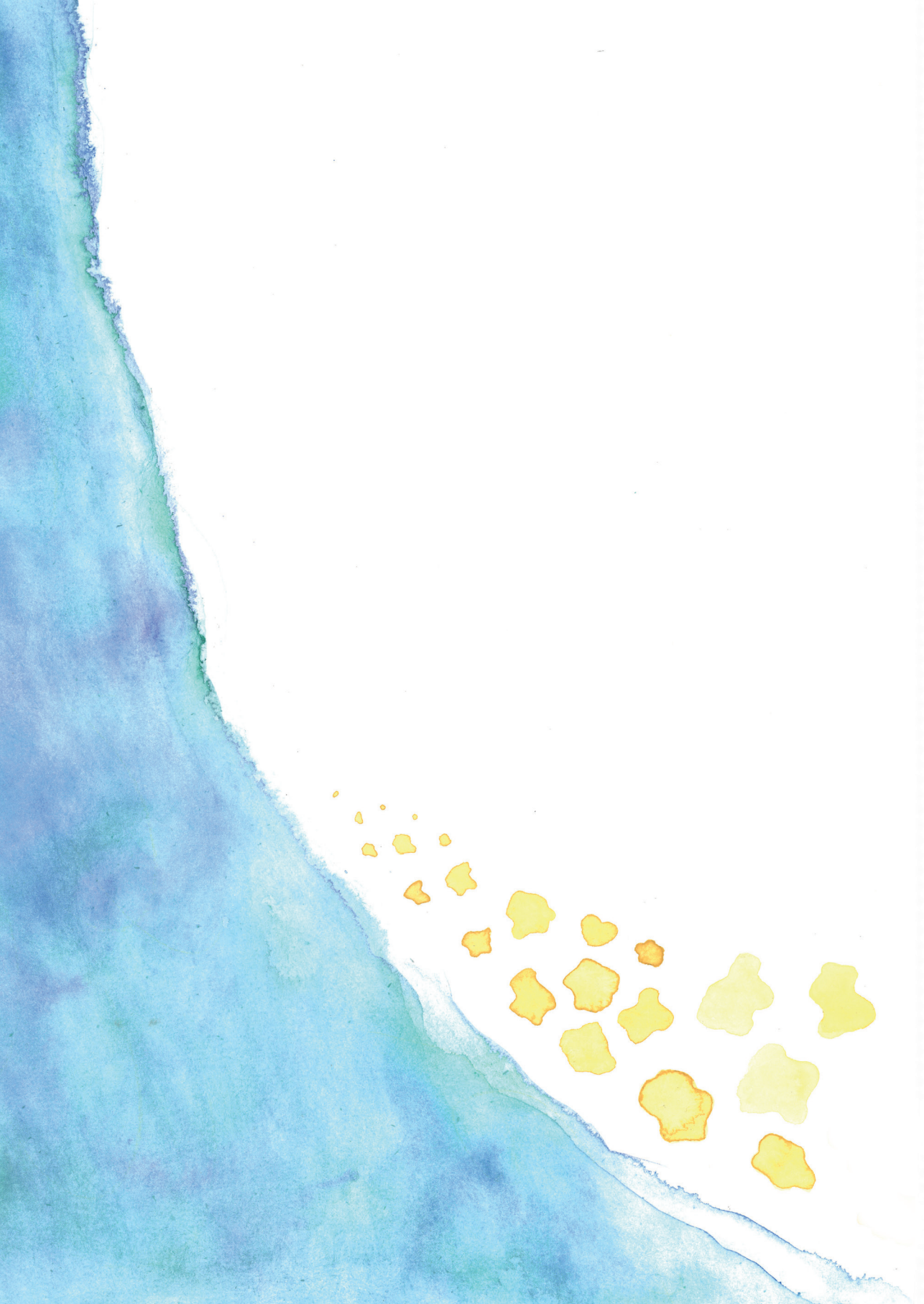
Abbreviations: MBL, mannose-binding lectin; HPA, human platelet antigen; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PBS, phosphate buffered saline; BSA, bovine serum albumin; HRP, horseradish peroxidase.

SUPPLEMENTAL TABLE 2. Clinical data and immunohistopathology per case

	Case	Age mother	G/P	GA	αHPA	αHLA	Sex	SGA	Bleed	Plt.	C1q syn	C4d syn.	MAC syn.	MBL syn.
Newly diagnosed FNAIT	1	34	G1P0	41+0	1a	No	F	No	Skin	44	D/F	F/D	A/F	A/F
	2	35	G2P0	40+1	1a	Yes*	M	Yes	None	17	D/D	D/D	D/A	A/A
	5	28	G2P1	37+1	1a+5b	Yes	M	Yes	Skin	5	D/D	D/D	A/A	A/A
	7a	30	G3P1	32+2	1a	Yes	F	NT	ICH	11	D	A	D	A
	11	33	G1P0	34+3	1a+3a	No	M	No	Skin	41	A	A	D	A
	12	31	G2P1	38+3	1a	Yes	F	Yes	Skin	10	D	F	A	A
	13	32	G1P0	35+1	1a	No	M	No	Skin	40	F/D	A/F	A/A	A/A
	14	21	G2P0	41+4	1a	Yes*	M	No	ICH	8	F/F	A/F	F/F	A/A
	15	29	G2P1	32+6	1a+5b	Yes	F	Yes	None	68	F	D	F	A
FNAIT (IVIg)	7b	32	G4P2	35+5	1a	Yes	F	No	None	22	F/D	A/A	F/F	A/A
	8	31	G3P2	38+1	1a+3a	Yes	M	No	None	266	D	A	A	-
	11	35	G3P1	37+2	1a	No	F	No	None	169	F	A	A	A
	37	37	G2P1	38+0	1a	Yes	M	No	None	78	D	A	D	A
	38	31	G5P1	39+2	1a	No	M	Yes	None	50	A	A	D	A
	39	33	G2P1	38+5	1a	NT	M	No	None	30	D	F	A	A
	40	33	G2P1	39+0	1a	No	M	No	None	85	F	A	D	A
	41	31	G3P1	38+6	1a	No	M	No	None	18	F/F	A/A	A/F	A/-
	42	30	G2P1	38+4	1a	Yes*	M	No	None	24	A/F	F/A	F/D	A/A
	43	35	G5P2	38+5	1a	No	F	No	None	8	F	A	A	A
	46	38	G3P2	37+6	1a	Yes*	F	No	None	271	A/F	A/A	D/D	A/A
	47	30	G4P1	36+0	1a	Yes*	F	No	ICH	6	-/A	A/A	D/D	A/A
	48	37	G4P2	39+2	1a	NT	F	No	None	172	A/A	A/A	D/D	A/A
	50	26	G2P1	37+0	1a	Yes	F	No	None	164	A	A	A	A

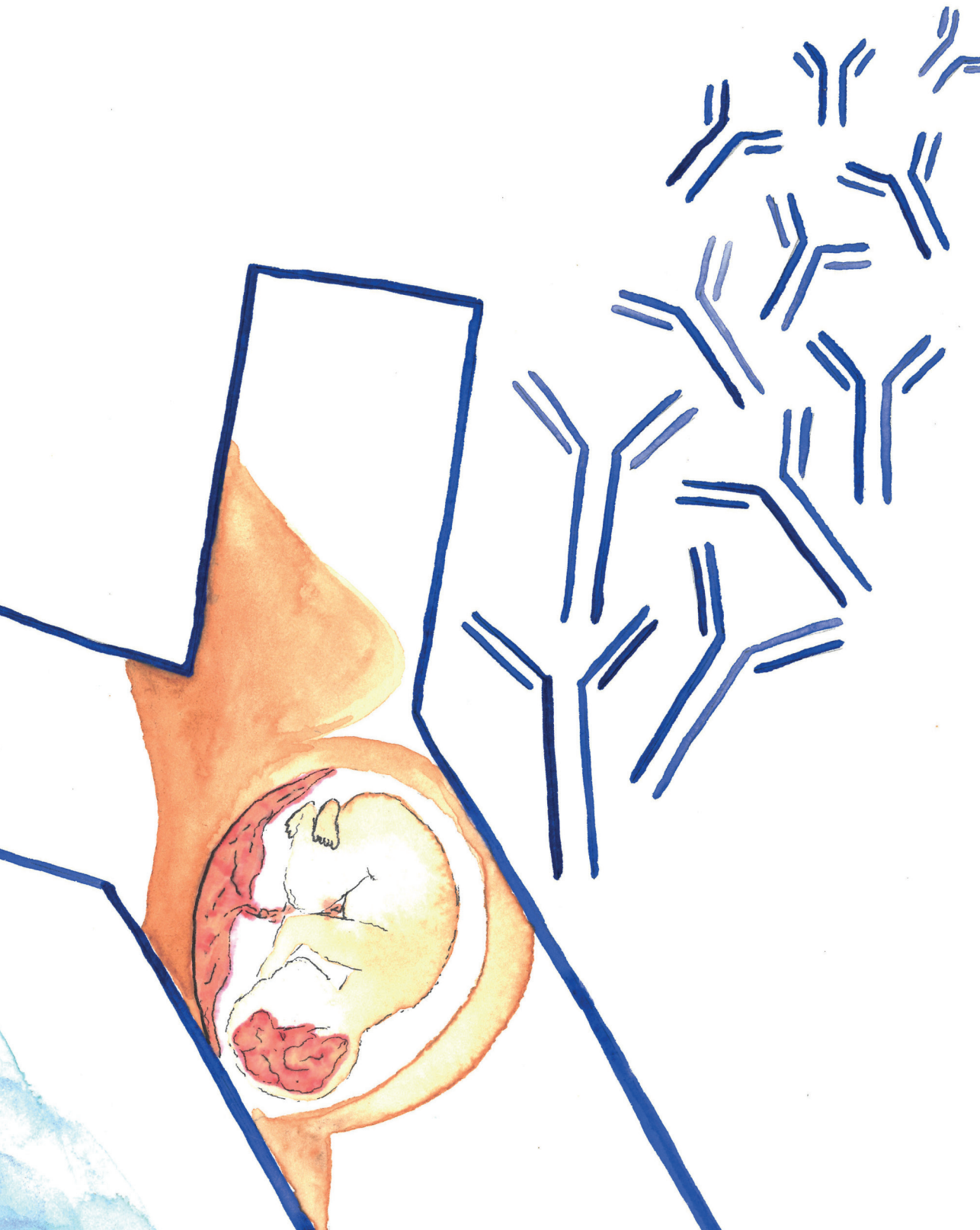
Abbreviatios: G, gravidity; P, parity; GA, gestational age; αHPA, anti-human platelet antigen directed antibodies; αHLA, anti-human leukocyte class I antibodies; SGA, small for gestational age; Bleed, bleeding symptoms; Plt, lowest platelet count; syn., syncytiotrophoblast; MAC, membrane attack complex; MBL, mannose binding lectin; f.v. fetal vessels; PW, placenta weight; MVM, maternal vascular malperfusion; FVM, fetal vascular malperfusion; VUE, villitis of unknown origin; F, focal; D, diffuse; A, absent; *Specificity of antibodies could not be determined; Corresp. GA, corresponding with gestational age; NT, not tested; ICH, intracranial hemorrhage; AV, avascular villi; Low, low grade focal; DVH, distal villous hypoplasia, IS, ischemia.

C1q f.v.	C4d f.v.	MAC f.v.	MBL f.v.	BW p<10	Maturation	MVM	FVM	Infection	VUE	Fetal hypoxia
F/F	A/A	A/A	A/F	No	Delayed	Infarct	-	-	-	-
A/F	A/A	A/A	A/F	Yes	Corresp. GA	-	AV	-	Low	-
F/F	F/D	A/F	A/A	No	Corresp. GA	-	-	-	-	-
F	F	A	A	No	Delayed	-	-	-	-	-
F	F	F	A	Yes	Accelerated	Infarct abrupt	-	Stage 1	-	Mild
D	F	A	A	Yes	Delayed	-	-	-	Low	-
D/F	D/D	A/A	A/A	No	Corresp. GA	DVH	-	-	-	-
D/F	D/F	A/A	A/A	No	Delayed	-	-	Stage 1	-	-
F	F	A	A	Yes	Accelerated	Infarct	-	-	Low	Mild
F/D	F/F	A/F	A/A	No	Corresp. GA	-	-	-	-	-
D	D	A	-	No	Delayed	-	-	-	Low	-
F	A	F	A	Yes	Corresp. GA	-	-	-	-	-
A	F	A	A	NT	Corresp. GA	DVH	-	-	-	-
F	F	D	A	No	Corresp. GA	-	-	-	-	-
A	A	A	A	No	Corresp. GA	-	-	Stage 1	-	-
A	F	A	A	NT	Delayed	IS	-	-	Low	-
D/F	F/F	A/A	A/-	NT	Delayed	-	-	-	-	-
F/F	F/F	F/A	F/F	NT	Corresp. GA	-	-	-	-	-
F	F	A	A	NT	Delayed	-	-	-	Low	-
D/D	D/F	A/A	F/F	No	Corresp. GA	-	-	-	-	Mild
-/F	A/F	A/A	F/F	No	Delayed	DVH	-	-	-	-
A/F	A/F	A/A	A/F	No	Corresp. GA	DVH, IS	-	-	-	-
A	A	A	A	No	Corresp. GA	-	-	-	-	-



PART THREE

Clinical relevance of HPA-5b antibodies



CHAPTER 5

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

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SUMMARY

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

INTRODUCTION

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

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

METHODS

STUDY POPULATION

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

CLINICAL DATA COLLECTION

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

DEFINITIONS

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

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

OUTCOME MODIFIERS

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

PRIMARY AND SECONDARY OUTCOME

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

STATISTICAL ANALYSIS

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

RESULTS

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

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

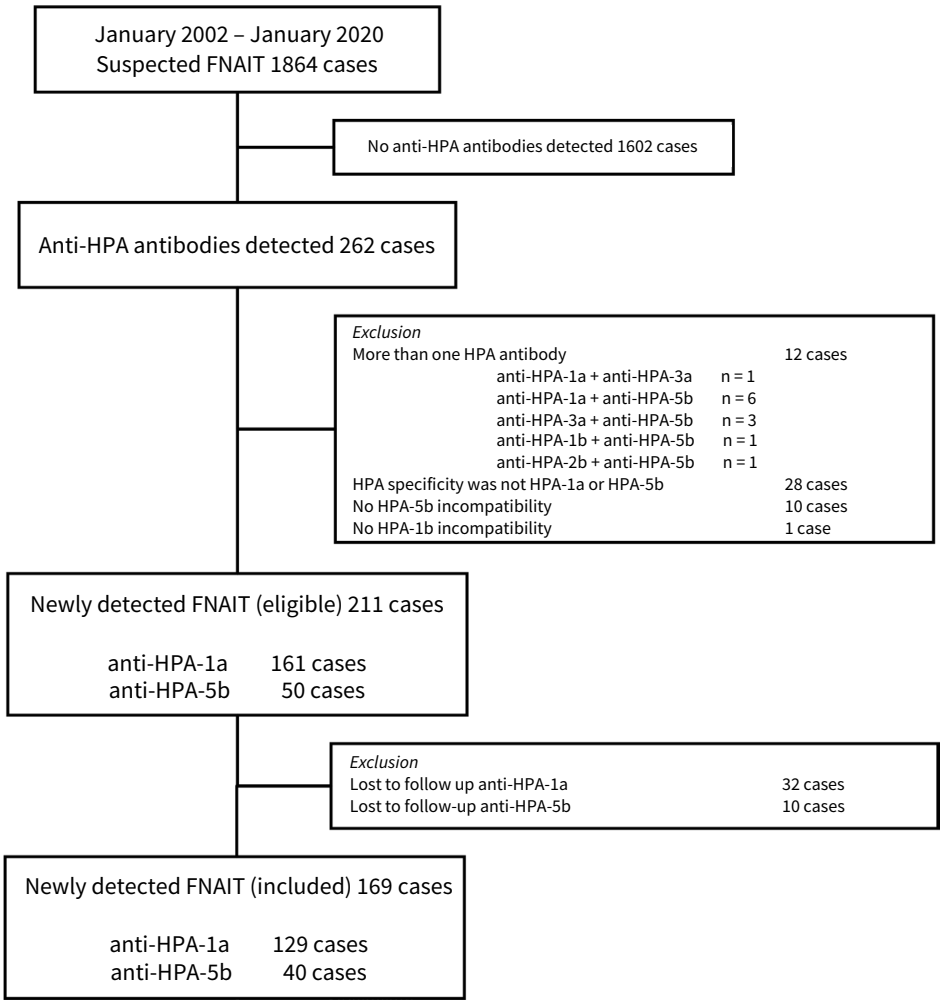

FIGURE 1. Flowchart study population

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

CLINICAL CHARACTERISTICS

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

TABLE 1. Clinical characteristics of the FNAIT cases

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

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

TABLE 2. Clinical outcome of the FNAIT cases

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

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

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BLEEDING

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

PLATELET COUNT, BLEEDING AND POSTNATAL TREATMENT

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

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

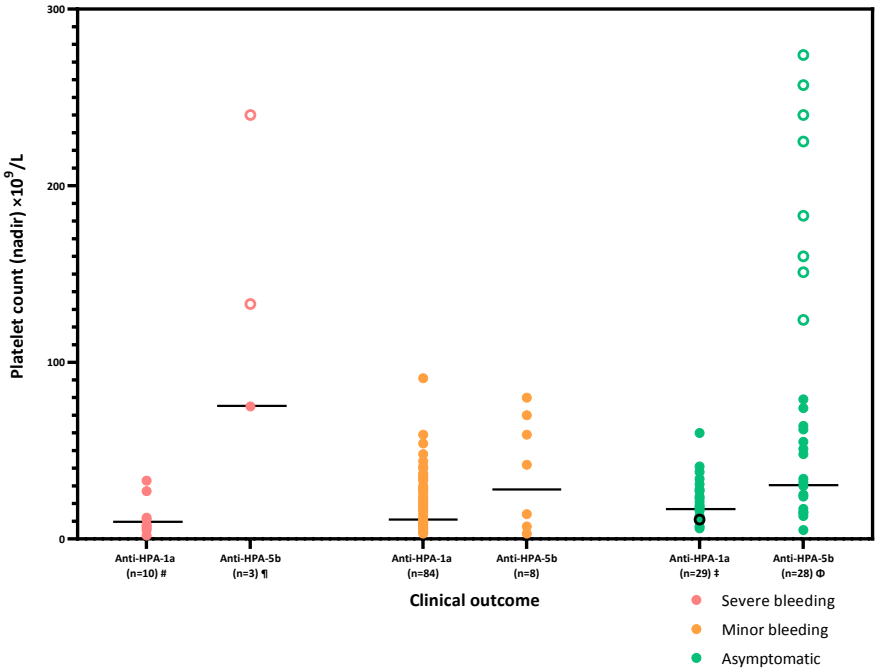

FIGURE 2. Platelet count and clinical outcome

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

Missing values for 4 cases due to mortality.

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

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

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

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

ANTENATALLY SUSPECTED FNAIT CASES

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

OBSERVED VERSUS EXPECTED RATE OF FETAL–MATERNAL INCOMPATIBILITY

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

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

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

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

DISCUSSION

MAIN FINDINGS

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

INTERPRETATION

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

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

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

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

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

STRENGTHS AND LIMITATIONS

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

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

CONCLUSION

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

DECLARATIONS

CONTRIBUTION TO AUTHORSHIP

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

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DISCLOSURE OF INTERESTS

There are no conflicts of interest to disclose.

DETAILS OF ETHICAL APPROVAL

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

FUNDING

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

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

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

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

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

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

SUPPLEMENTAL INFORMATION TABLE 3. Description of antenatally detected FNAIT cases

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

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

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

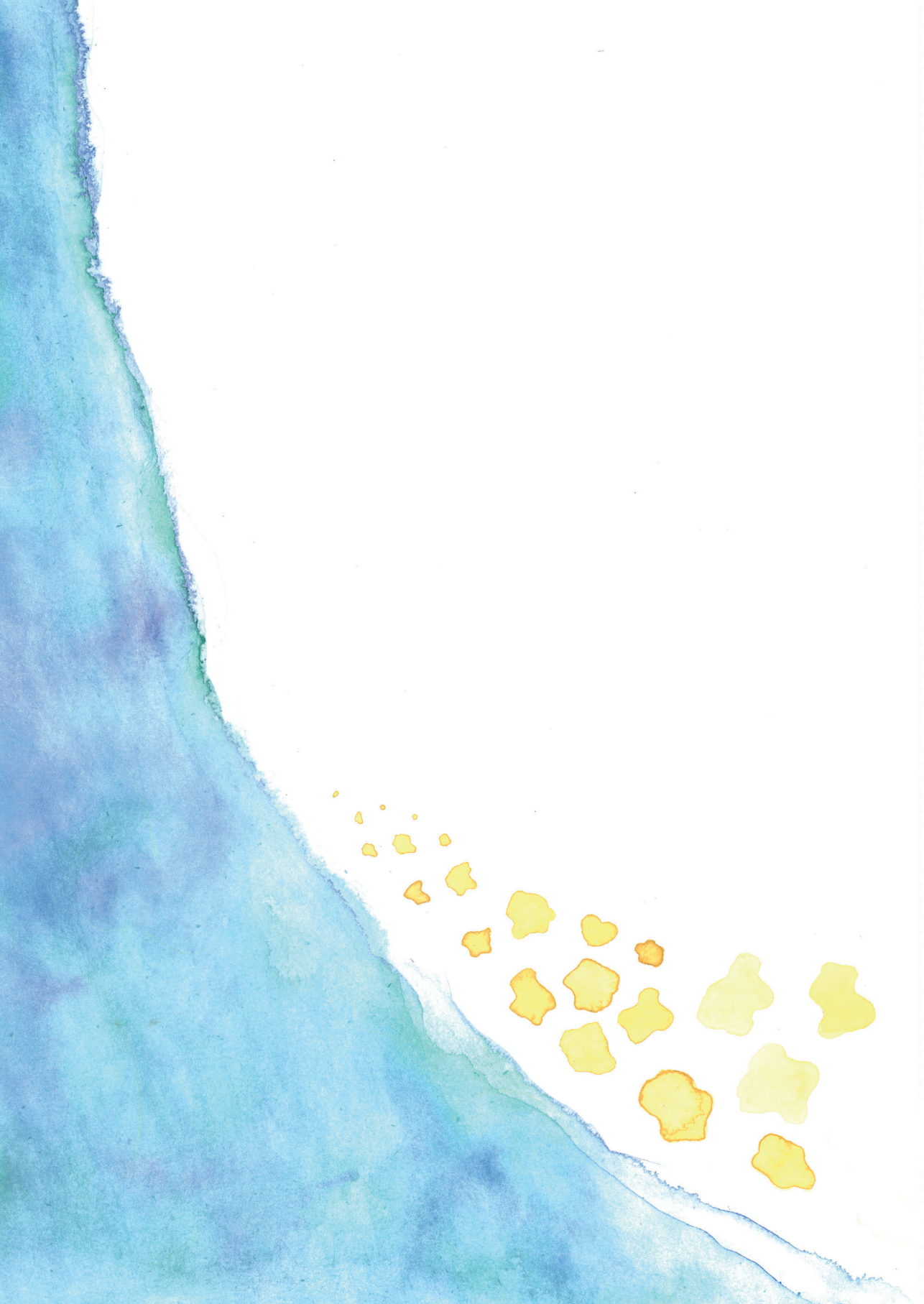
HPA-1		HPA-5	
Genotype		Genotype	
HPA-1aa	= 0.7056	HPA-5aa	= 0.84
HPA-1ab	= 0.2688	HPA-5ab	= 0.1472
HPA-1bb	= 0.0256	HPA-5bb	= 0.0064
Calculation or rates of paternal genotypes assuming that father is HPA-1a or HPA-5b positive.*			
HPA-1aa	= 0.7056 / (0.7056 + 0.2688) = 0.7241	HPA-5ab	= 0.1472 / (0.1472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

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

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

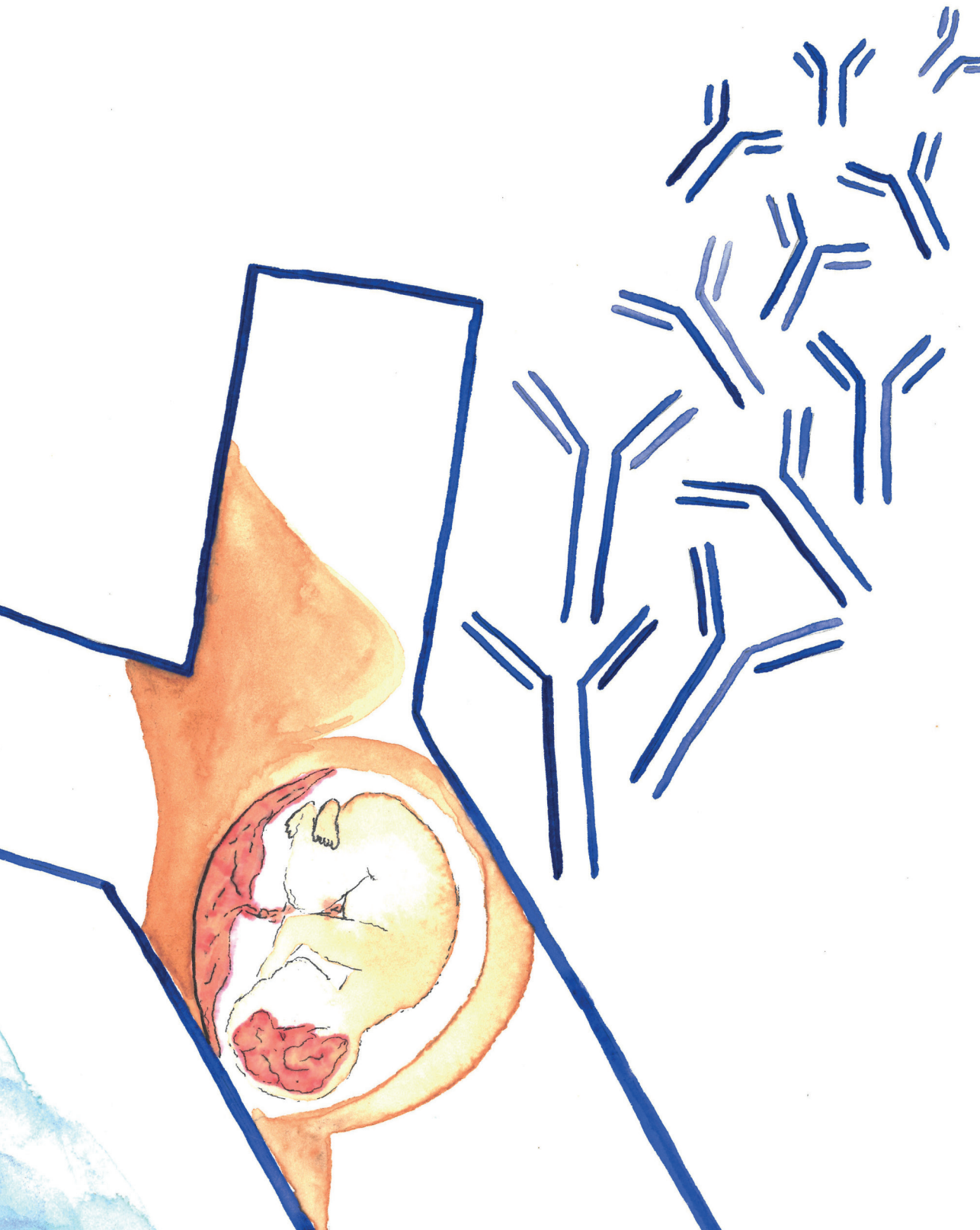
#	G/P	HPA antibody	MAIPA OD 10G11#	Genotype child/ father	Clinical features and reason suspicion	Bleeding	Platelet count (×10⁹/L)	Other risk factors	Treatment
I		5b	>3.000	5aa/5aa*	Cerebral abnormalities, deformities of extremities on antenatal ultrasound	ICH possible?	-	Congenital abnormalities	TOP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

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



PART FOUR

Neonatal management



CHAPTER 6

Postnatal treatment for children with fetal and neonatal alloimmune thrombocytopenia: a multicentre, retrospective cohort study

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SUMMARY

Background

Children affected by fetal and neonatal alloimmune thrombocytopenia (FNAIT) are at risk for severe intracranial haemorrhage. Management in the postnatal period is based on sparse evidence. We aimed to describe the contemporary management and outcomes of patients with FNAIT in high-income countries.

Methods

In this multicentre, retrospective, cohort study, we set up a web-based registry for the collection of deidentified data on the management and course of neonates with FNAIT liveborn between January 1, 2010, and January 1, 2020. Eight centres from seven countries (Australia, Norway, Slovenia, Spain, Sweden, the Netherlands, and the USA) participated. Eligibility criteria comprised anti-human platelet antigen (HPA) alloantibodies in maternal serum, confirmed maternal and fetal HPA incompatibility, and bleeding detected at antenatal ultrasound, neonatal thrombocytopenia (<150 × 10^9 platelets per L), or both in the current or previous pregnancy. Clinical data were retrieved from local medical records of the first neonatal admission and entered in the registry. The key outcome was the type of postnatal treatment given to neonates with FNAIT. Other outcomes were daily median platelet counts in the first week of life, median platelet count increment after first unmatched versus first matched transfusions, and the proportion of neonates with mild or severe bleeding.

Findings

408 liveborn neonates with FNAIT were entered into the FNAIT registry, of whom 389 from Australia (n = 74), Norway (n = 56), Slovenia (n =19), Spain (n = 55), Sweden (n = 31), the Netherlands (n = 138), and the USA (n = 16) were included in our analyses. The median follow-up was 5 days (IQR 2–9). More neonates were male (241 [64%] of 379) than female (138 [36%]). Severe thrombocytopenia (platelet count <50 × 10^9 platelets per L) was reported in 283 (74%) of 380 neonates, and extreme thrombocytopenia (<10 × 10^9 platelets per L) was reported in 92 (24%) neonates. Postnatal platelet count nadir was higher in the no-treatment group than in all other groups. 163 (42%) of 389 neonates with FNAIT received no postnatal treatment. 207 (53%) neonates received platelet transfusions, which were either HPA-unmatched (88 [43%] of 207), HPA-matched (84 [41%]), or a combination of both (35 [17%]). The proportion of neonates who received HPA-matched platelet transfusions varied between countries, ranging from 0% (Slovenia) to 63% (35 of 56 neonates; Norway). Postnatal intravenous immunoglobulin treatment was given to 110 (28%) of 389 neonates (alone [n = 19] or in combination with platelet transfusions [n = 91]), with the proportion receiving it ranging from 12% (17 of 138 neonates; the Netherlands) to 63% (ten of 16 neonates; the USA) across countries. The median platelet increment was 59 × 10^9 platelets per L (IQR 35–94) after HPA-unmatched platelet transfusions and 98 × 10^9 platelets per L (67–134) after HPA-

matched platelet transfusions (*P*<0.0001). Severe bleeding was diagnosed in 23 (6%) of 389 liveborn neonates, with one having a severe pulmonary haemorrhage and 22 having severe intracranial haemorrhages. Mild bleeding was diagnosed in 186 (48%) neonates.

Interpretation

Postnatal management of FNAIT varies greatly between international centres, highlighting the absence of consensus on optimal treatments. Our data suggest that HPA-matched transfusions lead to a larger median platelet count increment than HPA-unmatched transfusions, but whether HPA matching is also associated with a reduced risk of bleeding remains unknown.

Funding

Sanquin

RESEARCH IN CONTEXT

- **EVIDENCE BEFORE THIS STUDY**

  We searched PubMed without language restrictions for studies published between database inception and April 27, 2022 reporting on postnatal treatment and outcomes in patients with fetal and neonatal alloimmune thrombocytopenia (FNAIT). Search terms related to postnatal management and outcomes of patients with FNAIT were used. We identified four prospective and ten retrospective cohort studies. Available studies had methodological limitations including small numbers of patients, no randomisation and no analyses of confounding factors. Optimal postnatal management is not known; current guidelines are based on sparse qualitative evidence owing to the rarity of the condition. None of the studies we found compared the differences in management between different countries. The standard of care and whether postnatal management varies between international referral centres is unknown.

- **ADDED VALUE OF THIS STUDY**

  To our knowledge, this multicenter study is the first to investigate postnatal treatment strategies and outcomes of neonates affected by FNAIT in different countries. We gathered data from seven different countries and 389 FNAIT cases and found great variation in postnatal management strategies, particularly in the use of human platelet antigen (HPA)-matched platelet transfusions and intravenous immunoglobulins.

- **IMPLICATIONS OF ALL THE AVAILABLE EVIDENCE**

  This study shows variation in postnatal treatment strategies for FNAIT. HPA-matched or HPA-unmatched platelet transfusions and intravenous immunoglobulins are frequently administered postnatally; however the efficacy of these treatment strategies is unknown. Our findings could motivate international collaboration trough multicentre, randomised trials aimed at improving the management and outcomes of patients with FNAIT. Data from this study can serve as a basis from which future clinical trials can be designed.

INTRODUCTION

Children affected by fetal and neonatal alloimmune thrombocytopenia (FNAIT) face an increased risk of bleeding during pregnancy and after birth. Incompatibility in human platelet antigens (HPAs) between mother and fetus can lead to a maternal alloimmune response, with the formation of HPA-alloantibodies. Platelet-directed antibodies (IgG) are actively transported across the placenta into the fetal circulation. These alloantibodies bind to platelets and possibly endothelial cells resulting in fetal thrombocytopenia and an increased risk of bleeding.[1-3] FNAIT is the leading cause of severe thrombocytopenia in otherwise healthy term neonates and occurs in approximately one in 1500 pregnancies.[4, 5]
The main goal of antenatal and postnatal management of FNAIT is to prevent severe fetal and neonatal intracranial bleeding and its long-term sequelae. Administration of intravenous immunoglobulin (IVIg) to the mother during pregnancy is often used as a first-line treatment in mothers during pregnancies subsequent to one where FNAIT was diagnosed.[6] However, quantitative evidence supporting postnatal treatment in FNAIT is sparse and there is no international consensus on neonatal management.[7] Given the rarity of this disease, large prospective randomised trials are not available. Guidelines are mostly based on small observational studies and expert opinion.[6] We therefore aimed to evaluate the international practices in postnatal treatment and the outcomes of patients with FNAIT.

MATERIAL AND METHODS

STUDY DESIGN AND PARTICIPANTS

For this retrospective cohort study, on September 1, 2020, we set up a multicentre, web-based registry for the collection of deidentified data on the postnatal management and course of liveborn neonates with FNAIT. Eight centres from seven countries with specific interest and expertise in FNAIT agreed to participate: the Australian Neonatal Alloimmune Thrombocytopenia registry Monash University, Melbourne, VIC, Australia), the Arctic University of Norway (Tromsø, Norway), University Medical Centre Ljubljana (Ljubljana, Slovenia), Blood and Tissue Bank (Barcelona, Spain), Karolinska University Hospital (Stockholm, Sweden), Leiden University Medical Center (Leiden, the Netherlands), Sanquin Diagnostics (Amsterdam, the Netherlands), Levine Children's Hospital (Charlotte, NC, USA), and Boston Children's Hospital (Boston, MA, USA; Supplemental Table 1). Investigators were supplied with personal credentials to enter clinical data into a secured online database (Castor Electronic Data Capture 2019). Participants were eligible if they were liveborn between January 1, 2010, and January 1, 2020, their mothers had anti-HPA alloantibodies in their serum, incompatibility between the maternal and fetal HPAs was confirmed,[8] and they (or a previous pregnancy) had bleeding detected at an antenatal ultrasound, neonatal thrombocytopenia ($<150 \times 10^9$ platelets per L), or both. The medical ethical committee of

Leiden-Delft-DenHaag provided a waiver of consent for the initiating country (G20.074). The requirement for informed consent was waived. International investigators obtained ethical consent according to national laws and regulations.

PROCEDURES

Between September 1, 2020 and September 1, 2021, the following information was retrieved from local medical records of the first neonatal admission and entered in the online registry: time of diagnosis (antenatal or postnatal), reason for suspecting FNAIT, anti-HPA alloantibody specificity, method of HPA-alloantibody detection, gravidity, parity, the presence of maternal thrombocytopenia, antenatal treatment, gestational age at birth, delivery mode, sex (as determined by caregiver directly after birth), birthweight (including percentile and small for gestational age), neonatal skin or organ bleeding, intracranial haemorrhage (including neuroimaging reports), neonatal mortality, lowest platelet count per day (up to nine platelet counts per participant), postnatal treatment per day during the first admission, and bleeding complications after start of treatment.

We defined five postnatal treatment groups: no treatment, platelet transfusion from HPA-unmatched donors, platelet transfusion from HPA-matched donors, HPA-unmatched and HPA-matched platelet transfusions, and postnatal intravenous immunoglobulin. Participants who received an HPA-matched platelet transfusion did so from donors who were HPA-typed and selected on the absence of the implicated HPA. Participants who received both a platelet transfusion and postnatal intravenous immunoglobulin treatment or steroids were not analysed separately but included in a treatment group on the basis of the platelet transfusion received. Platelet transfusion thresholds for participants with and without bleeding and the recommended transfusion doses from clinical guidelines for FNAIT postnatal treatment were reported for each centre.

Antenatal diagnosis of FNAIT was defined as the detection of anti-HPA alloantibodies in the mother's serum during the current or previous pregnancy, with confirmed HPA incompatibility between the mother and fetus in the current pregnancy. Postnatal diagnosis was defined as the detection of anti-HPA alloantibodies after birth. Small for gestational age was defined as a birthweight of less than the 10th percentile according to local or national growth charts. Neonates born from pregnancies during which mothers received antenatal treatment (intravenous immunoglobulins, corticosteroids, intrauterine platelet transfusions, or a combination thereof) are called antenatally treated cases.

Bleeding symptoms were divided into mild and severe bleeding. Mild bleeding was defined as any uncomplicated haemorrhage (eg, petechiae, haematomas, or a grade 1–2 intraventricular haemorrhage [grading system was adapted from Papile *et al*[9] and Inder *et al*[10]]. Severe bleeding was classified by a severe intracranial haemorrhage (a grade 3 intraventricular haemorrhage,

an intraventricular haemorrhage of any grade in combination with parenchymal involvement, a parenchymal haemorrhage or cerebellar haemorrhage, a subdural haemorrhage causing parenchymal compression, a subarachnoid haemorrhage, or an epidural haemorrhage), severe organ bleeding (life-threatening bleeding associated with shock or requiring volume boluses, red blood cell transfusions, or inotropes), or both. Asymptomatic cases were participants without any bleeding symptoms. In participants with a severe intracranial haemorrhage, we attempted to estimate the timeframe in which the intracranial haemorrhage could have occurred by recording the date of the latest (antenatal) ultrasound without intracranial haemorrhage and the date at which the intracranial haemorrhage was diagnosed.

OUTCOMES

Our primary aim was to describe current practice in the postnatal treatment of neonates with FNAIT per country. Our key endpoint was postnatal treatment during first admission, described in the five treatment groups. Other aims were to describe neonatal outcomes and platelet count increments. Endpoints were the daily median postnatal platelet counts in the first week of life in neonates with FNAIT per treatment group, the median change (increment) in platelet count after the first platelet transfusion in recipients of unmatched versus matched platelet transfusions, and the proportion of participants with mild or severe bleeding per treatment group.

STATISTICAL ANALYSES

Study sample size was not based on statistical hypothesis testing. To obtain an overview of contemporary treatment and differences in postnatal treatment between countries we aimed to recruit at least 200 neonates from at least six centres. Endpoints were analysed in a population of liveborn neonates with FNAIT who had available information on postnatal treatment. Median platelet counts per day are presented by treatment group and additionally stratified by antenatal treatment status.

To calculate the platelet count increment per transfusion type we subtracted the minimum platelet count on the day before transfusion and the day of transfusion (whichever was least) from the maximum platelet count on the day of transfusion or the day after transfusion (whichever was most). This choice was made because platelet counts exactly before and after transfusion and after transfusion were not available (only the lowest platelet count per day was documented). Participants who received transfusions the same day or had missing platelet counts for the days of interest were not included in these analyses. Only first transfusions were included in these analyses, subsequent transfusions were not included, because their effects could not be determined without taking into account the previous transfusion. We compared median platelet count increments after transfusion using Mann-Whitney *U* test (an unadjusted analysis). Because data on confounding variables such as the volume, concentration and duration of transfusions were not available, adjusted

analyses were not done. Platelet count increments after transfusion were calculated shown separately. We did a subgroup analysis of clinical outcomes and treatment by country to assess differences in FNAIT severity.

Statistical analyses were done in Stata (version 16) and SPSS (version 26.0). Data are presented as the numbers of participants and percentages or medians and IQRs. The distributions of postnatal treatment strategies are shown as pie charts and platelet counts are shown as dot plots with medians and IQRs. Figures were made with GraphPad Prism (version 9).

ROLE OF THE FUNDING SOURCE
The funder of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report.

RESULTS

408 liveborn neonates with FNAIT were entered in the FNAIT registry. We excluded 19 neonates (5%) who had missing information on postnatal treatment, leaving 389 (95%) in our analyses (Figure 1). 255 (66%) of 389 cases of FNAIT were diagnosed postnatally (Table 1). Postnatal suspicion of FNAIT was due to skin bleeding in 127 (33%) of 389 neonates, thrombocytopenia detected as a chance finding in 118 (30%), and severe bleeding in ten (3%). 134 (34%) neonates were diagnosed antenatally, of whom 117 were diagnosed after a diagnosis of FNAIT in the mother's previous pregnancy. FNAIT was diagnosed antenatally in a screening study or because of a family history of FNAIT in 12 (3%) of 389 neonates. For the remaining five (1%) neonates, FNAIT was suspected due to severe antenatal bleeding. The method of detecting anti-HPA antibodies differed between countries (Supplemental Table 2). Antenatal treatment was given in 105 (78%) of 134 pregnancies that were antenatally diagnosed; the maternal administration of intravenous immunoglobulin was started in all 105 pregnancies, intravenous immunoglobulin was combined with steroids in 12, and intravenous immunoglobulin and steroids were combined with intrauterine platelet transfusions in two. There was an over-representation of male neonates and small-for-gestational-age neonates (Table 1). Baseline characteristics, particularly the prevalence of antenatal treatment, varied between countries (Supplemental Table 3).

The median follow-up during admission was 5 days (IQR 2–9). 163 (42%) of 389 neonates with FNAIT received no postnatal treatment (Table 2). 207 (53%) neonates received platelet transfusions, which were either HPA-unmatched platelet transfusions (88 [43%] of 207), HPA-matched platelet transfusions (84 [41%]), or a combination of both (35 [17%]). In the no-treatment group, 77 (47%) of 163 mothers were treated antenatally. The median number of postnatal transfusions given in the total population was 1 (IQR 1–2). The proportion of neonates

who received HPA-matched platelet transfusions varied between countries, ranging from 0% (Slovenia) to 63% (Norway; Figure 1; Supplemental Figure 1).

TABLE 1. Clinical characteristics of liveborn neonates with FNAIT

Variable	Total (n = 389)
HPA specificity, n (%)	
HPA-1a	291 (75)
HPA-1b	3 (1)
HPA-2b	3 (1)
HPA-3a	3 (1)
HPA-5a	7 (2)
HPA-5b	46 (12)
HPA-15a	3 (1)
HPA-15b	5 (1)
HPA-1a and HPA-3a	1 (<1%)
HPA-1a and HPA-5b	8 (2)
HPA-1b and HPA-5b	2 (1)
Other	11 (3)
Unknown	6 (2)
Antenatal diagnosis, n (%)†	134 (34)
Postnatal diagnosis, n (%)	255 (66)
Antenatal treatment, n (%)	105/134 (78)
First pregnancy (primigravida), n (%)	82 (21)
Male sex, n (%)	241 (64)
Gestational age at birth (weeks) - median (IQR; min-max)	38 (37–40; 24–42)
Birthweight (g) - median (IQR; min-max)	3060 (2584–3441; 737–4520)
Small for gestational age (SGA), n (%)	77 (21)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing.
† 117 cases of FNAIT diagnosed antenatally after a diagnosis of FNAIT in a previous pregnancy diagnosed FNAIT pregnancy, 17 cases were newly diagnosed in this current pregnancy.
§ 82 (31%) of 272 women who had not been previously diagnosed with FNAIT were primigravida.
Abbreviations: HPA, human platelet antigen; IQR, interquartile range; g, grams.

Postnatal intravenous immunoglobulin treatment was given to 110 (28%) of 389 neonates (alone [n = 19] or in combination with platelet transfusions [n = 91]), with the proportion receiving it ranging from 12% to 63% across countries (Supplemental Figure 1). Four (4%) of 93 neonates with dosing information received a dose of 0.5 g/kg of bodyweight per day of postnatal intravenous immunoglobulin treatment, 85 (91%) received 1.0 g/kg per day, and four (4%) received 2.0 g/kg per day. The dose of postnatal intravenous immunoglobulin treatment was not known for 17 neonates. Steroid treatment was given to only four neonates from two countries (Spain and the Netherlands). Platelet transfusion guidelines varied between centres, with transfusion thresholds ranging from 20×10^9 platelets per L to 50×10^9 platelets per L in neonates without bleeding and from 50×10^9 platelets per L to 100×10^9 platelets per L in neonates with bleeding (Supplemental Table 4). Transfusion doses varied from 10 mL/kg to 20 mL/kg (Supplemental Table 4).

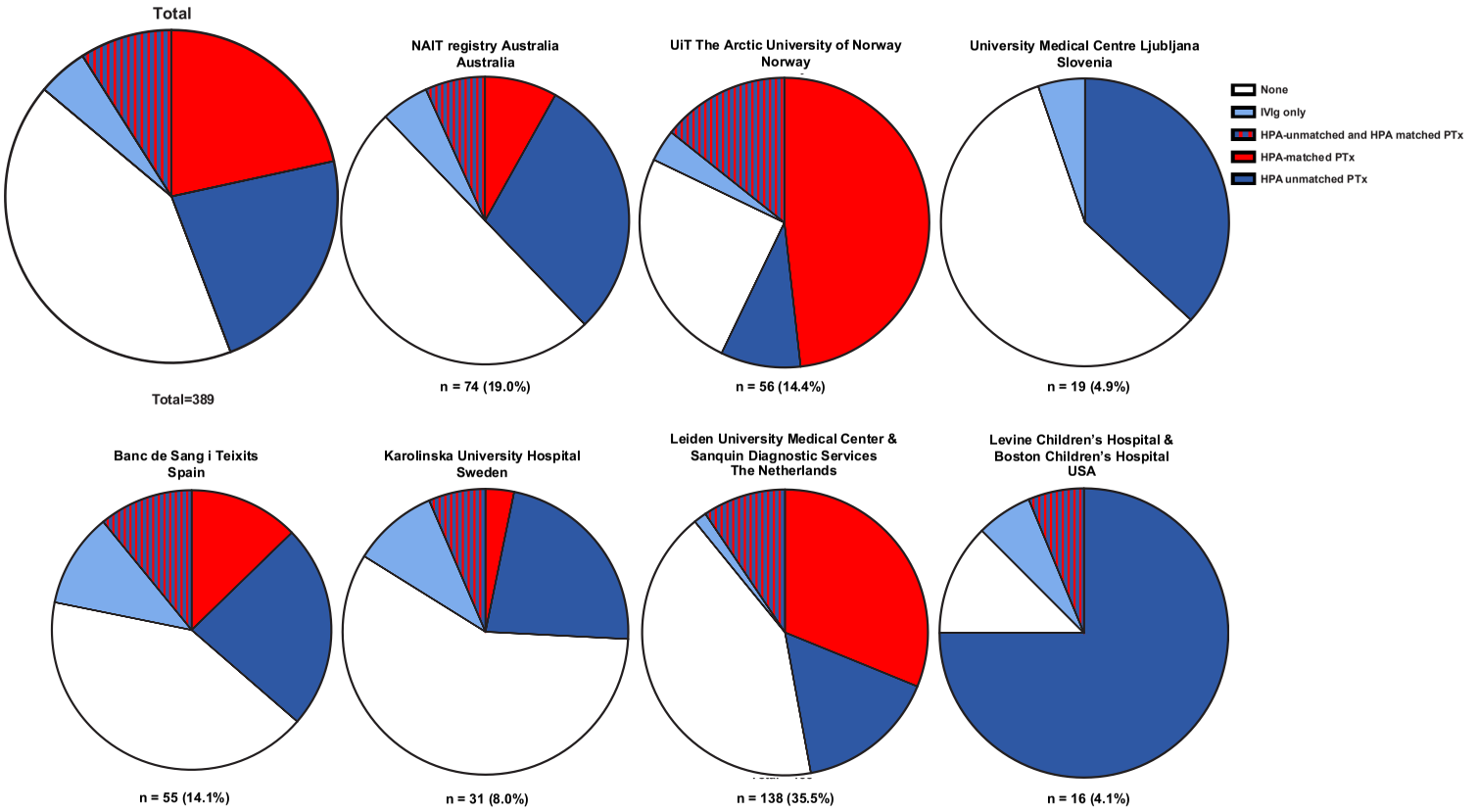

FIGURE 1. Postnatal treatment strategy per country

The distribution of treatment strategies applied in all cases and per centre. Cases that received no postnatal treatment were allocated in the no postnatal treatment group. Cases treated with HPA-unmatched donor platelets were allocated in unmatched platelet group (unmatched PTx). Cases treated with HPA typed and matched donor platelet transfusions were allocated in the matched platelet transfusion group (matched PTx). Cases that received both matched and HPA-unmatched donor platelets were allocated in both HPA-unmatched and HPA-matched platelet transfusion group (both HPA-unmatched and HPA-matched PTx). Cases that were treated with intravenous immunoglobulin (IVIg) only were allocated in the IVIg only group.

TABLE 2. Treatment and outcome of liveborn neonates with FNAIT per postnatal treatment strategy

Characteristic	No postnatal treatment (n = 163)	HPA-unmatched platelet transfusion (n = 88)	HPA-matched platelet transfusion (n = 84)	HPA-unmatched and HPA-matched platelet transfusions (n = 35)	Intravenous immunoglobulin only (n =19)	Total (n = 389)
Antenatal treatment - (n, %)	77 (47)	3 (3)	21 (25)	0	4 (21)	105 (27)
Postnatal treatment						
Additional IVIg treatment - (n, %)	-	42 (48)	24 (29)	25 (71)	19 (100)	110 (28)
Additional steroids - (n, %)	-	1 (1)	1 (1)	2 (6)	-	4 (1)
Platelet count						
Platelet count nadir (× 10^9 platelets per L) - median (IQR)	65 (34-162)	12 (8-19)	12 (7-18)	6 (3-14)	23 (12-38)	21 (10-51)
Thrombocytopenia (Platelet count <150 × 10^9 platelets per L) - (n, %)	111 (72)	88 (100)	84 (100)	35 (100)	19 (100)	337 (89)
Severe thrombocytopenia (Platelet count <50 × 10^9 platelets per L) - (n, %)	58 (38)	88 (100)	84 (100)	35 (100)	18 (95)	283 (74)
Very severe thrombocytopenia (Platelet count <25 × 10^9 platelets per L) - (n, %)	13 (8)‡	74 (84)	75 (89)	33 (94)	11 (58)	206 (54)
Extreme thrombocytopenia (Platelet count <10 × 10^9 platelets per L) - (n, %)	1 (1)‡	33 (38)	31 (37)	23 (66)	4 (21)	92 (24)
Severity of bleeding symptoms†						
Mild bleeding	39 (24)	57 (65)	58 (69)	24 (69)	8 (42)	186 (48)
Severe bleeding	2 (1)	10 (11)	5 (6)	6 (17)	0	23 (6)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing.
† Severe bleeding was defined as cases with severe organ bleeding and/or severe ICH, mild bleeding are cases with skin bleeding, mild organ bleeding and/or intraventricular haemorrhage (IVH) grade I of II.
‡ Reason why no treatment was given in these thrombocytopenic infants is unknown. Platelet count was below 25 × 10^9 per L for one day in all 14 cases.
Abbreviations: IVIg, intravenous immune globulin; IQR, interquartile range.

The median age at detection of platelet count nadir was 0 days (the day of birth; IQR 0–1). In 348 (92%) of 380 neonates with complete data, platelet count nadir was detected at 3 days or earlier. Severe thrombocytopenia was detected in 283 (74%) of 380 and extreme thrombocytopenia was detected in 92 (24%; Table 2). Postnatal platelet count nadir was higher in the no-treatment group than in all other groups (Table 2). Treatment groups differed in the proportion of neonates who were antenatally treated and in the proportion of neonates who were treated with (additional) postnatal intravenous immunoglobulin (Table 2). In all treatment groups, median platelet count increased in the first week after birth (Figure 2). The platelet counts of neonates receiving HPA-unmatched platelets were not different from those of neonates receiving HPA-matched platelets after day 3 (Figure 2). At day 4, the median platelet count of neonates receiving HPA-unmatched platelets (87 × 10^9 platelets per L [IQR 48–142]) did not differ from the median platelet count of neonates receiving HPA-matched platelets (125 × 10^9 platelets per L [70–172]; $P = 0.15$).

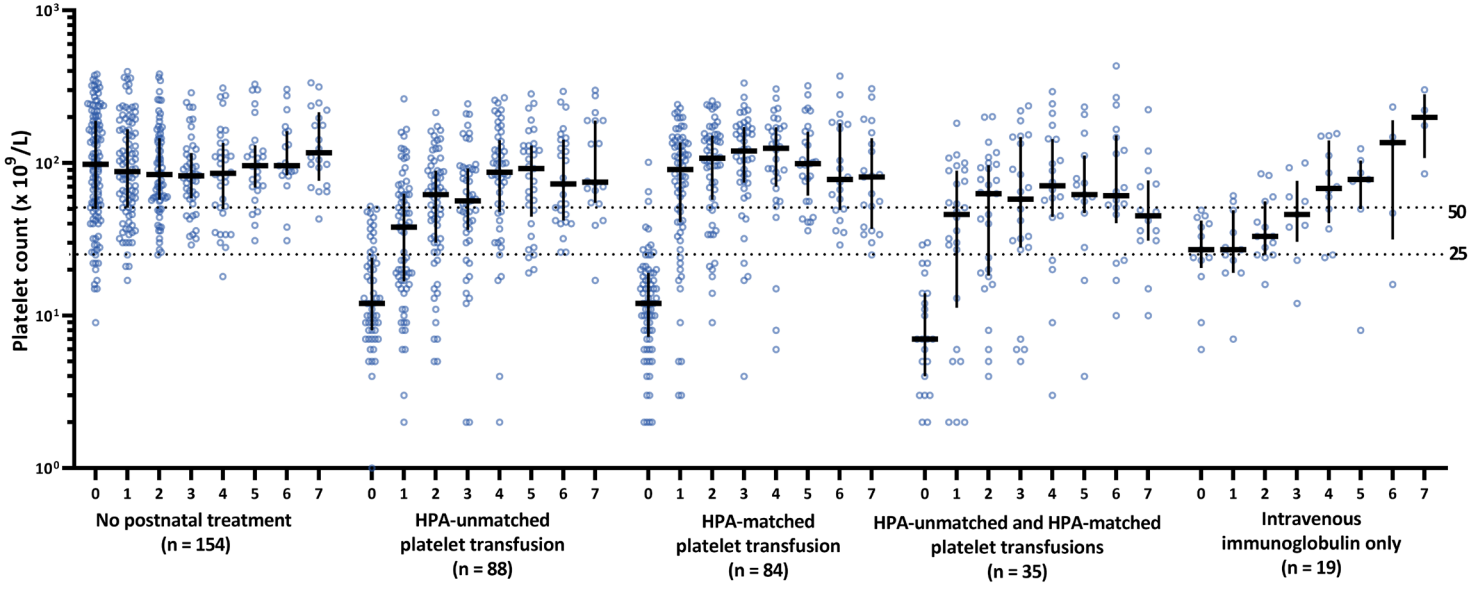

FIGURE 2. Platelet count per treatment strategy

The dot plot shows the lowest neonatal platelet count per day per treatment strategy. The dots represent individual platelet counts, the solid, black horizontal lines represent median values, ant the vertical solid black lines represent the IQRs. Dashed lines represent platelet counts of 50×10^9 per L and 25×10^9 per L.

50 neonates who did not receive antenatal or postnatal treatment had severe thrombocytopenia (platelet count <50 × 10^9 platelets per L) and 11 had very severe thrombocytopenia (platelet count <25 × 10^9 platelets per L), but none developed bleeding. Platelet counts during the first week of life stratified by antenatal treatment status are shown in Supplemental Figure 2. The median nadir platelet count was 110 × 10^9 platelets per L (IQR 33–191) for neonates who were antenatally treated and 17 × 10^9 platelets per L (8–20) for neonates who did not receive antenatal treatment. The median nadir platelet counts of neonates were 19 × 10^9 platelets per L (IQR 9–40) if their mothers had anti-HPA-1a antibodies, 55 × 10^9 platelets per L (28–146) if their mothers had anti-HPA-5b antibodies, and 39 × 10^9 platelets per L (16–46) if their mothers had anti-HPA-15b antibodies (the most commonly involved antigens).

207 neonates, of whom 24 (12%) were antenatally treated with intravenous immunoglobulin, received 367 postnatal platelet transfusions. We excluded 81 (39%) neonates from our analysis of post-transfusion platelet count increments, because they either received a second transfusion on the same day as the first transfusion (n = 15) or on the day after the first transfusion (n = 29) or had missing data (n = 37). The platelet increment after the first transfusion was calculated and compared between HPA-unmatched platelet transfusions (n = 60) and HPA-matched platelet transfusions (n=66). The median platelet increment was 59 × 10^9 platelets per L (IQR 35–94) after HPA-unmatched platelet transfusions and 98 × 10^9 platelets per L (67–134) after HPA-matched platelet transfusions (P<0.0001; Figure 3). Our results were similar when stratified by antenatal treatment status (Figure 3).

Severe bleeding was diagnosed in 23 (6%) of the 389 liveborn neonates. One neonate had a severe pulmonary haemorrhage; the other 22 neonates were diagnosed with severe intracranial haemorrhages (Supplemental Table 5). Three (14%) cases of intracranial haemorrhage were detected antenatally by ultrasound, 17 (77%) were detected postnatally, and the timepoint of detection was unknown for two (9%). In two of the three neonates who were diagnosed antenatally, antenatal intravenous immunoglobulin treatment was started after the detection of anti-HPA antibodies in the mother's serum at 20 weeks' gestational age and 32 weeks' gestational age, respectively. Estimating the timepoint of severe intracranial haemorrhage development was not possible in 16 (73%) of 22 neonates owing to missing data and the lack of serial ultrasound examinations during pregnancy and after delivery. In one neonate with a severe intracranial haemorrhage diagnosed postnatally, the bleeding worsened postnatally on MRI after the initial diagnosis. In this neonate, platelet count nadir was 19 × 10^9 platelets per L on day 5 after birth and the implicated antibody was directed against HPA-15b. No other worsening of severe bleeding was reported for the other neonates.

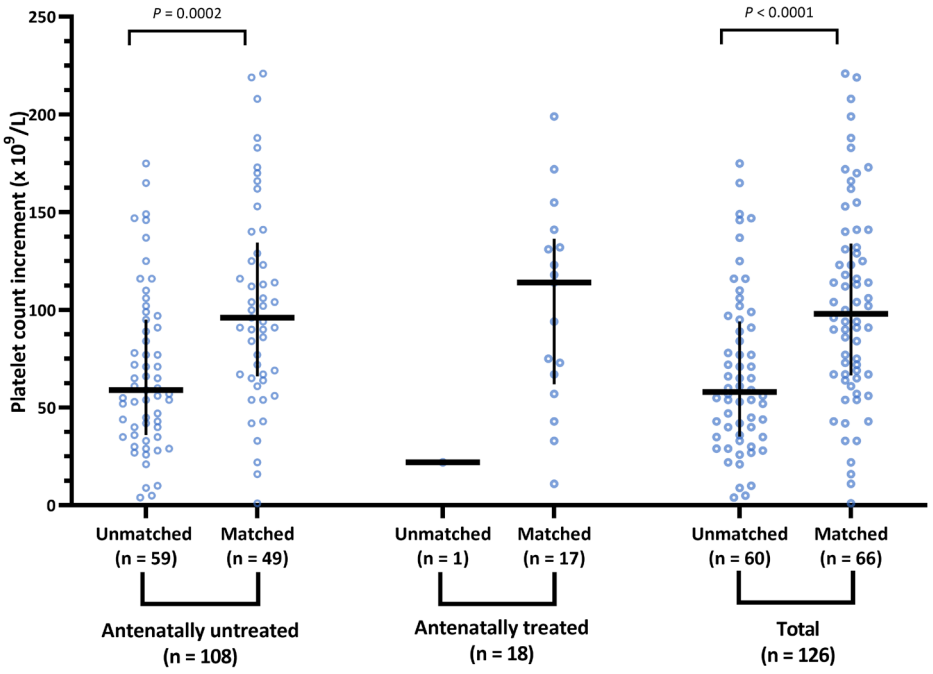

FIGURE 3. Platelet increment after first platelet transfusion

Platelet count increment was calculated of the first matched or the first HPA-unmatched platelet transfusion. The first set of plots shows cases that did not receive antenatal treatment (antenatally untreated). The second set of plots shows cases that received antenatal treatment during pregnancy (antenatally treated). The last set of plots shows platelet increment of all cases. Black lines represent medians with interquartile ranges. Median platelet count increments were compared by performing Mann Whitney U test (unadjusted analysis), statistical testing in the antenatally treated group was not possible; the HPA-unmatched group in this subgroup included 1 case only.

Mild bleeding was diagnosed in 186 (48%) of 389 neonates, of whom eight (4%) had a mild intracranial haemorrhage, eight (4%) had mild organ bleeding, and 170 (91%) had skin bleeding. Of the eight neonates who had a mild intracranial haemorrhage, five (63%) had a grade 1 intraventricular haemorrhage and three (38%) had an intracranial haemorrhage that was not specified. Five of the eight neonates who had mild organ bleeding had gastrointestinal bleeding, two had umbilical cord bleeding, and one had a retinal haemorrhage. None of the neonates who had a previous sibling diagnosed with FNAIT were diagnosed with intracranial haemorrhage. In our subgroup analysis assessing treatment and clinical outcome by country, differences were observed in the proportion of neonates who were antenatally treated, ranging from three (5%) of 56 in Norway to 47 (64%) of 74 in Australia (Supplemental Table 3). The proportion of neonates with severe bleeding was similar across all countries (Supplemental Table 3).

DISCUSSION

This multicentre, retrospective cohort study of 389 neonates with FNAIT born in a 10-year inclusion period in seven countries highlights the large variation in contemporary postnatal treatment strategies. Postnatal management strategies included frequent use of matched or unmatched platelets for transfusion, intravenous immunoglobulin, or a combination of treatments. Analysis of one of our outcomes showed differences in platelet count increments between treatment types, with the median platelet increment being significantly larger after matched platelet transfusions than after unmatched platelet transfusions. We could not analyse associations between treatment type and the occurrence of severe bleeding due to missing data on the timing of bleeding. This study presents new information on platelet responses to postnatal treatment for FNAIT, providing a starting point for a comparative clinical trial.

In a previous national cohort study in the Netherlands, we found a great diversity in postnatal treatment strategies in neonates with FNAIT, despite the availability of a national guideline.[11] To our knowledge, this is the first international study addressing postnatal treatment in neonates affected by FNAIT. More than half of the neonates were treated with platelet transfusions, which were either HPA-matched or HPA-unmatched, or a combination of both. Differences in the treatment strategy might be partly explained by variation in treatment guidelines between centres and in the availability of HPA-matched platelets per centre. For example, HPA-matched platelets are not routinely available in many countries (eg, Slovenia) and intravenous immunoglobulin is recommended as a first-line treatment in other countries (including some centres in the USA). Differences in treatment strategy might also be related to variation in clinical characteristics of the cases per country, although we did not investigate this possibility.

53% of the neonates received platelet transfusions, which is in line with a systematic review reporting that 51% of neonates with FNAIT receive postnatal platelet transfusions.[7] Unmatched platelet transfusion products are often readily available, whereas platelets matched for HPA-1, HPA-5, or both are not always available from hospital stocks or from blood centres due to donor availability and logistical challenges. With 98% HPA-1a positivity and 20% HPA-5b positivity in the population,[12] there is a high chance that HPA-unmatched platelet products will be incompatible. Whether matched platelet products yield better clinical outcomes than unmatched platelet products is still unclear. No randomised clinical trials have investigated the differences in clinical outcome and bleeding risk between the two treatments. We observed significantly larger median platelet count increment in patients receiving matched versus unmatched platelet transfusions. These data confirm results from previous smaller studies showing that the median platelet increment ranged from 116-170×10^9 per L after matched transfusions and from 27-68×10^9 per L after unmatched donor

transfusions.[13-16] As in these previous studies, we did not adjust for possible confounding factors between the two groups. However, our cohort had a large sample size and we used only data from first transfusions in patients who received no further transfusions on the following day. First matched platelet transfusions led to larger platelet increments than first unmatched platelet transfusions, but platelet counts after day 3 were similar. However whether matched platelets prevent severe bleeding better than unmatched platelets remain undocumented. Circulating maternal HLA-antibodies in the neonate may also cause the destruction of transfused platelets.[17] These HLA-antibodies were not considered in this study. Approximately a third of neonates received intravenous immunoglobulin postnatally, either alone or in combination with platelet transfusions. Administration of intravenous immunoglobulin to the mother during pregnancy decreases pathogenic IgG transport from the mother to the fetus due to competition at the receptor level in the placenta and has been shown to reduce the risk of fetal intracranial haemorrhage.[6] The mechanism of action of postnatal intravenous immunoglobulin treatment in FNAIT is not clear. Although our study was not designed to compare the outcomes of treatment regimens, platelet counts in the groups receiving platelet transfusions increased more rapidly than that in the intravenous immunoglobulin only group, in line with previous smaller studies.[18, 19]

We found a lower prevalence of severe bleeding (6%) compared with other cohort studies, which have reported rates from 10% to 25%.[5, 20] Several explanations are possible. First, we included only liveborn neonates with FNAIT and therefore, unlike other studies, did not include fetuses that had died or pregnancies that had been terminated due to intracranial haemorrhage. As a result, calculating mortality rates was also not possible. Second, about a quarter of neonates in our study received antenatal intravenous immunoglobulin treatment, which could possibly have prevented the occurrence of intracranial haemorrhage. Finally, the definitions of bleeding differed between studies; we classified eight cases of intracranial haemorrhage as mild bleeding, whereas the other studies reported on all neonates with intracranial haemorrhages without stratifying by severity.

In our study, worsening of the intracranial haemorrhage after postnatal diagnosis was reported in only one neonate, suggesting that this event is rare. This finding is consistent with earlier studies suggesting that intracranial haemorrhage develops predominantly during pregnancy rather than postnatally.[21] Given the small proportion of neonates with FNAIT and thrombocytopenia who develop intracranial haemorrhage, it is unlikely that thrombocytopenia is the sole cause of intracranial haemorrhage—another factor might potentially increase the risk of bleeding. In a 2022 cohort study,[22] genetic screening was done in 194 fetuses antenatally diagnosed with intracranial haemorrhage. Pathogenic variants of *COL4A* and *COL4A2* (encoding basement membrane proteins) were found in 36 (19%) of 194 fetuses, emphasising the heterogeneity in the causes of fetal intracranial haemorrhage. Additionally, most HPAs are expressed by endothelial cells.[23, 24] Several studies using *in vitro*

and murine models have shown that HPA-antibodies can bind to the endothelium which might increase the risk of ICH.[1-3]

This study yielded two additional interesting findings. First, we found an over-representation of male neonates with FNAIT, confirming a similar finding in a previous study.[25] In maternal RhD alloimmunisation, male neonates are also reported to be more severely affected than female neonates.[26] The reason for the difference in sex distribution in neonates affected by FNAIT is not clear. Possible explanations can be found in transplantation medicine. Studies in this field have shown that sex-mismatch is a risk factor for transplant rejection, possibly due to recognition of Y chromosome-encoded peptides by the maternal immune system.[27] Second, we found that a large proportion of neonates were born small for gestational age, consistent with a previous report.[28] This may partly be due to selection bias because additional routine diagnostics tests, including a full blood count, are often done in neonates born with low birthweight, which can lead to the detection of thrombocytopenia. Alternatively anti-HPA-1a could have bound to placental cells that express HPA-1a leading to placental damage and dysfunction, and leading hence to fetal growth restriction.[29, 30]

There is no international consensus on the optimal postnatal treatment strategy for FNAIT. Different platelet transfusion thresholds and volumes were recommended in the guidelines of the participating centres. The safety and benefits of platelet transfusions in neonates have been questioned after a large, randomised trial in preterm neonates showed that a restrictive transfusion policy (transfusion at $<25\times10^9$ platelets per L) was associated with an improved outcome compared to a more liberal strategy (transfusion at $<50\times10^9$ platelets per L).[31, 32] Whether platelet transfusions in neonates with FNAIT could also have deleterious effects, is not known and requires further investigation.

As shown in this study, platelet counts in most neonates with FNAIT increased to more than 50×10^9 platelets per L in the first week of life. Neonates with FNAIT in the no-treatment group were less likely to have severe disease than those in the treatment groups, because guidelines recommend the administration of platelet transfusions to neonates with very low platelet counts, bleeding, or both. Platelet counts in neonates in the no-treatment group increased spontaneously within the first week of life. None of the 50 neonates with severe thrombocytopenia who did not receive antenatal or postnatal treatment developed bleeding and their platelet count increased.

Our study had several limitations. First, the results of our study should be interpreted in light of its retrospective design and probable selection bias, which is highlighted by the small numbers of neonates at several centres. Second, different antibody screening methods were used to detect HPA-antibodies. Differences in the sensitivity of these tests may have influenced the composition of our cohort.[8] Third, we were not able to analyse the association

between bleeding, the time of treatment initiation, and platelet transfusion thresholds, because we could not identify the time of bleeding onset in most neonates. Finally, it is difficult to compare treatment groups owing to confounding by indication, hampering the study of eventual causal effects of the treatments on clinical outcomes. To identify optimal postnatal treatment strategies, information on the timing of the intracranial haemorrhage or other types of severe bleeding in FNAIT is essential. In neonates who develop intracranial haemorrhage postnatally, the time at which bleeding develops can only be assessed if serial neuroimaging examinations are frequently done throughout pregnancy and throughout the neonatal period. However, because FNAIT is predominantly diagnosed after birth, these data might only become available if a screening program is in place to detect HPA-alloimmunised pregnancies.

The true effect of different postnatal treatments can only be reliably established with a randomised study design. In the absence of antenatal screening programmes to detect pregnancies at risk for FNAIT in a timely manner, FNAIT is hugely underdiagnosed, hampering inclusion rates for such a study.[33] However, this large, multicentre cohort study of neonates with FNAIT evaluated postnatal treatment strategies in seven countries, which varied greatly, provides valuable information for clinicians and researchers on FNAIT treatments and outcomes, and shows the potential of international collaboration in a future clinical trial. Although our data suggest that HPA-matched transfusions lead to higher platelet count increment than HPA-unmatched transfusions, whether HPA matching reduces the risk of bleeding is unclear. This study highlights the urgent need for further trials to establish evidence-based guidelines for the management of neonates with FNAIT.

DATA SHARING STATEMENT

Requests for data can be send to the corresponding author and will be reviewed by the scientific committee of the initiating centre and primary investigators of participating centres. If approval is given, data will be shared via a secure portal. Data sharing requests can be sent to the corresponding author beginning 3 months and ending 36 months after publication. The study protocol will be made available upon reasonable request to the corresponding author.

AUTHORSHIP CONTRIBUTIONS

TWdV contributed to data curation, investigation, project administration, writing – original draft DW contributed to investigation and writing – review & editing, CCD contributed to formal analysis, methodology and writing – review & editing, VA, MZ, VY, HBH contributed to investigation and project administration, JGvdB, DO, CEvdS, MSV, ET, EMW contributed to writing – review & editing, CCS, ED, HEH, JLK, ZKM, EMD, NN, LP, MS, HT, contributed to investigation and writing – review & editing, MH contributed to conceptualisation and writing – review & editing, EL contributed to methodology, supervision and writing – review

& editing. TWdV, DW, VA, CCS, ED, HEH, HBH, JLK, NN, MS, MSV, LP, ET, HT, VY, MZ had access to, verified and interpreted the data of their centre and entered the data in the secured online database. TWdV and CCD had access to the complete database and verified and analysed the data. All authors read and approved the manuscript. TWdV and EL had final responsibility to submit the manuscript for publication.

DECLARATION OF INTERESTS

JGvdB reports an unrestricted research grant from Novo Nordisk and previous payment for teaching by Bayer, both were paid to the institution. DO is funded as a research consultant by Janssen Pharmaceuticals Inc and participates on the Advisory board of Janssen Pharmaceuticals Inc. HT reports previous payment from Prophylix AS related to a patent on a monoclonal anti-HPA-1a antibody and is funded as a research consultant by Janssen Pharmaceuticals Inc since 1st of August 2021. HT will be a local study site principal investigator in a planned multicentre natural history study on FNAIT sponsored by Rallybio. ET and EL report a consultancy fee from Janssen Pharmaceuticals Inc as members of advisory board on FNAIT. All other authors report no conflict of interest.

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SUPPLEMENTARY INFORMATION

SUPPLEMENTAL TABLE 1. Participating sites.

Country	Centre	Principle Investigator	Patients enrolled
The Netherlands	Leiden University Medical Centre, Leiden & Sanquin Diagnostic Services, Amsterdam	Enrico Lopriore & Masja de Haas	138
Australia	NAIT registry, Monash University, Melbourne	Erica Wood	93
Norway	UiT The Arctic University of Norway, Tromsø	Heidi Tiller	56
Spain	Blood and Tissue Bank, Barcelona	Núria Nogués	55
Sweden	Karolinska University Hospital, Stockholm	Emöke Deschmann	31
Slovenia	University Medical Centre Ljubljana, Ljubljana	Jana Lozar Krivec	19
United States	Levine Children's Hospital, Charlotte	Matthew Saxonhouse	13
United States	Boston Children's Hospital, Boston	Martha Sola-Visner	3

SUPPLEMENTAL TABLE 2. HPA-antibody detection method

	Australia (n = 74)	Norway (n = 56)	Slovenia (n = 19)	Spain (n = 55)	Sweden (n = 31)	The Netherlands (n = 138)	USA (n = 16)	Total (n = 389)
Antibody detection method - (n, %)								
MAIPA-assay	40 (54)	56 (100)	0	54 (98)	31 (100)	133 (98)	0	314 (91)
PIFT	25 (34)	0	11 (58)	2 (4)	0	127 (93)	0	165 (43)
Pak Lx assay (Immucor)	26 (35)	5 (9)	5 (26)	18 (33)	3 (10)	5 (4)	0	62 (16)
MACE	0	0	12 (63)	38 (69)	0	0	5 (31)	55 (14)
Flowcytometry	0	0	0	0	0	0	1 (6)	1 (1)
Unknown	12 (16)	0	5 (26)	0	0	0	10 (63)	27 (7)

Percentages do not add up to 100 because in 55% (214/389) of cases two or more antibody detection methods were used.
Abbreviations: HPA, human platelet antigen; USA, United states of America; MAIPA, monoclonal antibody specific immobilization of platelet antigen; PIFT, platelet immunofluorescence test; MACE, modified antigen capture ELISA.

SUPPLEMENTAL TABLE 3. Treatment and outcome of liveborn neonates with FNAIT per country

Characteristic	Australia (n = 74)	Norway (n = 56)	Slovenia (n = 19)	Spain (n = 55)	Sweden (n = 31)	The Netherlands (n = 138)	USA (n = 16)	Total (n = 389)
HPA antibody specificity - (n, %)								
HPA-1a	56 (76)	54 (96)	16 (84)	37 (67)	24 (77)	98 (71)	6 (38)	291 (75)
HPA-5b	6 (8)	1 (2)	3 (16)	5 (9)	3 (10)	28 (20)	0	46 (12)
Other	12 (16)	1 (2)	-	13 (24)	4 (13)	12 (9)	10 (62)	52 (13)
Pregnancy characteristics								
First pregnancy (primigravida) - (n, %)	9 (12)	14 (25)	6 (32)	15 (27)	4 (13)	34 (25)	0	82 (21)
FNAIT diagnosed in previous pregnancy - (n, %)	30 (41)	18 (32)	3 (16)	12 (21)	12 (39)	39 (28)	3 (19)	117 (30)
Antenatally treated - (n, %)	47 (63)	3 (5)	5 (26)	11 (20)	12 (39)	44 (32)	3 (19)	105 (27)
Neonatal characteristics								
Male sex - (n, %)	39 (59)	34 (61)	14 (74)	38 (69)	24 (77)	83 (61)	9 (56)	241 (64)
SGA - (n, %)	5 (8)	16 (29)	2 (11)	13 (24)	15 (48)	22 (17)	4 (25)	77 (21)
Platelet count								
Platelet count nadir (× 10^9 per L) - median (min. – max.)	25 (1–305)	15 (2–106)	26 (6–375)	27 (2–322)	39 (5–353)	20 (3–382)	14 (4–297)	21 (1–382)
Very severe thrombocytopenia (PC<25×10^9 per L) - (n, %)	33 (50)	39 (70)	8 (50)	27 (49)	11 (35)	78 (57)	10 (63)	206 (54)
Severity of bleeding symptoms†								
Mild bleeding - (n, %)	33 (45)	36 (64)	11 (58)	22 (40)	14 (45)	64 (46)	6 (38)	186 (48)
Severe bleeding - (n, %)	3 (4)	3 (5)	1 (5)	5 (9)	2 (7)	8 (6)	1 (6)	23 (6)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing.

† Severe bleeding was defined as cases with severe organ bleeding and/or severe ICH, mild bleeding are cases with skin bleeding, mild organ bleeding and/or intraventricular haemorrhage (IVH) grade I of II.

Abbreviations: USA, United States of America; HPA, human platelet antigen; FNAIT, fetal neonatal alloimmune thrombocytopenia; SGA, small for gestational age; L, litre; PC, platelet count.

SUPPLEMENTAL TABLE 4. Transfusion guidelines per centre in neonates affected by FNAIT during the study period (2010-2020)

	Transfusion threshold in cases without bleeding	Transfusion threshold in cases with bleeding	Recommended platelet transfusion dosage
Monash University, Australia	30×10^9/L in a term infant 50×10^9/L in a preterm infant	100×10^9/L for intracranial bleeding 50×10^9/L for other sites of bleeding	10 – 20 mL/kg
UiT The Arctic University of Norway, Norway	25×10^9/L	50×10^9/L	15 mL/kg
University Medical Centre Ljubljana, Slovenia	30×10^9/L until 72 hours after birth 20×10^9/L after 72 hours after birth	100×10^9/L for intracranial bleeding 50×10^9/L for other sites of bleeding	15 – 20 mL/kg
Banc de Sang i Teixits, Spain	50×10^9/L	100×10^9/L	20 mL/kg
Karolinska University Hospital, Sweden	30×10^9/L 50×10^9/L in neonates with birthweight <1500 grams during the first week of life	100×10^9/L for intracranial bleeding 50×10^9/L for other sites of bleeding	10 – 15 mL/kg
Leiden University Medical Center & Sanquin Diagnostics, The Netherlands†	25×10^9/L	50×10^9/L	10 mL/kg
Levine Children's Hospital, United States of America	30×10^9/L in a term infant 50×10^9/L in a preterm infant	100×10^9/L for intracranial bleeding	15 mL/kg
Boston Children's Hospital, United States of America	30×10^9/L in a term infant 50×10^9/L in a preterm infant	100×10^9/L for intracranial bleeding	15 mL/kg

† Random donor platelet transfusions are composed of material from five donors. Matched HPA transfusion products are composed of a single donor. The concentration of platelets in HPA matched transfusions are not different from HPA unmatched donor transfusions.

SUPPLEMENTAL TABLE 5. Characteristics of neonates with severe intracranial haemorrhage

Variable	Total (n = 22)
First pregnancy - n (%)	13 (59)
HPA antibody specificity - n (%)	
HPA-1a	14 (63)
HPA-1b	1 (5)
HPA-3a	1 (5)
HPA-5a	1 (5)
HPA-5b	2 (9)
HPA-15b	2 (9)
Unknown	1 (4)
Type of ICH - n (%)	
Intraparenchymal	10 (45)
Intraventricular grade III or IV	5 (23)
Subarachnoid	5 (23)
Subdural	1 (4)
Subpial	1 (4)
Detection of ICH - n (%)	
Antenatal, 23 weeks' GA	1 (4)
Antenatal, 32-34 weeks' GA	2 (9)
Postnatal, day of birth	2 (9)
Postnatal, 1 day after delivery	9 (41)
Postnatal, 2 days after delivery	3 (14)
Postnatal, 3 or more days after delivery	3 (14)
Unknown	2 (9)
Skin bleeding - n (%)	12 (55)
Platelet count nadir, median (IQR; min-max)	11 (7 – 26; 2 – 158)

All statistics and percentages are calculated based on the valid numbers. i.e. excluded the missing

† Case with platelet count 158 × 10^9/L received antenatal treatment.

Abbreviations: HPA, human platelet antigen; ICH, intracranial haemorrhage; GA, gestational age; IQR, interquartile range.

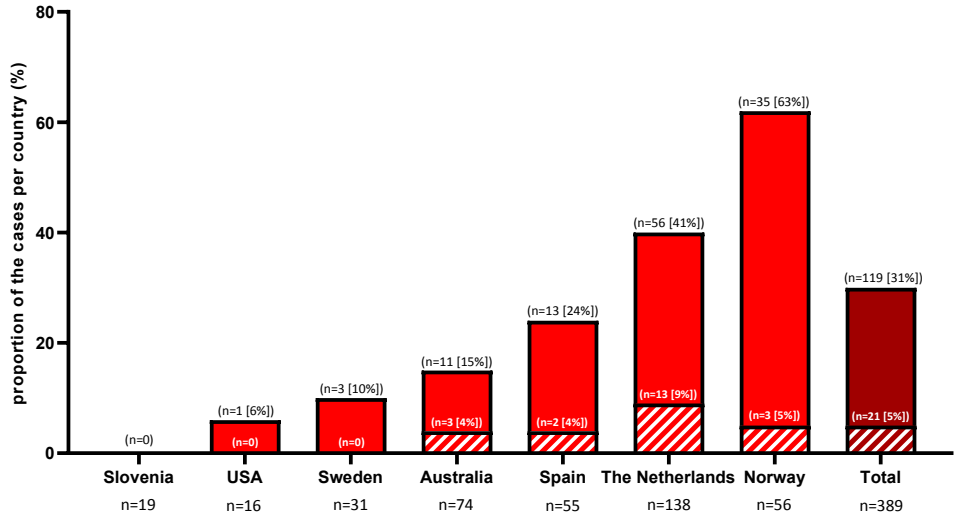


FIGURE 1A. Matched platelet transfusions

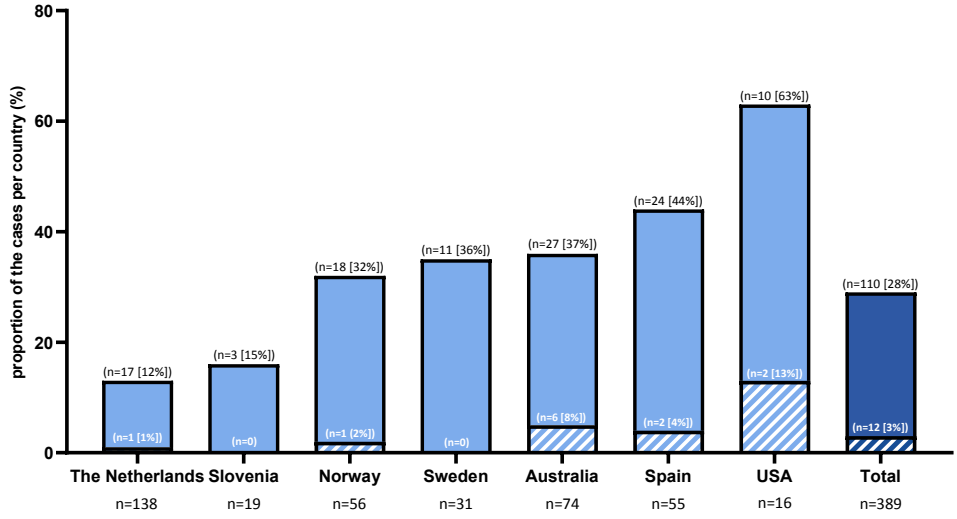


FIGURE 1B. Postnatal treatment with intravenous immune globulin (IVIg)

SUPPLEMENTAL FIGURE 1. Treatment with matched platelet transfusions and IVIg per country

Figure 1A shows the proportion of cases treated with HPA typed and matched donor platelet transfusions. **Figure 1B** shows the proportion of cases that were treated postnatally with intravenous immune globulin (IVIg) either in combination with platelet transfusions or IVIg alone. Striped parts in both graphs represent cases that were treated antenatally

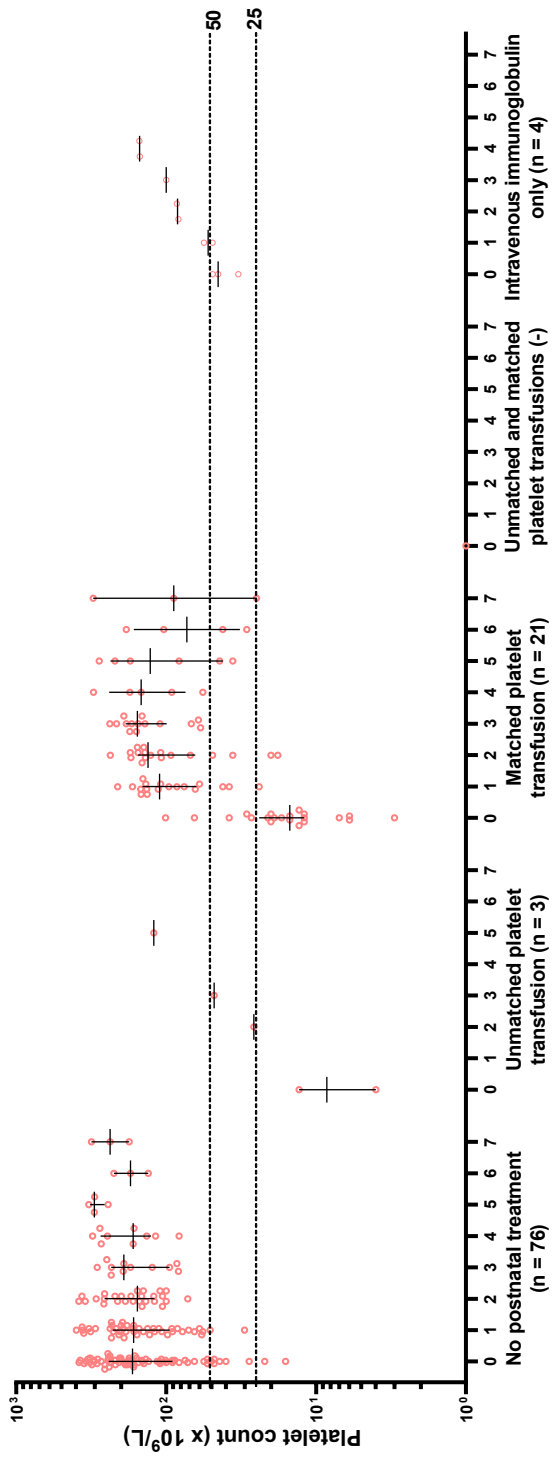


FIGURE 2A. Antenatally treated cases only

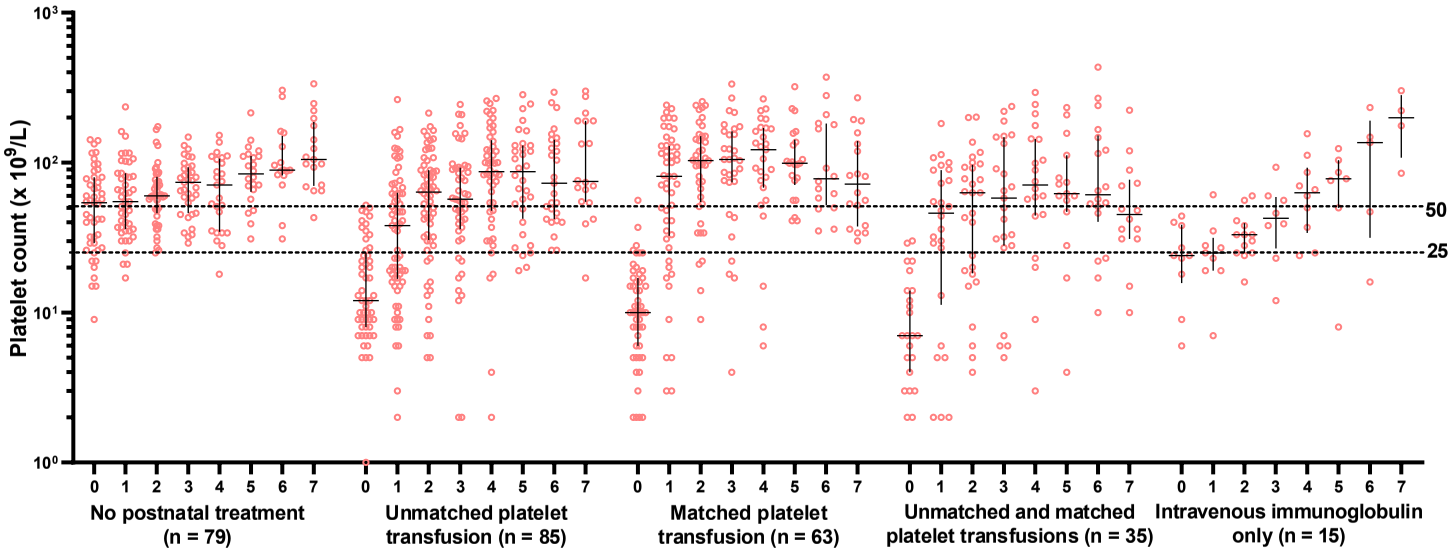
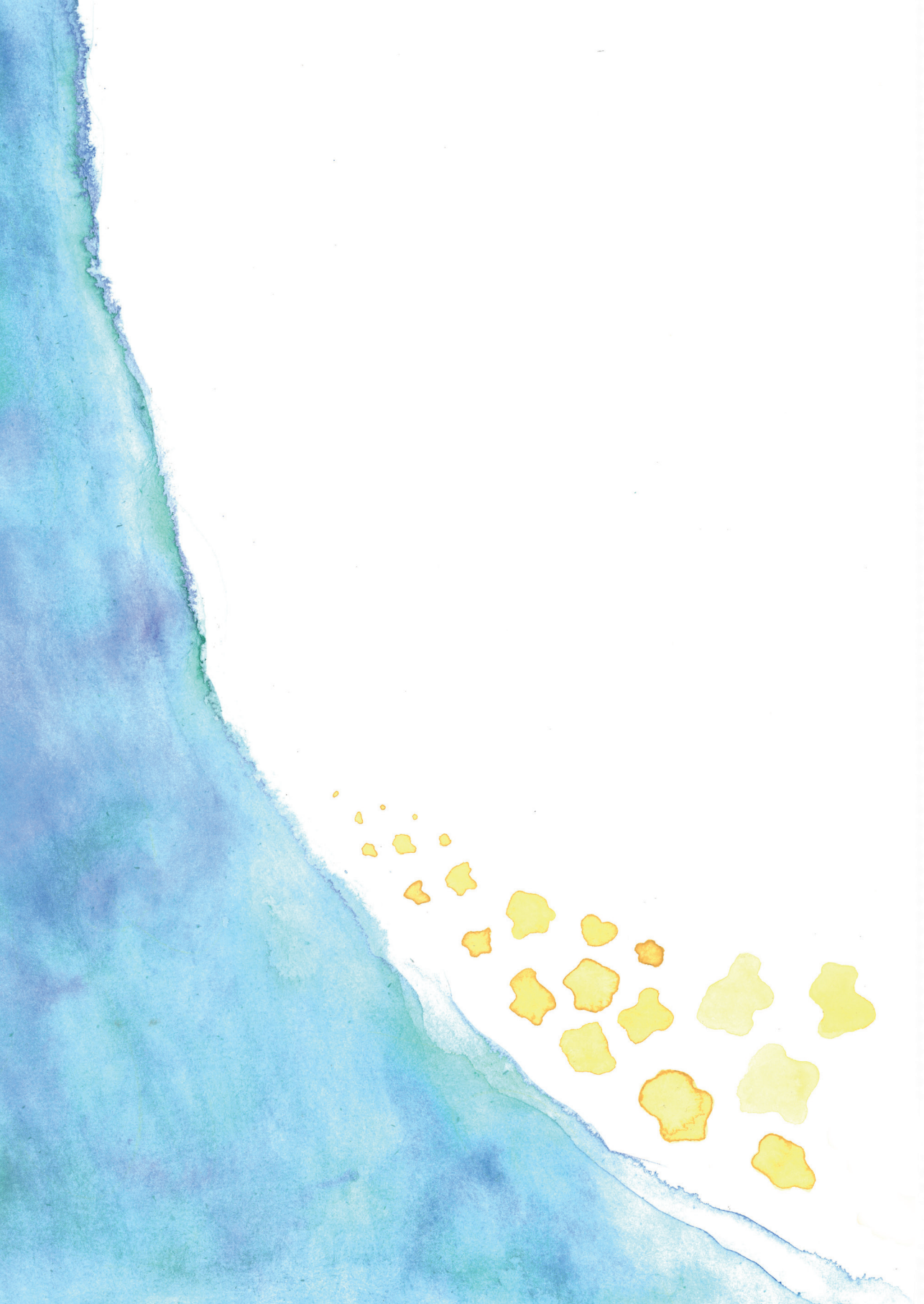


FIGURE 2B. Antenatally untreated cases only

SUPPLEMENTAL FIGURE 2. Platelet count per treatment strategy (stratified by antenatal treatment)

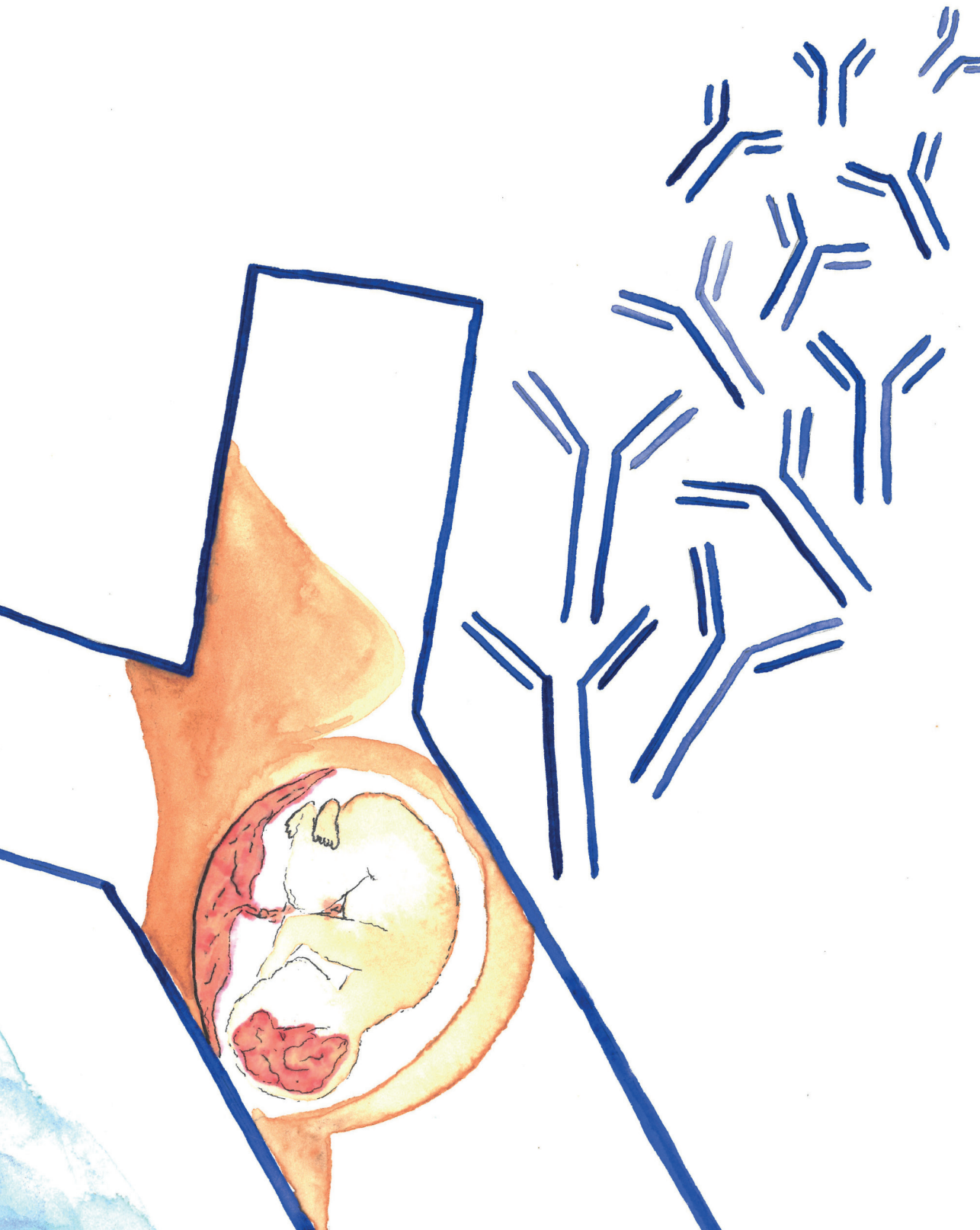
Supplemental Figure 2A shows the lowest neonatal platelet count per day per treatment strategy group of cases that were antenatally treated with IVIg. Missing data for one case in the no-treatment group. **Supplemental Figure 2B** shows the lowest neonatal platelet count per day per treatment strategy group of cases that were not antenatally treated with IVIg. Missing data for eight cases in the no treatment group. The horizontal lines in the graph represent platelet count of 50×10^9 per L and 25×10^9 per L for the upper and lower line respectively. Dots represent platelet counts, for each day the median and interquartile range are depicted with black lines.

Abbreviations: PTx, platelet transfusion; IVIg, intravenous immunoglobulin;



PART FIVE

Long-term outcome



CHAPTER 7

Children newly diagnosed with fetal and neonatal alloimmune thrombocytopenia: neurodevelopmental outcome at school age

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ABSTRACT

OBJECTIVE

To evaluate the neurodevelopmental outcome at school age in children newly diagnosed with fetal neonatal alloimmune thrombocytopenia (FNAIT).

STUDY DESIGN

This observational cohort study included children diagnosed with FNAIT between 2002 and 2014. Children were invited for cognitive and neurological testing. Behavioral questionnaires and school performance results were obtained. A composite outcome of neurodevelopmental impairment (NDI) was used, subdivided into mild-to-moderate and severe NDI. Primary outcome was severe NDI, defined as IQ < 70, cerebral palsy Gross Motor Functioning Classification Scale (GMFCS) level ≥ III or severe visual/hearing impairment. Mild-to-moderate NDI was defined as IQ 70-85, minor neurological dysfunction or cerebral palsy GMFCS level ≤ II or mild visual/hearing impairment.

RESULTS

In total, 44 children were included at a median age of 12 years (range 6-17 years). Neuroimaging at diagnosis was available in 82% (36/44) of children. High-grade intracranial hemorrhage (ICH) was detected in 14% (5/36). Severe NDI was detected in 7% (3/44); in two children with high-grade ICH and one with low-grade ICH and perinatal asphyxia. Mild-to-moderate NDI was detected in 25% (11/44); in one child with high-grade ICH; eight without ICH and in two children neuroimaging was not performed. Adverse outcome (perinatal death or NDI) was 39% (19/49). Four children (9%) attended special needs education, three of whom had with severe NDI and one with mild-to-moderate NDI. Total behavioral problems within the clinical range were reported in 12%, which is comparable to 10% in the general Dutch population.

CONCLUSION

Children who are newly diagnosed with FNAIT are at increased risk for long-term neurodevelopmental problems, even those without ICH.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT), the platelet equivalent of hemolytic disease of the fetus and neonate, can cause severe bleeding in children during pregnancy and shortly after delivery. This risk of bleeding is caused by maternal platelet directed alloantibodies, that are actively transported across the placenta during pregnancy. These antibodies result in platelet destruction and possibly interfere with endothelial cells[1] in the fetus/neonate, resulting in a risk of bleeding. ICH occurs in 10-25% of the cases with severe thrombocytopenia and can be effectively prevented by antenatal treatment.[2, 3] However, in the absence of antenatal screening for platelet alloantibodies, FNAIT is often diagnosed postnatally, if bleeding symptoms are present or thrombocytopenia is detected as a finding by chance. Effective antenatal treatment is currently only given in subsequent pregnancies.[3-5]

Knowledge on long-term outcome of children with FNAIT is scarce. Previous studies primarily addressed the neurodevelopment of children after antenatal treatment for FNAIT,[6] mainly focused on cases with ICH,[7-10] or based their conclusions on questionnaire surveys.[11] It is important to evaluate neurodevelopment of children only treated after diagnosis of FNAIT and who did not have signs of ICH. This knowledge is crucial to provide adequate follow-up care for children affected by FNAIT and to judge the potential need for an FNAIT screening program.

The present study evaluates the long-term neurodevelopmental outcome of children newly diagnosed with FNAIT. In addition, behavioral difficulties and school performance reports were assessed.

METHODS

PARTICIPANTS

All children newly diagnosed with FNAIT between 2002 and 2014 and referred to the Leiden University Medical Center (LUMC) and survived the neonatal period were eligible for study participation. The LUMC is the national clinical expertise center for FNAIT in The Netherlands. FNAIT was diagnosed based on clinical suspicion with a neonatal platelet level of $< 150\times10^9$/L and/or bleeding complications, confirmed fetal-maternal HPA incompatibility, and the presence of maternal HPA specific alloantibodies.[12] Children that died perinatally were excluded. Other exclusion criteria were congenital abnormalities not related to FNAIT and the family having moved abroad. The Medical Ethics Committee of Leiden-Delft-The Hague approved the study (P19.069). Written informed consent from all parents or caregivers was obtained. All children provided assent. If children could not assent, parents were asked for permission and the study was conducted unless children opposed participation. The study

was registered at ClinicalTrials.gov (Identifier: NCT04529382). When informed consent was obtained, a one-time follow-up examination was planned, consisting of taking the history, a neurological examination and a standardized intelligence test, either at home or at the outpatient clinic of the LUMC. Parents were asked to complete a questionnaire on their child's behavior and to share school performance results.

METHODS OF MEASUREMENT

The following maternal, obstetrical and neonatal characteristics were obtained from the medical records: gravidity, parity, gestational age at birth (weeks plus days), birth weight (grams), neonatal sex, lowest platelet count, specificity HPA alloantibody, reason for FNAIT suspicion, postnatal treatment, bleeding symptoms, neonatal morbidity including perinatal asphyxia (5-minute Apgar score < 7 or arterial blood cord pH < 7.0) and/or neonatal sepsis (clinical suspicion of infection and positive blood culture), and cerebral imaging report. If the report of the cerebral ultrasound described abnormalities, the images were retrieved and re-evaluated by neonatologists specialized in neonatal cerebral imaging (SJS and LSdV). All scans were scored for intracranial hemorrhage (ICH), including intraventricular hemorrhage (IVH) and/or parenchymal hemorrhage. IVH was classified as any blood in the ventricular system with a distinction between low-grade (small IVH without associated ventricular dilatation) or high-grade (large IVH with associated ventricular dilatation or periventricular hemorrhagic infarction). Parenchymal hemorrhage was classified with a distinction between low-grade parenchymal hemorrhage (hemorrhagic lesions in the brain parenchyma/cerebellum ≤ 4 mm) or high-grade parenchymal hemorrhage (lobar hemorrhages). High-grade ICH was defined as either high-grade IVH or high-grade parenchymal hemorrhage. Low-grade ICH was defined as low-grade IVH or low-grade parenchymal hemorrhage. Organ bleeding that required supportive care was classified as severe organ bleeding.

Cognitive development was assessed with the Wechsler Intelligence Scale for Children, fifth edition (WISC-V-NL).[13] The WISC generates a Full Scale Intelligence Quotient (FSIQ) score representing a child's general intellectual ability. The FSIQ is on a standard score metric with a mean of 100 and a standard deviation (SD) of 15. In the Dutch norm population, 13.6% has mild-to-moderate cognitive impairment (IQ 70–85, [-1 SD]) and 2.2% has severe cognitive impairment (IQ < 70, [-2 SD]).13 In case of problems in language and speech development and communication, the Snijders-Oomen nonverbal intelligence test (SON) was performed.[14] Neurological examination was performed according to the adapted version of the Touwen examination, which aims to detect minor neurological dysfunction (MND) and addresses eight neurological domains.[15] Before puberty, the severity of MND, simple or complex, is based on the number of abnormal domains, whereas after puberty it is based on specific abnormal domains.[16] The level of cerebral palsy (CP) was classified using the Gross Motor Functioning Classification System (GMFCS) 17 where a score of 2 or higher was categorized as CP. If lower, a child was categorized as minor neurologic dysfunction.

Behavioral functioning was assessed using the Child Behavior Checklist (CBCL) for 6-18 years.[18] In the present study, internalizing problems score (anxious and depressive symptoms, social withdrawal, and somatic complaints), externalizing problems score (rule-breaking and aggressive behavior), and total problems score were assessed. The CBCL scoring system creates a T-score based on a Dutch normative sample with a mean of 50 and SD of 10, which was interpreted as within the normal (T < 60, < 84th percentile), borderline (T = 60–63; 84th–90th percentile), or clinical range (T ≥ 64; ≥ 91st percentile).

School performance results were obtained for reading comprehension, spelling, and arithmetic/mathematics according to the Dutch National Pupil Monitoring System (Cito).[19] These results were compared to peers and graded as I through V. Grade I represents the 20% highest scoring children and grade V the 20% lowest scoring children. Additionally, the proportion of children that needed special education was reported.

OUTCOMES

The primary outcome measure was the prevalence of severe neurodevelopmental impairment (NDI), defined as at least one of the following: severe cognitive impairment (IQ < 70, [-2 SD]), CP GMFCS level ≥ III, bilateral blindness, and/or bilateral deafness requiring amplification. Secondary outcome measures were mild-to-moderate NDI, total behavioral problem score, school performance, and the overall adverse outcome defined as NDI or perinatal mortality. In addition, we compared the risk of severe NDI between cases with and without ICH. Mild-to-moderate NDI was defined as the presence of one of the following criteria: mild-to-moderate cognitive impairment (IQ 70–85, [-1 SD]), CP GMFCS level I or II, MND, vision loss, and/or hearing loss.

DATA ANALYSES

Data are presented as frequencies and percentages for categorical variables and as means with SD or medians with interquartile range (IQR) for continuous variables. Mean IQ score was compared to Dutch norm data using a one-sample T-test. Mean IQ score was compared between children with and without ICH using a two-sample T-test. The proportion of cases with cognitive impairment, behavior problems and levels of school performance scores were compared to the Dutch norm data using a binominal test. The risk of severe NDI in cases with severe ICH was compared to the risk of severe NDI in cases without severe ICH using the Fisher's Exact test. Clinical characteristics and risk factors for NDI (neonatal morbidity, SGA, gestational age at delivery and/or maternal educational level) were reported for cases with NDI and without NDI. Data were analyzed using IBM SPSS Statistics 26.0 (Chicago, IL, USA). Clinical characteristics of the included children and the children who did not undergo neurodevelopmental assessment were compared to assess selection bias.

RESULTS

STUDY POPULATION

Between 2002 and 2014, 67 cases with newly diagnosed FNAIT were referred to the LUMC (Figure 1). Perinatal mortality occurred in 5 (7%) children: one termination of pregnancy after the diagnosis of severe ICH and hydrocephalus, two children died in utero after ICH, and two in the neonatal period due to severe ICH. In total, 44/56 (79%) children were included for long-term neurodevelopmental assessment.

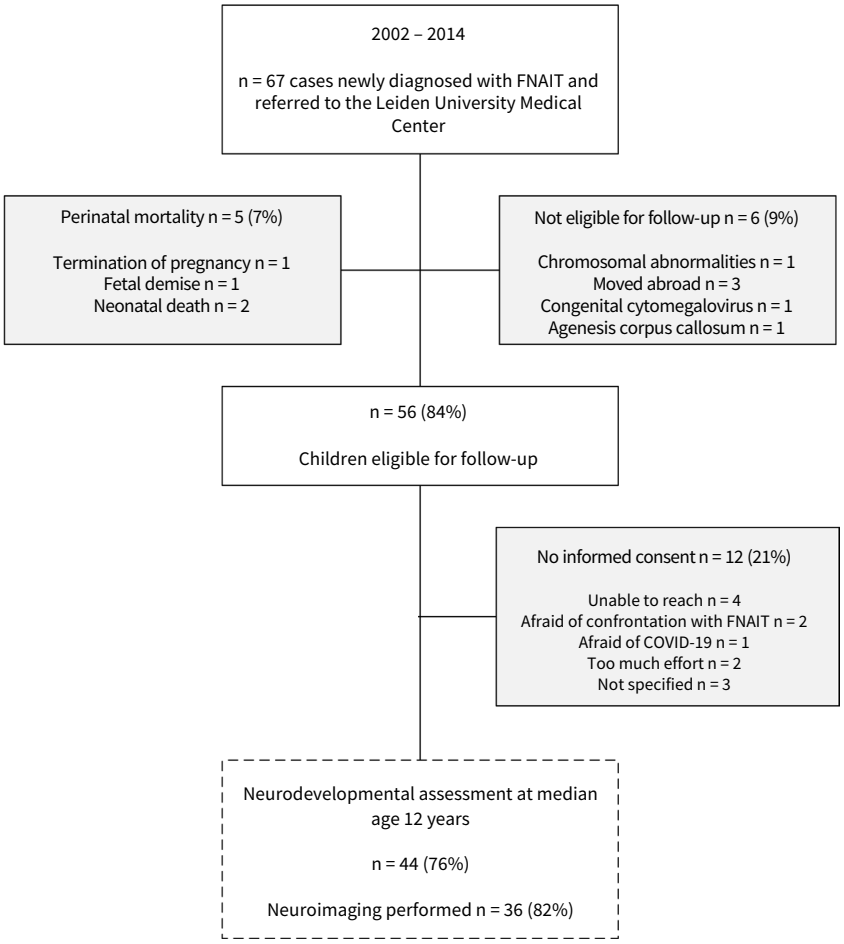

FIGURE 1. Study population

Abbreviation: FNAIT, fetal and neonatal alloimmune thrombocytopenia;

CLINICAL CHARACTERISTICS AND NEONATAL OUTCOME

Table 1 shows the clinical characteristics of the 44 school-aged children included for long-term follow-up. The majority (80%) had anti-HPA-1a alloantibodies. Children were born at a median gestational age of 38^{+5} (IQR 37^{+3} – 40^{+5}) weeks with a median birth weight of 3135 (IQR 2610 – 3649) grams. In total, 35 (80%) children were male. Neonatal morbidity occurred in four (9%) children; one child had early neonatal sepsis and three had perinatal asphyxia. The median nadir platelet count was 14 × 10^9/L (minimum 2 × 10^9/L and maximum 158 × 10^9/L), 31 (71%) children had a platelet count below 25 ×10^9/L. Characteristics of the included cases were comparable to the cases who did not undergo neurodevelopmental assessment (Supplemental Table 1).

TABLE 1. Clinical characteristics and neonatal outcome

Variables	n = 44
Diagnostics	
HPA specificity, n (%)	
HPA-1a	35 (80)
HPA-5a	2 (5)
HPA-5b	5 (11)
HPA-1a and HPA-3a	1 (2)
HPA-1a and HPA-5b	1 (2)
Reason for FNAIT suspicion, n (%)	
Skin bleeding	24 (54)
Organ bleeding	2 (5)
Hematological examination without clinical signs of thrombocytopenia	16 (36)
Antenatal ICH	2 (5)
Pregnancy	
First pregnancy, n (%)	23 (52)
Signs of fetal bleeding on ultrasound, n (%)	2 (5)
Maternal IVIg treatment, n (%) †	2 (5)
Neonatal	
Gestational age at delivery, weeks^{+days}, median (IQR)	38^{+5} (37^{+3} – 40^{+5})
Female sex, n (%)	9 (20)
Birth weight, gram, median (IQR)	3135 (2610 – 3649)
SGA (birth weight < 10^{th} percentile), n (%)	9 (20)
Apgar score, 5 minutes after birth, median (IQR)	10 (9 – 10)
Skin bleeding (hematoma or petechiae), n (%)	29 (66)
ICH, n/N (%) ‡	8/36 (22)
Low-grade ICH	3/36 (8)
High-grade ICH ‖	5/36 (14)
Organ bleeding, n (%)§	5 (11)
Of which severe	2 (5)
Platelet count nadir × 10^9/L, median (IQR)	14 (7 – 30)
Platelet count < 25 × 10^9/L, n (%)	31 (71)
Postnatal treatment given, n (%)	26 (59)

† In two children, ICH was observed during pregnancy after which FNAIT was diagnosed and IVIg treatment was started, in one case at 23 weeks' gestation and in the other child at 30 weeks' gestation (for details see Table 4).
‡ Neuroimaging was not performed in 8/44 (18%) of the children. In 29/44 children (66%) cerebral ultrasound was performed and in 7/44 children (16%) both cerebral ultrasound and MRI were performed.
§ One case with lung bleeding (severe), one case with gastro-intestinal bleeding (severe) two cases with retinal bleeding (mild) one case with scrotal hematoma (mild).
Abbreviations: HPA, human platelet antigen; FNAIT, fetal neonatal alloimmune thrombocytopenia; ICH, intracranial hemorrhage; IVIg, intravenous immunoglobulins; IQR, interquartile range; SGA, small for gestational age; L, liter.

Neonatal cerebral imaging was performed in 36/44 children (82%). ICH was reported in eight children (22%). Two were classified as high-grade IVH, three as high-grade parenchymal hemorrhage, one as low-grade IVH and, two as low-grade parenchymal hemorrhage. In two children, high-grade ICH was detected antenatally upon routine ultrasound examination (at 23 weeks and 30 weeks of gestational age). In both cases antenatal IVIg treatment was started after HPA-antibodies were diagnosed.

LONG-TERM NEURODEVELOPMENTAL OUTCOME

Long-term neurodevelopment was assessed at a median age of 12 years (minimum 6 years and 5 months and maximum 17 years and 4 months (Table 2)). Overall, NDI was present in 14 (32%) children of which 3 with severe NDI (7%, 3/44, 95% CI: -0.8 – 14%) and 11 with mild-to-moderate NDI (25%, 11/44, 95% CI: 12 – 38%). The overall adverse outcome, NDI or perinatal death, was 39% (19/49, 95% CI: 25 – 54%).

Cognitive assessment with the WISC-V was performed in 41/44 children. The mean IQ score was 100 (SD 14). Due to severe problems in speech and language development, two children were assessed with the SON. IQ scores of these two children were 49 and 60. For one child, the parents did not consent to cognitive testing. Overall, the mean IQ score (98 ± 17) was not different from the general Dutch population norm (100 ± 15; P = 0.420). Mild-to-moderate cognitive impairment was present in 16% (7/43, 95% CI: 5.0 – 28%) and severe cognitive impairment in 5% (2/43, 95% CI: -1.8 – 11%).

Neurological testing was completed in 41 children (93%). MND was detected in eight children of which four children presented with simple MND (10%, 95% CI: 0.5–19%) and four with complex MND (10%, 95% CI: 0.5–19%). CP was observed in two children (5%, 2/41, 95% CI: -1.9 – 12%), one with spastic diplegia and one with spastic tetraplegia, both classified as GMFCS level IV. One child (2%) was diagnosed with bilateral deafness requiring hearing amplification, related to perinatal asphyxia. The cause of perinatal asphyxia in this child remained unclear.

Table 3 presents the details of the children with NDI. Of the three children with severe NDI, one was diagnosed with high-grade IVH and one with high-grade parenchymal hemorrhage. The third child with severe NDI was diagnosed with bilateral deafness, postnatal MRI showed cerebral edema related to perinatal asphyxia and (low-grade parenchymal hemorrhage). Of the 11 children with mild-to-moderate NDI, one was diagnosed with high-grade IVH, eight children did not have cerebral hemorrhage and in two children no brain imaging was reported. Two cases with high-grade ICH had a normal neurodevelopmental outcome. Both cases that had no NDI despite high-grade ICH had an unilobular parenchymal hemorrhage whereas in the group of children that had NDI and high-grade ICH, two had periventricular hemorrhagic infarction and one had a multilobular parenchymal hemorrhage. An overview of the neurodevelopmental outcome of the 44 FNAIT cases is shown in Figure 2.

TABLE 2. Neurodevelopmental outcome

Variables	n = 44	Dutch norm scores
Age, years and months, median (IQR)	12y0m (9y9m - 14y11m)	
Cognitive		
Full scale IQ, mean (SD) †	98 (17)	100 (15)
Verbal comprehension	104 (13)	
Visual spatial score	99 (16)	
Fluid reasoning scale	101 (13)	
Working memory score	97 (14)	
Processing speed	98 (13)	
Normal range (IQ > 85), n (%)‡V	35 (80)	
Mild-to-moderate cognitive impairment (IQ 70 – 85)	7 (16)	13.6%
Severe cognitive impairment (IQ < 70)	2 (5)	2.2%
Neurological		
MND, n/N (%)§		
Simple MND	4/41 (10)	15%
Complex MND	4/41 (10)	6%
Abnormal domain, n/N (%)		
Posture	4/41 (10)	
Reflexes	0	
Involuntary movements	1/41 (2)	
Coordination	6/41 (15)	
Fine manipulative ability	2/41 (5)	
Associated movements	0	
Sensory deficits	1/41 (2)	
Cranial nerve function	1/41 (2)	
CP, n (%) ||	2 (5)	0.4%
Bilateral deafness requiring hearing amplification, n (%)	1 (2)	
Bilateral blindness, n (%)	0	
Demographics		
Maternal education level, n/N (%)		
Low	4/42 (10)	
Intermediate	17/42 (40)	
High	21/42 (50)	
NDI		
NDI, n (%) #		
Normal	30 (68)	
Mild-to-moderate NDI	11 (25)	
Severe NDI	3 (7)	

† IQ was not available in 1/44 (2%). Verbal comprehension, visuospatial score, fluid reasoning scale, and working memory score were not available in 4/44 (9%). Processing speed was not available in 3/44 (7%).
‡ Based on the information of the school results and questionnaires that were completed by the parents or caregivers, we categorized the missing cognitive test scores as normal.
§ Neurological test was not performed in 3/44 (7%) children due to no permission.
|| Both children with CP were classified as a Gross Motor Functioning Classification Scale (GMFCS) level IV. One child had spastic diplegia, the other spastic tetraplegia.
Based on the information of the school results and questionnaires that were completed by the caregivers, we categorized the missing scores needed for NDI as normal.
Abbreviations: IQR, Interquartile range; IQ, intelligence quotient; SD, standard deviation; MND, minor neurological dysfunction; CP, cerebral palsy; NDI, neurodevelopmental impairment.

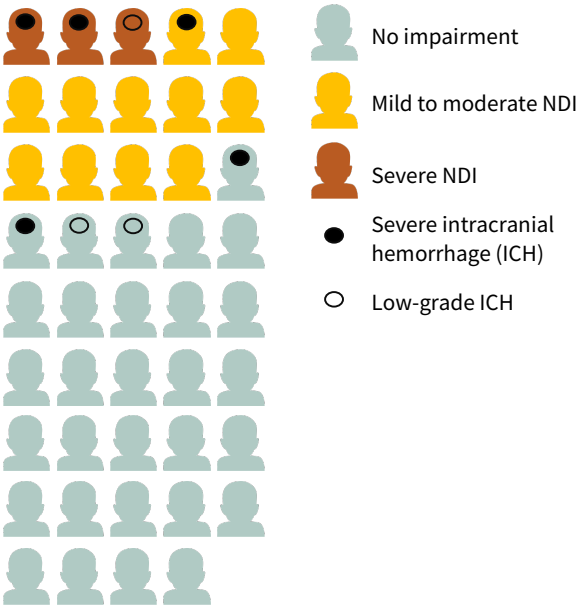

FIGURE 2. Neurodevelopmental outcome of 44 FNAIT cases

Abbreviations: NDI, neurodevelopmental impairment; ICH, intracranial hemorrhage.

Children with high-grade ICH had significantly lower cognitive scores compared to the children without high-grade ICH (median IQ 65 (range 49 – 106) versus 99 (range 76 – 129) respectively, $P = 0.027$). In total, 40% (2/5) of cases with high-grade ICH had severe NDI versus 3% (1/38) of cases without high-grade ICH (relative risk 15.6, 95% CI 1.7 – 142.6, $P = 0.030$). In 50% (7/14) of the cases with NDI, other risk factors for NDI were present compared to 20% (6/30) in the cases without NDI. Clinical characteristics of the cases with and without NDI are shown in Supplemental Table 2. Within the group of cases that had NDI, the majority (71%, 10/14) were affected by anti-HPA-1a. Of the four children affected by other HPA specific antibodies two had high-grade ICH, one was affected by perinatal asphyxia and one was born SGA.

BEHAVIORAL FUNCTIONING AND SCHOOL PERFORMANCE

Five out of 41 children (12%) scored in the clinical range (T score ≥ 91st percentile) on the total problem score of the behavioral questionnaire (Supplemental Table 3). School performance scores were available for 43/44 (98%) children (Supplemental Table 3). The prevalence of the children scoring the lowest level V range did not differ significantly from the Dutch norm population. Four children (9%, 4/44) attended special needs education, of which three had high-grade ICH.

TABLE 3. Characteristics of the children with neurodevelopmental impairment

	Sex	GA birth (wks)	Other risk factor for NDI†	PC< 25×10^9/L	ICH	Neuroimaging ‡	Other bleeding symptoms	HPA	Age at test (y)	IQ	Neurological examination; abnormal domain	Behavior total problem score	School performance; Reading comprehension, spelling, arithmetic §	NDI
1	F	40	Perinatal asphyxia, SGA	No	Low-grade parenchymal hemorrhage	MRI: White matter: cerebral edema (related to perinatal asphyxia). Parenchymal hemorrhage left parietal, ⌀ 2 mm	None	5b	16	85	Simple MND, bilateral deafness	ADHD	V, III, V	Severe
2	M	35	None	No	High-grade IVH	MRI: Bilateral PVHI and severe ventriculomegaly (VP shunt)‖	Antenatal ICH	5b	13	60 #	CP spastic diplegia GMFCS IV	NA	V, V, V Special needs	
3	M	41	Perinatal asphyxia, SGA	Yes	High-grade parenchymal hemorrhage	MRI: Bilateral hemorrhage: multilobular left and temporal lobe right	None	1a	16	49 #	CP spastic tetraplegia GMFCS IV	NA	V, V, V Special needs	
4	M	37	None	No	High-grade IVH	MRI: PVHI and cerebellar hemorrhage††	None	5a	10	70	Complex MND	Clinical	V, V, V Special needs	Mild-to-moderate
5	M	40	SGA	Yes	No	cUS: No abnormalities	Gastro-intestinal bleed	5a	10	76	Normal	Clinical	V, V, V Special needs	
6	M	35	None	No	Unknown	Not performed	Petechiae	1a	11	80	Simple MND	Normal	V, V, IV	
7	M	38	Low maternal education level	Yes	No	cUS: No abnormalities	Hematoma, petechiae	1a	16	81	NA	Normal	IV, IV, V	
8	M	39	SGA	Yes	No	cUS: No abnormalities	Petechiae	1a	8	84	Normal	Borderline	III, II, II	
9	M	41	None	Yes	No	cUS: Echogenicity grade I	Petechiae	1a	14	84	Normal	Normal	V, V, IV	
10	M	38	None	Yes	Unknown	cUS: Not performed	Petechiae	1a	9	89	Complex MND	Clinical	IV, I, II	
11	M	40	SGA	Yes	No	cUS: No abnormalities	Petechiae	1a	14	118	Complex MND	Normal	II, I, I	
12	M	41	None	Yes	No	cUS: No abnormalities	Petechiae	1a	13	129	Complex MND	Normal	I, I, I	
13	M	37	SGA	Yes	No	cUS: No abnormalities	Hematoma, petechiae	1a	10	95	Simple MND	Clinical	V, V, II	
14	M	39	None	Yes	No	cUS: No abnormalities	Hematoma, lung bleed	1a	6	95	Simple MND	Normal	IV, IV, I	

† Risk factors for NDI were defined as either one of the following: perinatal asphyxia, neonatal sepsis, SGA, prematurity < 32 weeks gestational age and/or low maternal educational level.
‡In 14% (2/14 with NDI) neuroimaging was not performed, in 57% (8/14) cerebral ultrasound was performed, and in 29% (4/14) both cerebral ultrasound and MRI were performed.
§ The scoring of the school results is according to the Dutch National Pupil Monitoring System (CITO).[28] The scores range from I to V, in which grade I represents the 20% best scoring children and grade V the 20% lowest scoring children.
‖ ICH was detected during the pregnancy at ultrasound; after which IVIG treatment was initiated at 23 weeks of gestational age.
IQ was assessed using the Snijders-Oomen nonverbal intelligence test because of problems in language and speech development and communication.
†† ICH was detected during the pregnancy at ultrasound, after which IVIG treatment was initiated at 30 weeks of gestational age.
Abbreviations: GA, gestational age; wks, weeks; PC, platelet count; L, liter; ICH, intracranial hemorrhage; HPA, human platelet antigen; IQ, intelligence quotient; NDI, neurodevelopmental impairment; F, female; M, male; SGA, small for gestational age, MRI, magnetic resonance imaging; MND, minor neurological dysfunction; ADHD, Attention Deficit Hyperactivity Disorder, PVHI, periventricular hemorrhagic infarction; VP, ventriculoperitoneal; CP, cerebral palsy; GMFCS, Gross Motor Function Classification System; NA, not assessed; cUS, cranial ultrasound.

DISCUSSION

This study shows that children newly diagnosed with FNAIT and who survive the neonatal period have a high risk of long-term neurodevelopmental impairment (NDI). Severe NDI was present in 7% and mild-to-moderate NDI in 25%, thus combined nearly one-third of the children had long-term neurodevelopmental problems. The overall adverse outcome, perinatal mortality or NDI, was 39%.

The risk of severe NDI in children with newly diagnosed FNAIT is especially high among those with high-grade ICH. In our cohort severe NDI was observed in two children with high-grade ICH and one child with low-grade ICH and cerebral injury related to perinatal asphyxia. This is in line with a previous study from our group that reported on the neurodevelopmental outcome after ICH due to FNAIT. In this study, severe and mild-to-moderate NDI was diagnosed in 60% (6/10) and 10% (1/10) of the FNAIT survivors with ICH, respectively.[7] In an international cohort study of 43 children with FNAIT-related ICH, 82% of the children that survived had severe neurological disabilities.[10] In our cohort, two cases with high-grade ICH had normal neurodevelopmental outcome, this finding fits in with previous literature in which children can have normal neurodevelopment despite severe brain hemorrhage.[20]
We hypothesized that the neurodevelopmental outcome of newly diagnosed children would be worse than the general population, independent of the presence of ICH. This expectation was based on increasing evidence that the maternal alloantibodies in FNAIT cause platelet destruction and possibly interfere with endothelial cells.[1, 21] Possibly, this leads to small cerebral bleeding and/or impaired (cerebral) angiogenesis that remain subclinical directly after birth, but affect brain development leading to developmental delay on the long term.[22] Alternatively it could be that fetal thrombocytopenia irrespective of the presence of anti-HPA-1a influences brain development.

Within our study, we found mild-to-moderate NDI in 25% of the children of whom only one was diagnosed with high-grade ICH. This percentage was higher than one would expect in the normal population. Half of the children that were classified as mild-to-moderate NDI were diagnosed with MND. In general, the proportion of complex MND (10%) in our study group was slightly higher compared to the 6% in Dutch school aged children.[15] Possibly, the high proportion of children with MND is related to subclinical cerebral damage, that remained undiagnosed short after delivery but led to mild and/or multiple neurodevelopmental problems in the long-term. Alternatively, the higher proportion of children with MND could be explained by the unequal sex distribution in our study group. In accordance with previous cohort studies on FNAIT,[23, 24] we observed an overrepresentation of boys in our cohort. However, these previous studies found a more balanced gender distribution compared to our cohort. Both simple and complex MND are diagnosed two times more often in male children compared to female children. Possibly the high rate of NDI could be related to the

relative higher risk of MND in male infants; 13 of the 14 children with NDI were male. Besides the overrepresentation of male sex, we observed that in 50% of the cases with NDI other risk factors for NDI were present whereas in the cases without NDI this was only in 20%. Unfortunately, an independent risk factor analysis to identify these factors was not possible due to our limited sample size. In addition, SGA, neonatal morbidity and low maternal education level are closely intertwined. HPA-1a immunization was reported to be associated with reduced birthweight in other cohort studies.[24, 25]

Previous studies have described the neurodevelopmental outcome of newly diagnosed children with FNAIT. Ward et al.[11] concluded that newly diagnosed children had a worse long-term outcome than their IVIg-treated siblings, but this was based on a behavioral questionnaire taken over the phone with a loss to follow-up of 32%. Knight et al.[26] conducted a study based on obstetric and pediatric surveillance data in the United Kingdom on children with FNAIT. In this study, 8% of the children newly diagnosed with FNAIT had a disability. However, this study was limited by a follow-up to only one year of age. The percentage of 8% disability seems lower than in our study yet was based on national surveillance data and not on standardized individual assessments and the definition of disability was not clearly described.

The proportion of children diagnosed with cerebral palsy in our study was higher than in the general population, 5% versus 0.2%[27] Both children with cerebral palsy had an antenatally acquired ICH. In total, 9% of the children were in special needs education, which is more than the regular population.[28]

This study shows that children with FNAIT without ICH may be at risk of mild-to-moderate long-term impairment, yet the risk of mortality and severe impairment is especially high for children with ICH. These findings stress the importance of preventing severe bleeding in FNAIT and therefore the development of assays that can identify pregnancies at risk for bleeding within HPA alloimmunized pregnant women. By identification of these pregnancies, antenatal treatment could prevent the occurrence of ICH and cerebral injury and thereby the associated adverse outcome.

The knowledge provided by this study will be of help for obstetricians and neonatologists counselling parents of a child affected by FNAIT. FNAIT survivors are at risk of neurodevelopmental problems, in particular children affected by ICH. To improve the neurodevelopment of children affected by FNAIT that were not antenatally treated with IVIg, adequate follow-up care should be provided. Additionally, the results of our study underline the importance of performing neuroimaging in children newly diagnosed with FNAIT.

A limitation of this study is the absence of a control group. Yet, by using standardized tests based on a normative sample, it is possible to compare the results to the population norms. Another limitation is that not all eligible children were included in the study since 21% of the eligible children did not undergo neurodevelopmental assessment. However, when comparing the included children to those who did not undergo neurodevelopmental assessment, the clinical characteristics were similar. Although most parents or caregivers did not specify the reason for not wanting to participate, some indicated that it had to do with being confronted with the disease again or participation asking too much effort. One further constraint of our study was that it was a single center study including only children referred to the LUMC. Between 2002 and 2014 195 children were diagnosed with FNAIT at our national reference laboratory (Sanquin) of which 67 (34%) were referred to the clinical expertise center (LUMC). A strength of the current study is that it is the first to assess the long-term neurodevelopmental outcome of children newly diagnosed with FNAIT using standardized psychometric tests, neurological tests and incorporating school performance results. In addition, our study included children at an older age than in previous studies, thereby providing a more reliable and accurate view of the long-term development of these children.

In conclusion, children newly diagnosed with FNAIT who survive the neonatal period are at high increased risk of long-term neurodevelopmental problems and therefore should have postnatal neuroimaging and be monitored adequately in a standardized follow-up program. Severe neurodevelopmental impairment and mortality is predominantly observed among children with ICH. In addition, a quarter of the children in this cohort suffer from mild-to-moderate impairment suggesting that the risk of NDI is high also in children without ICH.

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AUTHORSHIP CONTRIBUTIONS

TWdV contributed to methodology, investigation, formal analysis and writing – drafting the initial manuscript, MvZ contributed to investigation and writing – drafting the initial manuscript, MdH, contributed to conceptualization funding acquisition and writing – review or editing the manuscript, DO, CEvdS contributed to conceptualization and writing – review or editing of the manuscript, RNGBT, SJS, LSdV contributed to investigation and writing – review or editing of the manuscript, EL contributed to conceptualization, methodology and writing – review or editing of the manuscript, JMMvK contributed to conceptualization, supervision, and writing – review or editing of the manuscript.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE 1. Comparison of characteristics of children undergoing and excluded from neurodevelopmental testing

Variables	Included children n = 44	No neurodevelopmental assessment n = 12
Gestational age at delivery, weeks^{+days}, median (IQR)	38^{+5} (37^{+3} – 40^{+5})	37^{+6} (36^{+5} - 38^{+3})
First pregnancy, n (%)	23 (52)	4 (33)
Female sex, n (%)	9 (21)	3 (25)
Birth weight, gram, median (IQR)	3135 (2610 – 3649)	2953 (2288 – 3266)
SGA, (birth weight < 10th percentile), n (%)	9 (21)	2 (17)
Platelet count nadir, median (IQR)	14 (7 – 30)	29 (19 - 54)
Skin bleeding, n (%)	29 (66)	6 (50)
ICH, n/N (%)†	7/36 (19)	0
Postnatal treatment given, n/N (%)‡	26/44 (59)	4/9 (44)

Characteristics of the children included in the study were compared to the surviving FNAIT children who did not undergo neurodevelopmental assessment. Analysis was performed using the Mann Whitney *U* test (gestational age, birth weight, and platelet count) or with the Fisher's Exact Test (categorical variables). No statistically significant differences were found.
† Data available for 36/44 (82%) children. From the children who did not undergo neurodevelopmental assessment it was not known whether neuroimaging was performed or not.
‡ Data available for 9/12 (75%) children.
Abbreviations: IQR, interquartile range; SGA, small for gestational age; ICH, intracranial hemorrhage.

SUPPLEMENTAL TABLE 2. Clinical characteristics of the children with and without NDI

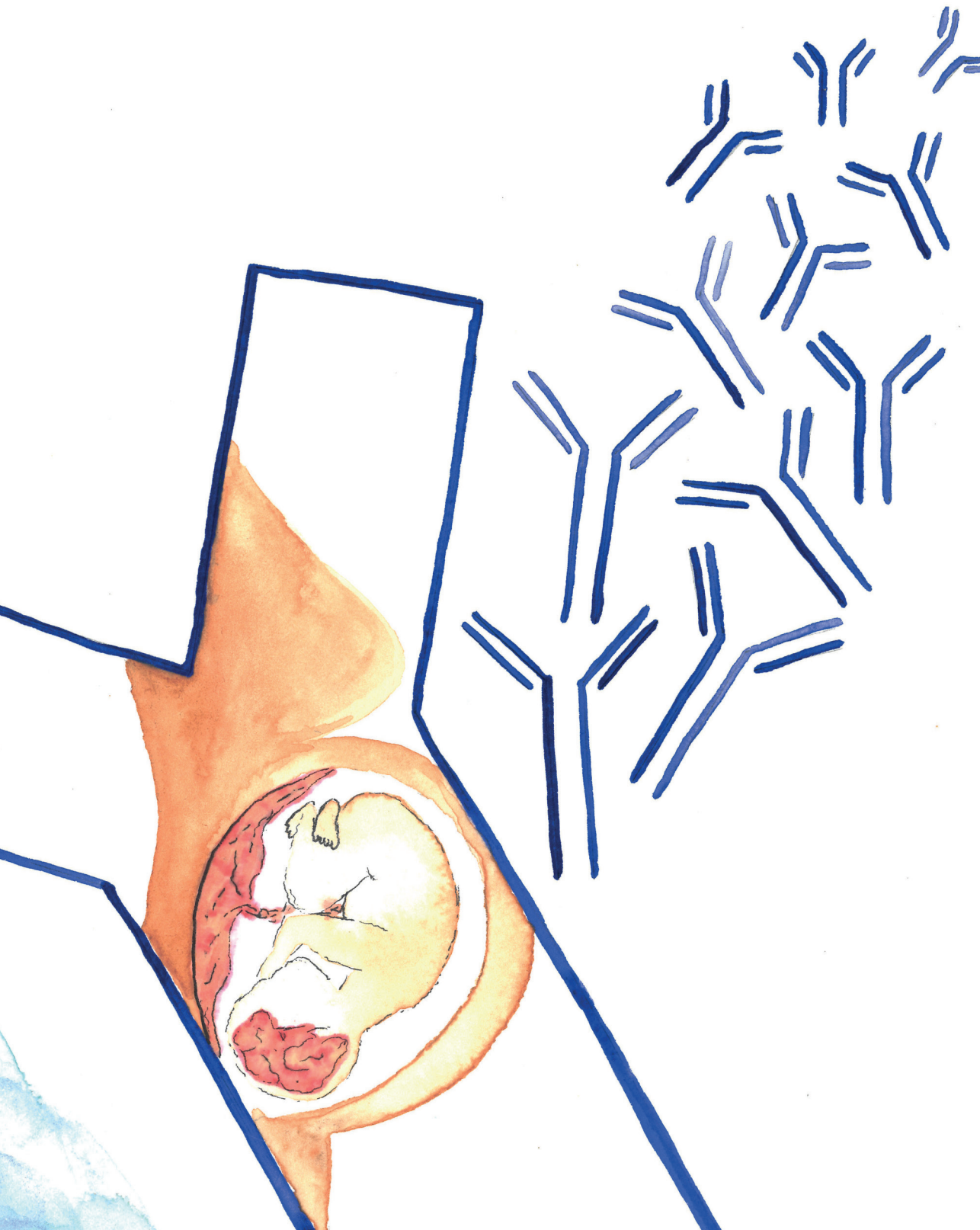
Variables	Children with NDI n = 14	Children without NDI n = 30
HPA specificity, n (%)		
HPA-1a	10 (71)	25 (84)
HPA-5a	2 (14)	0
HPA-5b	2 (14)	3 (10)
HPA-1a + HPA-3a	0	1 (3)
HPA-1a + HPA-5b	0	1 (3)
First pregnancy, n (%)	7 (50)	16 (53)
Gestational age at delivery, weeks^{+days}, median (IQR)	39^{+5} (37^{+5} – 40^{+6})	38^{+3} (36^{+3} – 40^{+3})
Prematurity (< 37 weeks gestational age), n (%)	2 (14)	5 (17)
Female sex, n (%)	1 (7)	8 (27)
Apgar score 1 minute after birth, median IQR	9 (8 – 9)	9 (8 – 9)
Apgar score 5 minutes after birth, median (IQR)	10 (9 – 10)	9 (9 – 10)
Perinatal asphyxia, n (%)	1 (7)	2 (7)
SGA (birth weight < 10th percentile), n (%)	6 (43)	3 (10)
Neonatal sepsis, n (%)	1 (3)	0
Perinatal asphyxia, n (%)	2 (14)	1 (3)
ICH, n/N (%) ‡		
Low-grade ICH §	1 (7)	2 (7)
High-grade ICH ‖	3 (21)	2 (7)
Platelet count < 25 × 10^{9}/L, n (%)	10 (71)	21 (70)
Low maternal education level, n (%)	2 (17)	2 (7)

Abbreviations: HPA, human platelet antigen; SGA, small for gestational age; ICH, intracranial hemorrhage; L, liter.

SUPPLEMENTAL TABLE 3. Behavioral functioning and school performance results

	Variables	n = 44
Behavioral functioning †	Clinical behavior problems, n/N (%)	
	Total	5/41 (12)
	Internalizing	6/41 (15)
	Externalizing	0
School performance results ‡	Reading comprehension score, n/N (%)	
	I	10/43 (23)
	II	9/43 (21)
	III	5/43 (12)
	IV	9/43 (21)
	V	10/43 (23)
	Spelling score, n/N (%)	
	I	16/43 (37)
	II	8/43 (19)
	III	7/43 (16)
	IV	4/43 (9)
	V	8/43 (19)
	Arithmetic/mathematics score, n/N (%)	
	I	14/43 (33)
	II	8/43 (19)
	III	2/43 (5)
	IV	6/43 (14)
	V	13/43 (30)

† Behavior questionnaire was not applicable in 2/44 (5%) children due to severe developmental problems and missing in one (2%) child.
‡ School performance results were not available for 1/44 (2%). Four children (4/44, 9%) attended special needs education.



CHAPTER 8

Long-term neurodevelopmental outcome in children after antenatal intravenous immunoglobulin treatment in fetal and neonatal alloimmune thrombocytopenia

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ABSTRACT

BACKGROUND

Children with fetal and neonatal alloimmune thrombocytopenia (FNAIT) face increased risk of intracranial hemorrhage (ICH) potentially leading to developmental impairment. To prevent ICH, pregnant women with alloantibodies against fetal platelets are often treated with intravenous immunoglobulin (IVIg). IVIg appears effective in vastly reducing the risk of fetal or neonatal bleeding complications. However, information on long-term neurodevelopment of these children is lacking.

OBJECTIVE

To evaluate long-term neurodevelopmental outcome in children with FNAIT who were treated with IVIg antenatally.

STUDY DESIGN

An observational cohort study was performed including children of mothers who were treated with IVIg during pregnancy because a previous child was diagnosed with FNAIT. Children were invited for a follow-up assessment including standardized cognitive and neurologic tests. The parents were asked to complete a behavioral questionnaire and school performance reports. The primary outcome was severe neurodevelopmental impairment (NDI), defined as severe cognitive impairment (IQ < 70), cerebral palsy with Gross Motor Function Classification System (GMFCS) Level ≥3, bilateral blindness, and/or bilateral deafness (requiring amplification). The secondary outcome was mild to moderate NDI, defined as either mild to moderate cognitive impairment (IQ < 85), cerebral palsy with GMFCS Level ≤ 2, minor neurologic dysfunction, vision loss, and/or hearing loss.

RESULTS

Between 2003 and 2017, 51 children were liveborn after antenatal IVIg treatment. One family moved abroad and was therefore not eligible for inclusion. In total, 82% (41/50) of the eligible cases were included for neurodevelopmental assessment at a median age of 9 years and 8 months. Severe NDI was not detected. The incidence of mild to moderate NDI was 14% (6/41, 95% confidence interval: 6%–29%). The children's mean cognitive score, behavioral scores, and academic achievement were not different from the Dutch norm groups. Neuroimaging was performed in 90% (37/41) of cases. Severe ICH had been diagnosed in two cases (5%), one antenatally before the start of IVIg and the other case 1 day after birth. Both cases had a normal neurodevelopmental outcome.

CONCLUSION

The risk of NDI in children whose mothers were treated for FNAIT with antenatal IVIg is comparable to that in the general population

AJOG AT A GLANCE

Why was the study conducted?

In fetal and neonatal alloimmune thrombocytopenia (FNAIT), administration of intravenous immune globulin (IVIg) to the mother during pregnancy is widely accepted for preventing the occurrence of antenatal or perinatal intracranial hemorrhage (ICH) in the child. However, knowledge about the long-term neurodevelopmental outcome of these children is lacking.

What are the key findings?

Mild to moderate neurodevelopmental impairment was present in 6/41 (14%) children. Two children were diagnosed with severe ICH. Both had normal neurodevelopmental outcomes.

What does the study add to what is already known?

Neurodevelopmental outcome of children with FNAIT that were born after a pregnancy in which their mother was treated with IVIg is comparable to that in the general population.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a disease defined by maternal human platelet antigen (HPA)-directed alloantibodies. The maternal antibodies can induce severe thrombocytopenia and possibly damage the endothelial cell layer with an increased risk of fetal or neonatal intracranial hemorrhage (ICH), potentially leading to irreversible brain damage or perinatal death.[1-3] The recurrence rate of ICH in subsequent HPA-incompatible pregnancies is up to 72%.[4] The mainstay of antenatal treatment of FNAIT to reduce the bleeding risk in the unborn child is weekly administration of intravenous immunoglobulin (IVIg) to the pregnant woman.[5, 6] In the absence of a screening program, this treatment is almost exclusively given to women who had a previous pregnancy complicated by FNAIT.[7] The exact mechanism of maternal IVIg treatment is not completely understood. It is hypothesized that IVIg treatment leads to lower pathogenic IgG transport from mother to fetus.[8, 9]

Although virtually every guideline worldwide recommends IVIg treatment, the use of IVIg during pregnancy is currently still off-label.[6] The efficacy on reduction of the risk of ICH appears to be very high,[5] although no placebo-controlled studies have been done. The lack of understanding of the mechanism of action of IVIg, raises some concerns about its widespread use and safety in FNAIT. Adverse maternal effects of IVIg include but are not limited to, headache, rash, fatigue, hemolytic anemia, renal failure, pancytopenia and aseptic meningitis.[10, 11] Whether IVIg may also have adverse effects in fetuses, including long-term side effects and neurodevelopmental impairment, is not well known.

Currently, only two small cohort studies have assessed the long-term neurodevelopmental outcomes of children with FNAIT after antenatal treatment.[12, 13] These studies concluded that long-term outcomes of these children were favorable. However, the interpretation of the studies was hampered by methodological limitations: substantial loss to follow-up, remote developmental assessment, and heterogeneous study population.[12, 13] Knowledge of long-term outcomes is essential to evaluate and improve the current quality of care and in evidence-based counselling of parents, particularly because children with FNAIT are at risk for ICH and its associated neurological sequelae. FNAIT survivors have a 70-82% risk of life-long sequelae (e.g., delayed development, cerebral palsy, cortical blindness, or seizures) as a result of the ICH.[14, 15]

This study aims to assess long-term neurodevelopmental outcome in children with FNAIT who were treated with IVIg during pregnancy. In addition, we assessed behavioral difficulties and school performance reports.

MATERIALS AND METHODS

STUDY POPULATION

Leiden University Medical Center (LUMC) is the national clinical expertise center in the Netherlands for platelet alloimmunization in pregnancy. Children of mothers referred to LUMC between 2003 and 2017 and who were treated with IVIg because of a risk of FNAIT were eligible for this study. FNAIT was diagnosed if there was a clinical suspicion in a previous pregnancy [neonatal platelet count < 150 × 10^9/L and/or (fetal) ICH or organ bleeding], confirmed fetal–maternal HPA incompatibility, and presence of HPA-directed antibodies in the maternal plasma.[16] Exclusion criteria for long-term follow-up examination were severe congenital abnormalities unrelated to FNAIT or if the family moved abroad. Weekly maternal IVIg treatment was administered according to the clinical guidelines: 0.5 g/kg/week from 28 weeks of gestation in standard-risk pregnancies (without history of ICH or organ bleeding) and 1.0 g/kg/week starting between 16 and 20 weeks gestation in high-risk pregnancies (with a history of ICH or organ bleeding)).[7] Cesarean section was not recommended as standard delivery mode in HPA immunized pregnancies. For standard-risk pregnancies with a previous vaginal delivery, planned induction of labor was considered to be safe. Between January 2005 and September 2007 treatment was given according to the study protocol of a randomized trial in standard-risk pregnancies comparing low-dose IVIg (0.5 g/kg/week) with standard-dose IVIg (1.0 g/kg/week) starting at 28 weeks of gestation.[17]

ETHICS

The medical ethical committee of Leiden-Delft-Den Haag provided ethical approval (P19.069). All parents and children (aged ≥12 years) gave written informed consent. This study was registered at ClinicalTrials.gov (identifier: NCT04529382).

CLINICAL DATA

The following obstetric data were obtained from medical records: gravidity, parity, antenatal treatment, mode of delivery, specificity of the HPA alloantibody, and gestational age at delivery. Data on the occurrence of ICH or organ bleeding in previous children was also noted. The following neonatal data were obtained: platelet count nadir, postnatal treatment and bleeding symptoms, birth weight, sex, and neonatal morbidity. Two experienced neonatologists specialized in neonatal neurology (SS and LV) reviewed cerebral imaging and cerebral imaging reports. Severe ICH was defined as intraventricular hemorrhage (IVH) grade III or IV or ICH with parenchymal involvement visible on cranial ultrasound. Minor ICH was defined as IVH grade I or II.[18] Severe organ bleeding was defined as organ bleeding requiring supportive care (e.g., ventilation in case of a pulmonary bleed). Neonatal morbidity was defined as the presence of one of the following conditions: perinatal asphyxia (5-min Apgar score < 7 or arterial cord blood pH < 7.0), neonatal sepsis (clinical suspicion of infection and positive blood culture), or necrotizing enterocolitis (NEC).[19] Small for gestational age

(SGA) was defined as birthweight below the 10th percentile.[20] Maternal education levels were obtained from a demographic questionnaire and categorized according to the Dutch Social and Cultural Planning Office (in Dutch: Sociaal Cultureel Planbureau).[21] All data were collected in a secure online database.[22]

PROCEDURES

We first sent introduction letters to the parents, explaining the purpose of the study followed by a phone call. If informed consent was obtained, an appointment for follow-up assessment either at home or at our outpatient clinic was made. Neurodevelopmental assessment consisted of a standardized cognitive test and neurological examination. Parents were requested to complete a questionnaire on their child's behavior and to obtain school performance scores from their child's teachers.

Cognitive development in children aged 3–6 years was assessed using the Dutch version of the Wechsler Preschool and Primary Scale of Intelligence, 4th edition (WPPSI-IV-NL).[23] Cognitive development in children between 7 and 17 years of age was assessed using the Dutch version of the Wechsler Intelligence Scale for Children, 5th edition (WISC-V-NL).[24]

Neurological functioning was examined with the adapted version of the Touwen examination for evaluating minor neurological dysfunction (MND).[25] This examination is divided into the following domains: posture, reflexes, involuntary movements, coordination, fine manipulative ability, associated movements, sensory deficits, and cranial nerve function. With one dysfunctional domain, outcome is classified as simple MND. If ≥ 2 clusters are dysfunctional, the outcome is classified as complex MND. The presence and grade of cerebral palsy (CP) was determined using the Gross Motor Function Classification System (GMFCS).[26]

To investigate behavioral problems, parents completed the Child Behavior Checklist for 1.5–5 years or 6–18 years.[27] Standard T scores were created using a Dutch normative sample. These scores compare the raw score to what would be "normal" responses for children of the same age and gender. The T scores of the normative sample are scaled with a mean of 50 and an SD of 10. Higher scores indicate a greater severity of problems. For each broadband scale of internalizing, externalizing, and total behavior problems, T scores can be interpreted as on the borderline (T = 60–63, 84th–90th percentile) or in the clinical range (T ≥ 64, ≥91st percentile).

For children > 6 years, school performance reports were obtained from the Dutch National Pupil Monitoring System (Cito) for the following categories: reading comprehension, spelling and arithmetic/mathematics.[28] Individual scores were compared with age-matched peers and categorized into levels, I to V, with level I being the top 20% scoring children and level V being the lowest 20% scoring children.

OUTCOMES

The primary outcome was the incidence of severe neurodevelopmental impairment (NDI). NDI is a composite outcome consisting of four different domains: cognitive functioning, vision, hearing, and neurologic functioning. Severe NDI was defined as the presence of one of the following criteria: severe cognitive impairment (IQ < 70 [-2 SD]), CP GMFCS level ≥ 3, bilateral blindness, and/or bilateral deafness (requiring amplification).[26] The secondary outcome was mild to moderate NDI, defined as the presence of one of the following criteria: mild to moderate cognitive impairment (IQ < 85 [−1 SD]), CP GMFCS level 1 or 2, MND, vision loss, or hearing loss.[26] Other outcomes were cognitive test scores (IQ) compared with Dutch norm scores and overall adverse outcome including severe NDI and/or perinatal mortality. In addition, we report the incidence of simple and complex MND, borderline and clinical behavior problems, and school performance scores.

STATISTICAL ANALYSES

Descriptive results are presented as the number of cases with percentages, mean with SD, or median with interquartile range (IQR) depending on the data type and distribution. Proportions of outcomes are presented with 95% confidence intervals (95% CI). The mean IQ scores were compared to Dutch norm data with a one-sample T test. The presence of behavioral problems and school performance scores were compared with the Dutch norm data using binomial tests. Data were analyzed using IBM SPSS Statistics software 26.0 (Chicago, IL, USA). Images were created with Microsoft Visio (Redmond, WA, USA). To examine selection bias, we compared the clinical characteristics of the included cases and the cases that were lost to follow-up.

RESULTS

STUDY POPULATION

Figure 1 shows the study population. One pregnancy complicated by HPA antibodies ended in fetal demise unrelated to FNAIT at 17 weeks of gestation. Autopsy revealed no signs of bleeding. Informed consent was obtained in 82% (41/50) of the eligible cases. Table 1 shows the characteristics of the included children (41 cases). In 12% (5/41) there was a history of severe ICH or organ bleeding in a previous pregnancy-three siblings were diagnosed with severe ICH, one with pulmonary bleeding and one with severe gastrointestinal bleeding. Clinical characteristics at birth of the included cases were comparable to those of children that were lost to follow-up (Supplemental Table). In 75% (30/40) of cases, the pregnant woman received standard-dose IVIg (0.5 g/kg/week) and in 25% (10/40) of the cases high-dosage IVIg (1 g/kg/week); in one case, the dose was not reported. None of the pregnant women were treated with corticosteroids. Fetal blood sampling was not performed. In total, 34% (14/41) of the children were delivered by cesarean section, in one case because of suspected placental

abruption at 31 weeks of gestation. The median gestational age at delivery was 37 weeks and 5 days with median birthweight of 3280 g. No neonatal morbidities occurred. The median platelet count nadir at birth was 65 × 10^9/L (minimum 6 × 10^9/L; maximum 382 × 10^9/L). In 14 cases (34%), the nadir was < 25 × 10^9/L and in 18 cases (43%) the nadir was < 50 × 10^9/L.

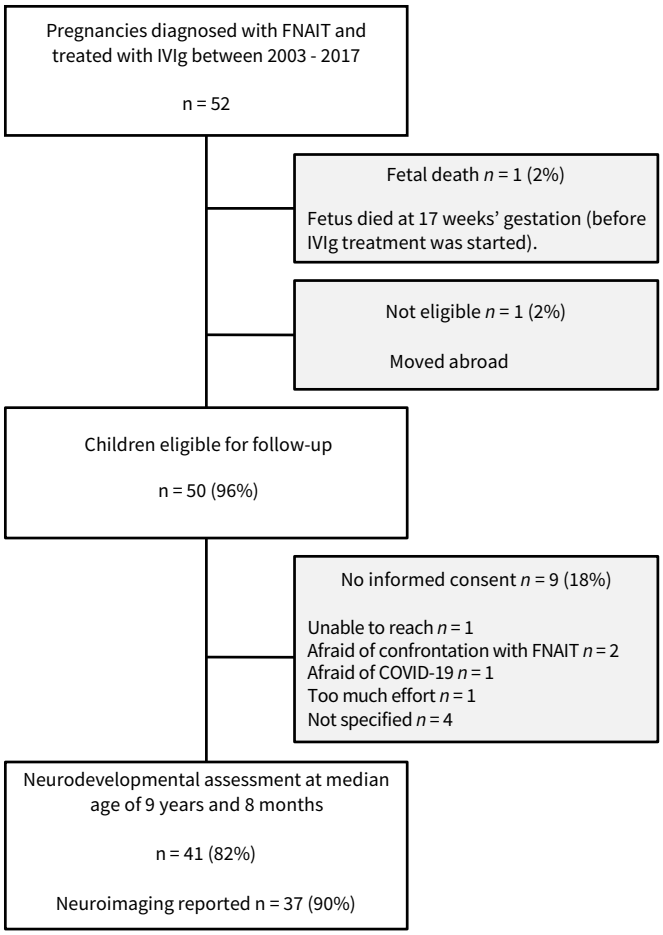

FIGURE 1. Flowchart of the study population

TABLE 1. Clinical and demographic characteristics of the FNAIT cases

Variable	n = 41
Diagnostics	
HPA specificity, n (%)	
HPA-1a	33 (80)
HPA-5b	4 (10)
Other	4 (10)
Pregnancy	
First pregnancy, n (%)	0
Severe hemorrhage in previous pregnancy, n (%)	5 (12)
Signs of fetal bleeding on ultrasound, n (%)	1 (2)
Maternal IVIg treatment, n (%)	41 (100)
Neonatal	
Gestational age at delivery, weeks+days, median (IQR)	37+5 (37+2 – 38+3)
Female sex, n (%)	21 (51)
Birthweight, gram, median (IQR)	3210 (2838 – 3427)
SGA (birthweight < 10th percentile), n (%)	2 (5)
Skin bleeding, n (%)	10 (24)
Intracranial hemorrhage, n/N (%)*	3/37 (8)
Minor: IVH grade I – II	1/37 (3)
Severe: Parenchymal	2/37 (5)
Platelet count nadir × 10^9/L, median (IQR)	65 (20 – 161)
Platelet count < 25 × 10^9/L, n (%)	14 (34)
Postnatal treatment given, n (%)	14 (34)
Demographics	
Maternal education, n (%)	
Primary and secondary school	3 (7)
Intermediate vocational education	13 (32)
High vocational education or university	25 (61)

*Neuroimaging was not performed in 4/41 (10%) of the cases
Abbreviations: HPA, human platelet antigen; SGA, small for gestational age; IVH, intraventricular hemorrhage; L, liter; PTx, platelet transfusion; IVIg, intravenous immunoglobulin.

NEUROIMAGING

Postnatal neuroimaging was performed in 90% (37/41) of the cases. Cranial ultrasound was performed in 92% (34/37) of these cases and 8% (3/37) underwent both cranial ultrasound and magnetic resonance imaging (MRI). Two infants (3%, 2/37) were diagnosed with severe ICH. In one such infant, parenchymal bleeding was detected on fetal ultrasound 1 week before the planned start of IVIg treatment at 27 weeks of gestation. Antenatal treatment (high-dose) was started directly and the bleeding remained stable on ultrasound. At 36 weeks of gestation a cesarean section was performed. Postnatally, the bleeding was classified as severe ICH. In the second case with severe ICH, a round intraparenchymal lesion in the left frontal lobe was detected on neonatal ultrasound. This lesion was suspect for intraparenchymal hemorrhage and therefore classified as severe ICH. The postnatal platelet count of this infant was 382 × 10^9/L. Neonatal MRI was not performed. A previous child in this family had severe gastrointestinal bleeding due to FNAIT. Besides this family in which the

mother delivered an infant with ICH after the previous child had a severe gastrointestinal bleeding, severe bleeding did not reoccur in other pregnancies. In addition to the two cases of severe ICH, minor ICH was diagnosed in one child (3%, 1/37); IVH grade II was suspected on cranial ultrasound and confirmed with MRI.

LONG-TERM NEURODEVELOPMENTAL OUTCOMES

Table 2 shows the long-term neurodevelopmental outcomes of 41 children at a median age of 9 years and 8 months (minimum: 4 years 5 months; maximum 16 years 2 months). Forty children underwent cognitive assessment. Six children (15%, 6/40) were tested using the WPPSI-IV-NL; the mean IQ score in this group was 106 (SD 6). Thirty-four children (85%, 34/40) were tested using the WISC-V-NL; the mean IQ score in this group was 103 (SD 11). The mean full IQ score of the total cohort was 104 (SD 11), higher than their Dutch peers (P = 0.042, mean difference: 3.6). Three children had mild to moderate cognitive impairment (IQ score 70-85). The neurological examination of the children showed that, simple MND was present in 10% (4/40) of the children. Severe NDI was not detected, nor was perinatal mortality. The incidence of mild to moderate NDI was 14% (6/41, 95% CI: 5.6%–29%). Table 3 shows the details of the cases with mild to moderate NDI. All three cases with ICH (two severe and one mild) had normal neurodevelopment.

BEHAVIORAL FUNCTIONING AND SCHOOL PERFORMANCE

The internalizing scores, externalizing scores, and total behavior scores of the included children were comparable to Dutch norm data. School performance scores were available for 85% (35/41) of the children; six children were <6 years-old. One child in our study population required special-needs education, this child had mild-to-moderate NDI. Compared with the Dutch norm population, fewer children scored in our cohort in the lowest (level V) range for reading comprehension [6% (2/35) vs. 20%; P = 0.019] and mathematics [3% (1/35) vs. 20%; P < 0.001]. Nine percent (3/35) of the children scored in the lowest range (level V) for spelling.

TABLE 2. Neurodevelopmental outcome of antenatally treated FNAIT cases

Variable	n = 41
Age, years months, median (IQR)	9y8m (7y5m – 11y8m)
Cognitive	
Full IQ scale, mean (±SD)*	104 (11)
Verbal comprehension	106 (14)
Visual spatial score	103 (12)
Fluid reasoning scale	102 (12)
Working memory score	99 (12)
Processing speed	103 (16)
Normal range (TIQ > 85), *n* (%)§	38 (93)
Mild-moderate impairment (TIQ 85 – 70)	3 (7)
Severe cognitive impairment (TIQ < 70)	0
Neurological	
Minor Neurologic Dysfunction, *n*/N (%)¶	
Simple MND	4/40 (10)
Complex MND	0
Abnormal domain, *n*/N (%)	
Posture	1/40 (3)
Reflexes	2/40 (5)
Involuntary movements	2/40 (5)
Coordination	2/40 (5)
Fine manipulative ability	0
Associated movements	1/40 (3)
Sensory deficits	1/40 (3)
Cranial nerve function	1/40 (3)
Cerebral Palsy, *n* (%)	0
Bilateral blindness or deafness	0
Behavior	
Total behavior problems (borderline to clinical), *n* (%)	2 (2)
Internalizing behavior problems (borderline to clinical), *n* (%)	3 (7)
Externalizing behavior problems (borderline to clinical), *n* (%)	2 (5)
NDI	
Neurodevelopmental impairment (NDI), *n* (%)	
Normal	35 (85)
Mild-moderate NDI	6 (15)
Severe NDI	0

*Cognitive test was not done in 1/41 (2%) anticipated FNAIT case.
§Based on the information of the school results and questionnaires that were completed by the caregivers case with missing cognitive test scores as normal.
¶Neurological test was not completed in 1/41 (2%) anticipated FNAIT case.
Abbreviations: IQ, intelligence quotient; MND, minor neurologic dysfunction; GMFSC, Gross Motor Function Classification Score.

TABLE 3. Characteristics of cases with mild-moderate neurodevelopmental impairment

Sex	GA at birth (weeks)	SGA	Platelet count $< 25\times10^9$/L	Neuroimaging (cranial ultrasound)	HPA	Age at evaluation (years)	TIQ	Neurological examination; abnormal domain	Behavior total problem score (CBCL)	School
Male	34	Yes	Yes	No abnormalities	HPA-1a	9	73	Simple MND; Sensory	Normal	Special needs education
Female	37	No	No	No abnormalities	HPA-1a	10	78	Normal	Normal	Regular education
Female	37	No	No	No abnormalities	HPA-1a	9	84	Normal	Normal	Regular education
Male	38	No	No	Not available	HPA-1a	8	89	Simple MND; Coordination	Normal	Regular education
Male	38	No	No	No abnormalities	HPA-1a	9	112	Simple MND; Coordination	Normal	Regular education
Female	37	No	No	No abnormalities	HPA-15a	10	112	Simple MND; posture	Normal	Regular education

Abbreviations: GA, gestational age; SGA, small for gestational age; G, gravidity; P, parity; L, liter; HPA, human platelet antigen; IQ, intelligence quotient; CBCL, Child Behavioral Checklist; MND, minor neurologic dysfunction; IVH, intraventricular hemorrhage.

COMMENT

PRINCIPAL FINDINGS

This is the first study to use standardized psychometric tests to assess the long-term neurodevelopmental outcomes of children whose alloimmunized mothers were treated with IVIg for FNAIT. Of the 41 children included, none had severe NDI. Mild to moderate NDI was diagnosed in three cases (7%) due to mild to moderate cognitive impairment. Simple MND was detected in 10% of the cases. All cases with ICH had normal neurodevelopmental outcomes. Behavior problem scores were within the normal range. Compared to Dutch norm scores for academic achievement, fewer children in our cohort scored at the lowest level for mathematics and reading comprehension.

RESULTS IN THE CONTEXT OF WHAT IS KNOWN

To date, only two cohort studies reported the long-term neurodevelopmental outcome of children born after antenatal FNAIT treatment. Ward *et al*.[13] suggested better neurodevelopmental outcomes in 71 children after antenatal treatment compared with 71 untreated siblings. However, the investigators performed telephone-surveys only and their conclusions were hampered by a substantial (37%) lost-to follow up rate. Another study evaluated the neurodevelopmental outcome in 37 children born with FNAIT after antenatal treatment. Neurodevelopmental outcome of these children was found to be similar to that in the normal population.[12] An important limitation of this study was the large heterogeneity in fetal management strategies, including intrauterine platelet transfusion, IVIg treatment, or both. Our lost-to-follow-up rate was low, and we avoided the important limitations of these two studies by studying a cohort that received similar antenatal treatment, and by performing standardized neurodevelopmental tests in all participants. Compared with the general Dutch population, our study cohort had higher cognitive test scores. However, the mean difference of 3.6 IQ points is not clinically relevant, as this difference is less than +0.5 SD (7 IQ points) compared to the national average.[17] The relatively high proportion of mothers with a high education level (61% *vs.* 41% in the Dutch general population) could explain the slightly higher IQ scores in our cohort.[14] The rate of simple MND (4/40; 10%) was comparable to the rates in the literature in a healthy population, with 10% at pre-school age and up to 15% at school age (9-years-old).[29]

An intriguing finding, in contrast to literature[22], was the absence of NDI in the three children who were diagnosed with ICH as a fetus or neonate. The most severe ICH occurred before IVIg treatment was started, at 27 weeks of gestation. In our current protocol, the standard-risk pregnancies start treatment with IVIg at 24 weeks. The other two children had milder forms of bleeding, possibly even unrelated to FNAIT. Whether the IVIg may have had a protective effect on the vascular endothelium, as suggested by some recent studies, cannot be concluded from our data.[1-3]

Despite weekly IVIg infusion, around forty percent of the children were born with a platelet count < 50 × 10^9/L. This was in line with previous retrospective studies that reported that up to 67% of neonates had a platelet count < 50 × 10^9/L after IVIg treated pregnancies.[5] These cases with severe thrombocytopenia despite antenatal IVIg treatment are sometimes referred to as 'non-responders,' while some colleagues suggest that this occurs because the dose of IVIg was too low in these cases.[30] The current study was not designed to provide explanations for this phenomenon.

CLINICAL IMPLICATIONS

This study used standardized tests to address the long-term neurodevelopmental outcome of children treated antenatally for FNAIT. It shows that the risk of severe NDI in this population is low. An earlier cohort[14, 15] study performed by our group reported severe and mild to moderate NDI in 60% and 10% of survivors with severe ICH due to untreated FNAIT, respectively.[22] These findings are in line with another study reporting neurological sequelae in 82% of surviving FNAIT cases with ICH.[28] Both studies underline the importance of preventing ICH in pregnancies complicated by FNAIT. Currently, FNAIT is predominantly diagnosed in cases with thrombocytopenia as a chance finding or in cases with unexpected fetal/neonatal bleeding. In subsequent pregnancies, antenatal IVIg therapy appears to work exceptionally well, although adequate placebo- controlled studies are lacking. To prevent all ICH and its associated neurodevelopmental injury it would be necessary to timely start IVIg treatment in those pregnancies at risk for FNAIT with a high risk of severe neonatal outcome. Perhaps this would be possible with a long-debated population-based screening program. However, the efficacy of IVIg in such a cohort of first immunizations has not been studied; this would require setting up a screening program first. In addition to evaluating the long-term outcomes of children that were treated with IVIg, it would be interesting to assess the outcomes of children newly diagnosed with FNAIT. A study from our research group on the long-term outcomes of children with newly diagnosed FNAIT both with and without ICH is in preparation.

As well as FNAIT, antenatal IVIg treatment is indicated for several other diseases such as for example, hemolytic disease of the fetus and neonate, gestational alloimmune liver disease, antiphospholipid syndrome, and immune thrombocytopenia.[31] Because of major differences in the pathophysiology of FNAIT and these other diseases it is not possible to generalize the favorable long-term outcomes of our study population to pregnancies with these conditions. However, the results from our study indicate that administration of IVIg during pregnancy did not have a negative impact on the cognitive, neurological and behavioral development of the children studied.

STRENGTHS AND LIMITATIONS

The major strength of our study is that all children underwent standardized assessment of cognitive, neurological, and behavioral development, including school performance scores,

which provides an integrated view of these children's neurodevelopmental outcomes. This cohort is the largest FNAIT cohort with antenatal IVIg treatment that has been assessed using standardized tests.

One limitation of this study is that we were unable to include all children in our follow-up (18% of the cases were lost to follow-up). However, our analysis showed that the clinical characteristics of the lost-to-follow-up group were similar to those of the children included in our study.

CONCLUSION

Normal distribution in cognitive, neurological, and behavioral development and school performance can be expected in children whose mothers have been treated with IVIg for FNAIT during pregnancy.

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Conflict of interest statement.
The authors report no conflict of interest.

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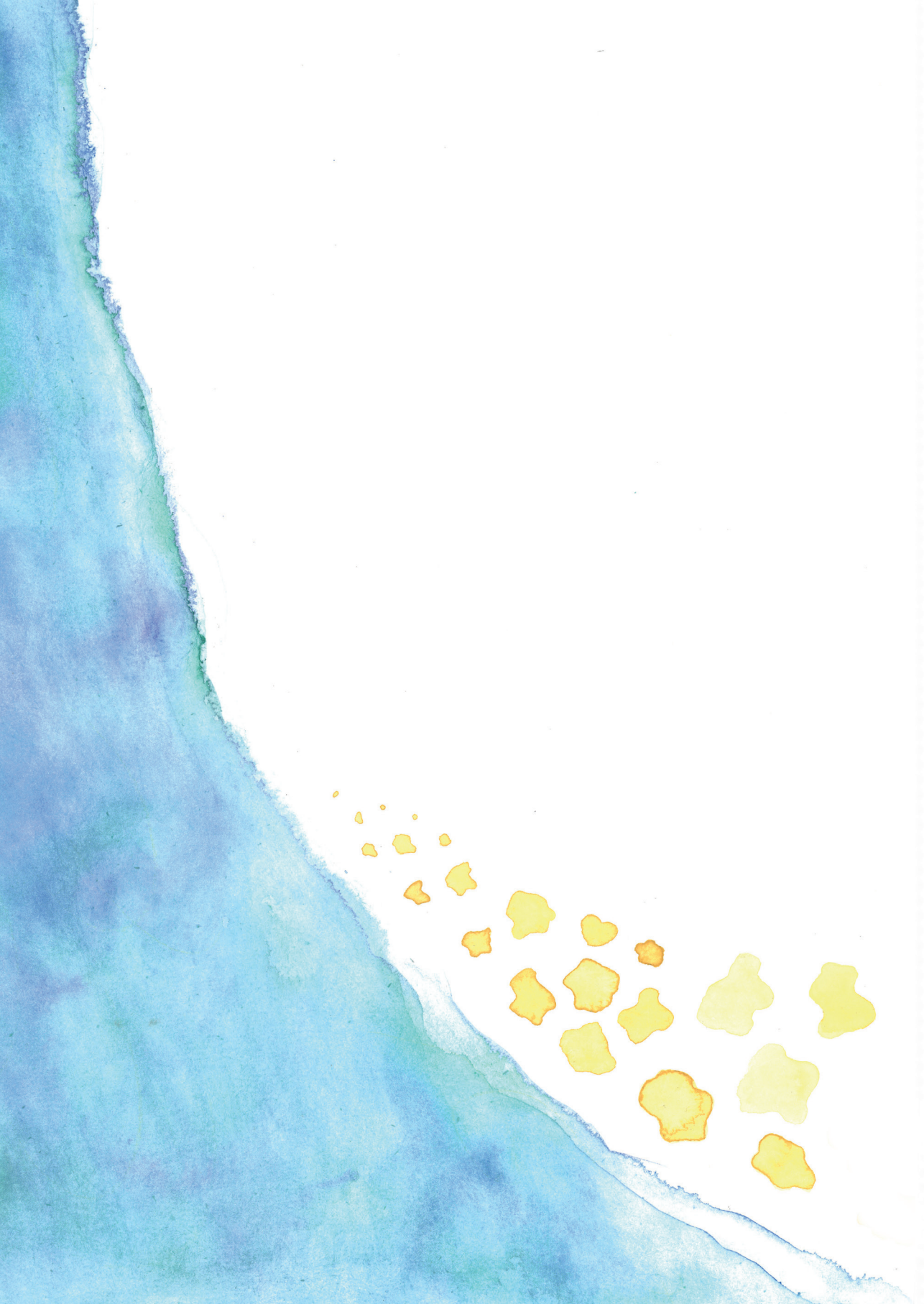
SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE. Comparison of the characteristics of cases that were included to cases that were lost to follow-up

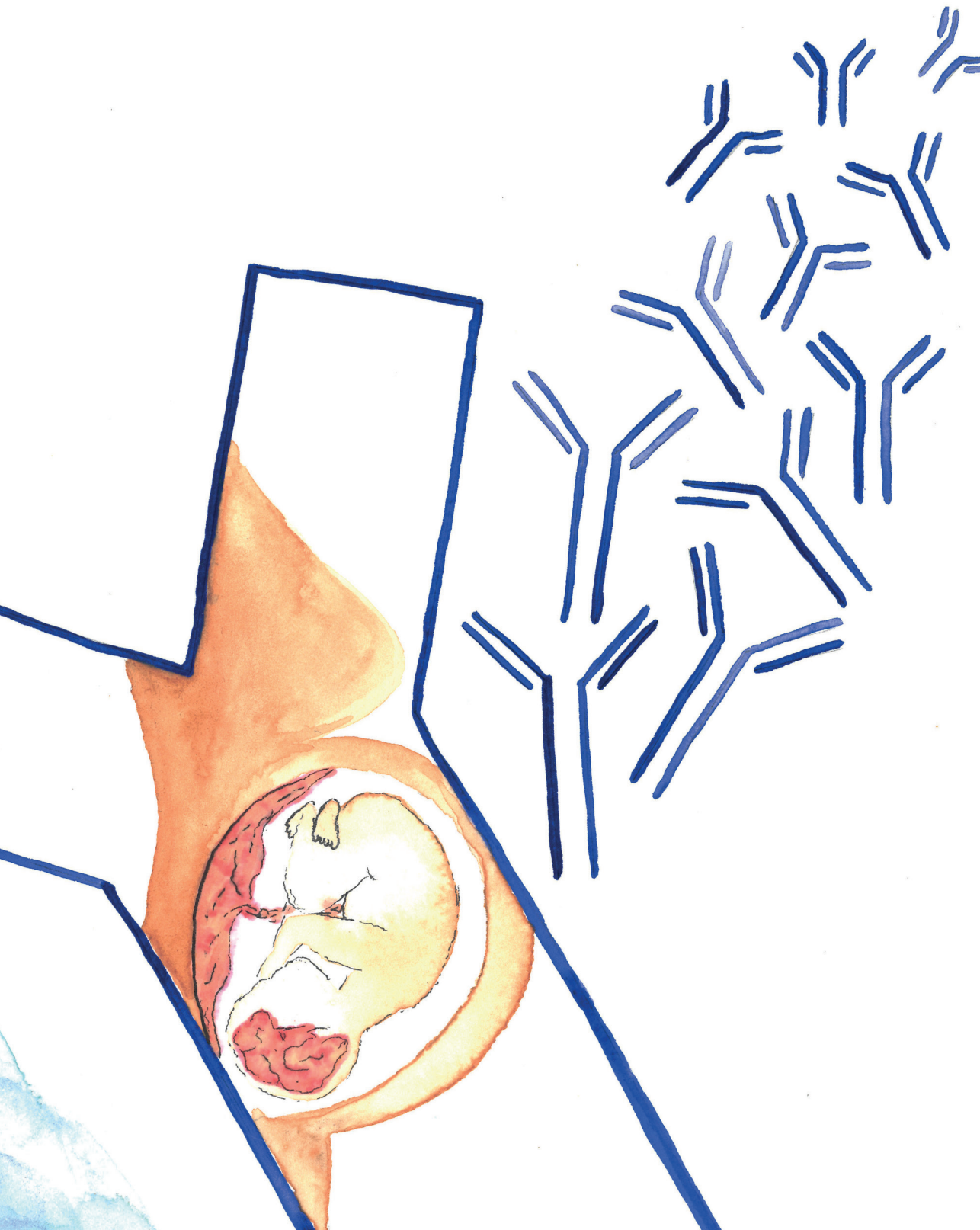
	Included cases n = 41	Lost to follow-up n = 9
HPA specificity, n (%)		
HPA-1a	33 (80)	6 (67)
HPA-5b	4 (10)	1 (11)
Other	4 (10)	2 (22)
Gestational age at delivery, median (IQR)§	37+5 (37+2 – 38+3)	38+0 (37+1 – 39+2)
First pregnancy, n (%)	0	0
Female sex, n (%)	21 (51)	6 (87)
Birthweight, gram, median (IQR)§	3210 (2838 – 3427)	3116 (2645 – 3385)
SGA, n/N (%)§	2/41 (5)	1/7 (14)
Platelet count nadir, median (IQR)§	65 (20 – 164)	88 (50 – 247)
Skin bleeding, n/N (%)§	10/41 (24)	0/7 (0)
Severe ICH, n (%)	2/41 (5)	0
Postnatal therapy, n (%)§	14/41 (34)	1/7 (14)

Characteristics of the included cases were compared to the cases that were lost to follow-up.
Analysis was performed using the Mann Whitney U test (gestational age, birthweight and platelet count) of with the Fisher's Exact Test (categorical variables). No statistically significant differences ($P < 0.05$) were found.
§ Data available for 7/9 (78%) of the cases that were lost to follow-up.
Abbreviations: HPA, human platelet antigen; SGA, small for gestational age; ICH, intracranial hemorrhage.



PART SIX

Cost effectiveness



CHAPTER 9

Cost-utility analysis of screening of pregnant women for fetal neonatal alloimmune thrombocytopenia

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ABSTRACT

BACKGROUND

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) results from maternal platelet-directed antibodies which can cause severe intracranial haemorrhage (ICH) in fetuses and new-borns. Screening for human platelet antigen-1a (HPA-1a) directed antibodies during pregnancy could allow for timely intervention with antenatal treatment and prevent the occurrence of severe ICH. As the incidence of severe ICH due to FNAIT is low, assessing the cost-effectiveness of adding screening for anti-HPA-1a to the prenatal screening program is relevant for decision making.

METHODS

A decision analysis model was developed to assess lifetime costs and effects of antenatal anti-HPA-1a screening with subsequent diagnostic and treatment interventions compared to the current situation without screening in the Netherlands. Model parameters were based on literature and expert opinions. One-way-sensitivity analysis and probabilistic sensitivity analysis were performed.

RESULTS

Adding of screening for HPA-1a to the current antenatal screening program of the Netherlands will lead to an additional cost of 4.7 million euro per year, and a gain of 226 Quality-Adjusted Life Years (QALY) per year, indicating an incremental cost-effectiveness ratio (ICER) of €20,782 per QALY gained. One-way-sensitivity showed that the uncertainty around the incidence of ICH, lifetime costs of disabled children and the probability of having antibody quantitation > 3.0 IU/ml at 20 weeks had the highest effect on the ICER.

CONCLUSION

Antenatal HPA-1a screening might be cost-effective. To obtain more knowledge and thereby reduce the uncertainty on risk stratification and the efficacy of intravenous immune globulin treatment in immunised pregnancies identified by screening, a pilot screening program is warranted.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare severe disease that may cause intracranial haemorrhage (ICH) and organ bleeding in fetuses and neonates. FNAIT results from maternal IgG antibodies directed against paternally inherited antigens on the fetal platelets. In the white population, the majority of FNAIT cases are caused by antibodies directed against human platelet antigen 1a (HPA-1a).[1] Implementation of population-based screening for FNAIT, in analogy to red blood cell antibody screening for secondary prevention of severe haemolytic disease of the fetus and neonate, is debated for decades.[2-4] It is argued that by screening, HPA-1a alloimmunised pregnancies can be identified and that timely antenatal intervention could prevent the occurrence of severe haemorrhage, and its life-long neurological sequelae.[2-8]

Over the last decades, cost-effectiveness studies on HPA-screening were performed in Canada,[5] France,[6] United Kingdom,[7] and Norway.[8] Gafni *et al.*[5] performed a hypothetical calculation assuming that prophylaxis would prevent all FNAIT related morbidity, however primary prophylaxis is not available yet. Another study[6] focused on the diagnostic costs of screening in new-borns to screening in primiparous women. However, antenatal treatment was only available in subsequent pregnancies in this screening design, whereas later became apparent that 63% of the ICH are diagnosed in first-born children.[9] Due to these limitations both studies were not further considered.[5, 6] In 1998, Williamson *et al.*[10] showed that screening of all pregnant women may indeed prevent severe bleeding and proposed to select high-risk pregnancies based on maternal HLA DRB3*01:01 status and antibody levels. The authors also proposed screening mid-pregnancy since women in their first ongoing pregnancy may produce clinically relevant HPA-1a antibodies, whereas antibody levels of multigravida women may decline during pregnancy to non-relevant quantities.[10] Based on these insights Turner *et al.*[7] performed a screening study and calculated the diagnostics test costs for antenatal screening, however their calculations were based on a study with a relatively limited sample size. Finally, Killie *et al.*[8] performed a cost-effectiveness study based on a large screening study including 100,448 pregnant women[11] with the assumption that near-term caesarean section would prevent the development of ICH.

We propose to treat HPA-1a alloimmunised women identified by a screening program with high risk of severe neonatal outcome with antenatal intravenous immunoglobins (IVIg) during pregnancy. We aimed to assess the cost-effectiveness of an antenatal screening program to timely detect HPA-1a antibodies during pregnancy in the Netherlands compared to the current situation without screening.

METHODS

We compared the lifetime costs and effects of antenatal anti-HPA-1a screening to the situation without screening in the Netherlands by developing a decision-analysis model. This model was built in Microsoft Excel (Microsoft Corporation, Redmond, WA). Because the proposed screening program aims to impact both the life expectancy and quality of life of children with FNAIT, outcome was expressed in Quality-Adjusted Life Years (QALYs). The incremental cost-effectiveness ratio (ICER) was expressed in terms of incremental cost per QALY. We assessed the costs and consequences of platelet antibody screening from a societal perspective, i.e., all costs and consequences were included, regardless of who incurs the costs and who obtains the effects. Costs have been discounted at a constant rate of 4% and effects at a constant rate of 1.5% according to the Dutch guidelines.[12] The price level of 2022 was used. Calculations were based on a population of 171,713 pregnant women.[13] Since the consequences of ICH can result in lifelong handicaps,[14] we applied lifetime horizon of the child.

PROBABILITIES

Situation without antenatal HPA-1a screening

The situation without antenatal anti-HPA-1a screening is summarised in Figure 1A (decision tree is shown in Supplemental Figure 1). In absence of anti-HPA-1a screening, FNAIT is often not recognised and therefore highly underdiagnosed.[15] In the base case, the probability of ICH due to undiagnosed FNAIT (5.5 cases/year in the Netherlands) was based on data from a screening study in The Netherlands (HIP study [HPA screening in pregnancy study], de Vos, Winkelhorst *et al*., manuscript in preparation) and the results of previous antenatal screening studies summarised in a systematic review.[16]

In the situation without screening, FNAIT is predominantly diagnosed postnatally. The probability of giving birth to a child diagnosed with FNAIT postnatally (9.3 cases/year in the Netherlands[17]) was based on a study of the national reference laboratory and clinical expertise centre in The Netherlands (2002-2019).[17] In most cases, FNAIT is suspected upon the detection of neonatal bleeding symptoms in combination with low platelet counts or if low platelet counts are detected as a finding by chance upon platelet count done for other reasons. Probabilities on the postnatal outcome (e.g. platelet count) of newly diagnosed FNAIT cases were retrieved from the FNAIT Registry 2020, an international database consisting of 408 FNAIT cases.[18]

A minority of the FNAIT cases is diagnosed during pregnancy after the detection of ICH in the fetus on ultrasound (1 case/year in The Netherlands[17]). When there is no fetal death related to ICH, IVIg treatment is started directly after FNAIT is diagnosed, or the child is delivered by caesarean section. In this model we assumed that all these antenatally diagnosed cases

were treated with antenatal IVIg treatment. Outcome of children with ICH was estimated on a case series of 21 children with FNAIT related ICH: 52% died, 33% were alive and had neurodevelopmental impairment (classified as disabled) and 14% were alive without neurodevelopmental impairment (classified as not disabled).[14]

Lastly, there is a group of women with follow-up pregnancies after a previous child was diagnosed with FNAIT (estimated on 4.2 cases/year in The Netherlands).[17] If fetal-maternal incompatibility is proven in the follow-up pregnancy, these women are offered IVIg treatment to reduce the risk of bleeding. Based on a recent study published by our group we assumed no disability in the group of children treated with IVIg in subsequent pregnancies.[19] Probabilities of the situation without screening are listed in Supplemental Table 1.

Antenatal screening

The situation with HPA-1a screening is visualised in Figure 1B (decision tree in Supplemental Figure 2). In the situation with HPA-1a screening, all pregnant women will be typed for HPA-1 early in pregnancy. If the mother is HPA-1a negative, maternal HLA typing is performed. This is done because women negative for HLA DRB3*01:01 rarely develop high levels of anti-HPA-1a.[20] HPA-1a negative women positive for HLA DRB3*01:01 are offered antibody screening at the 20th and 27th week in pregnancy. If anti-HPA-1a is detected, fetal typing is performed because multigravida pregnant women may carry an HPA-1a negative fetus not being at risk for FNAIT. HPA-1a immunised and incompatible pregnancies are subsequently classified as either high-risk or low-risk pregnancies using antibody quantitation according to the cut off values determined based on the Norwegian screening study.[21] If antibody quantitation is > 3 IU/ml, the pregnancy is considered high-risk, and the mother is treated by weekly administration of IVIg (dosage; 0.5 gram/kg/week) from the moment that the antibody is > 3 IU/ml.

The proportion of HPA-1a negativity was (2.4%) was based on the results of the HIP study (de Vos, Winkelhorst *et al*., manuscript in preparation). The probability of being HLA DRB3*01:01 positive (33%) was based on data of two cohorts of healthy blood donors.[22, 23] Data on the course of antibody quantitation and probabilities of having antibody quantitation > 3 IU/ml at 20th week and/or 27th week were based on the Norwegian screening study (additional data needed for the calculations of these probabilities were kindly provided by Jens Kjeldsen-Kragh and Mette Kjær).[21] Probabilities of the situation with HPA screening are shown in Supplemental Table 2.

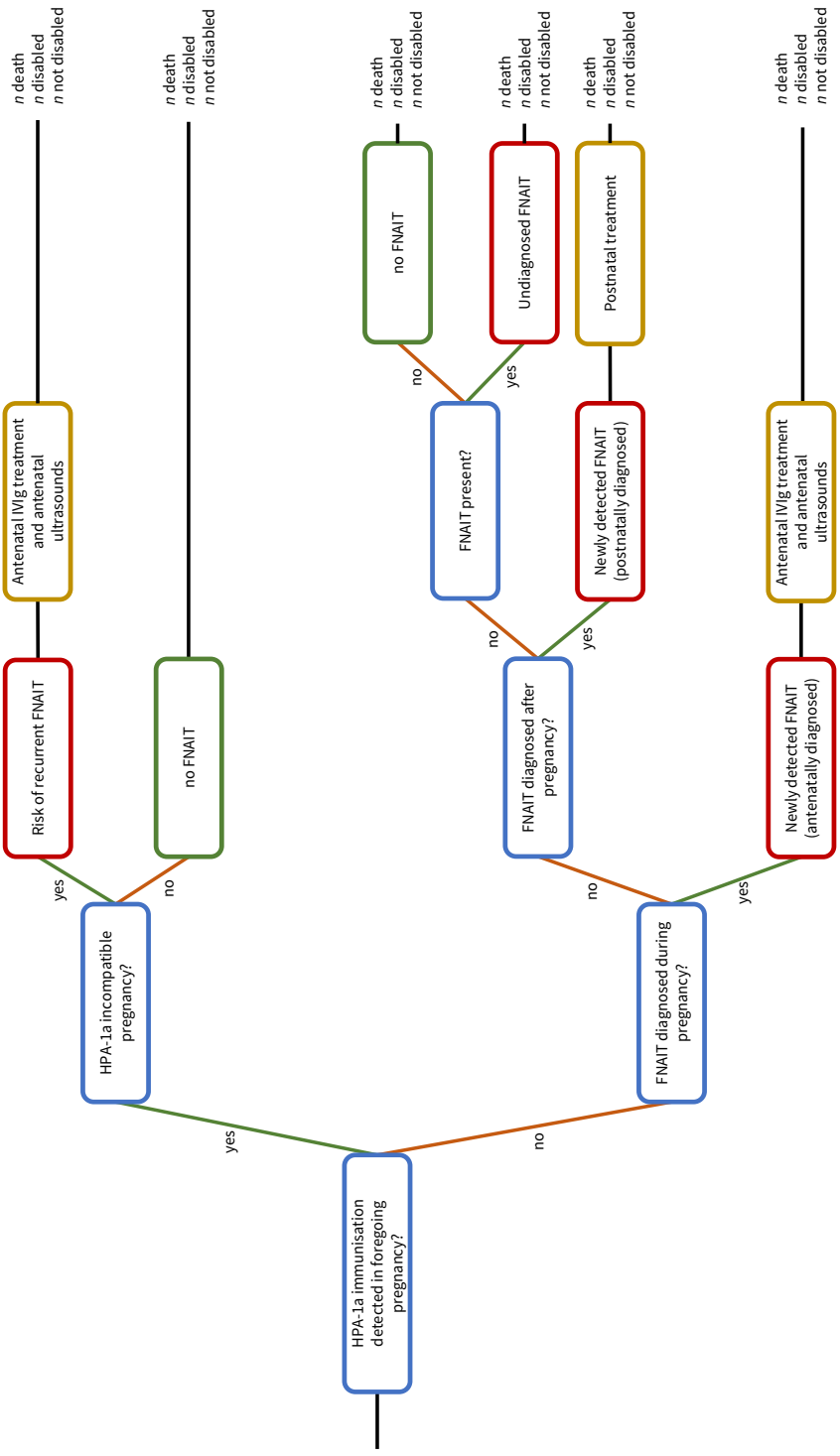

FIGURE 1A. Diagram of situation without screening

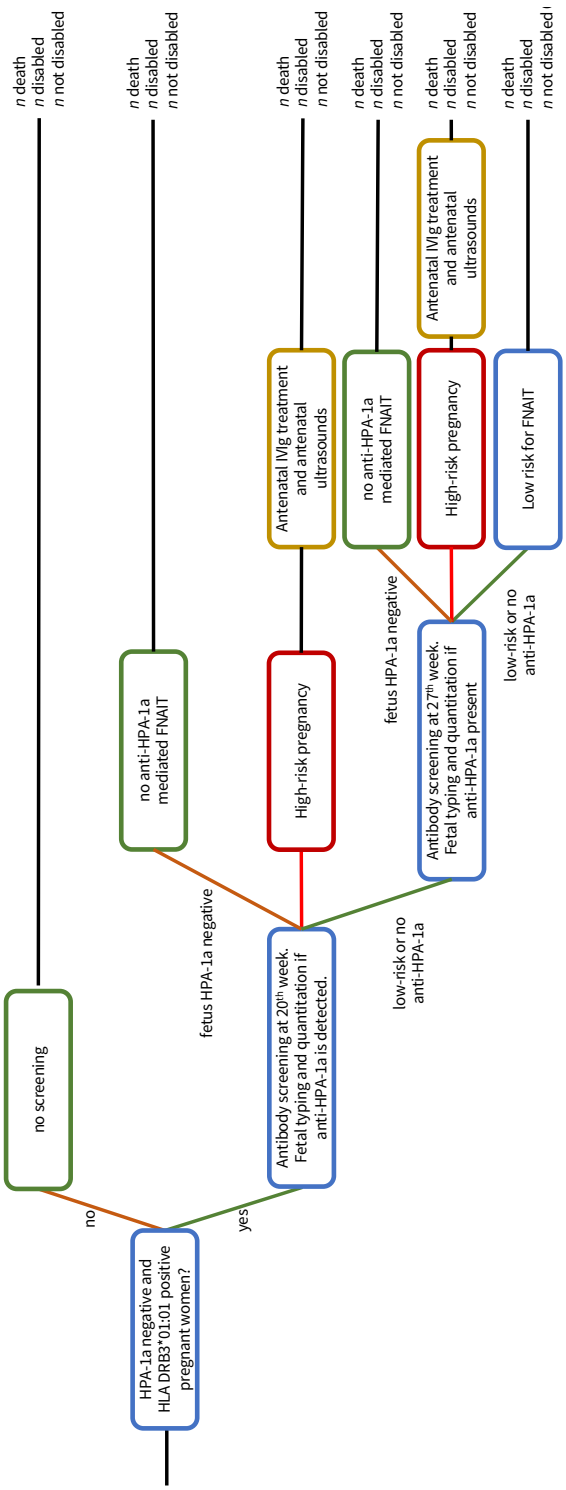

FIGURE 1B. Diagram of situation with HPA-1a screening.

Abbreviations: HPA, human platelet antigen; FNAIT, fetal and neonatal alloimmune thrombocytopenia; IVIg, intravenous immune globulin.

COSTS

Diagnostics test costs

Costs of diagnostic tests are shown in Supplemental Table 3. In the no-screening situation, FNAIT is diagnosed with maternal, paternal and neonatal (molecular) comprehensive HPA 1, 2, 3, 5 and 15-typing, HPA and HLA antibody identification and cross-matching paternal platelets with maternal serum (€1953). Costs of testing in the situation without screening were based on the prices of the nationwide reference laboratory.[24, 25] In case of antenatal screening for FNAIT, typing and antibody screening will be focused on HPA-1a, the sample throughput will increase which lowers the costs per sample. Costs for diagnostic tests in a screening setting were calculated by diagnostic experts on platelet antibody screening from Sanquin (Masja de Haas and Leendert Porcelijn). Costs used for the screening setting in the base case were €15 for maternal HPA-1 typing (including costs for drawing of the blood sample, sample logistics and report generation), €40 for maternal HLA typing, €75 for HPA antibody screening and €150 for antibody quantitation.

Treatment costs

Treatment costs are presented in Supplemental Table 4. Antenatal treatment costs consist of both administration costs and medication costs for weekly administration of IVIg (€223 per vial of 2.5 g[26]). Every first IVIg dosage during pregnancy is given in the hospital on day-care basis (€304[27]), subsequent dosages are administered by home care nurses (€200 per administration [personal communication Sanquin home service]). Additionally, costs for healthcare resource use were calculated including outpatient clinic visits[27] with costs of advanced fetal ultrasounds (€851[28]) at week at 21, 27, 31 and 35 weeks gestational age. These costs were calculated as additional costs compared to healthcare costs in the situation without screening. Travel costs and productivity costs of pregnant women were also taken into account.

Postnatal treatment costs depend on postnatal platelet counts, which were categorised in three groups. Neonates with platelet count > 100 × 10^9/L are regarded not at risk for bleeding and discharged, no additional costs were calculated for this group. Neonates with a platelet count 25-100 × 10^9/L will be admitted for clinical surveillance to the maternity ward (3 days, €449 per day[29]) including daily measurements of platelet counts. In addition, cranial ultrasound (€100[29]) will be performed to screen for ICH. Neonates with platelet count < 25 × 10^9/L will be admitted to the neonatology ward (high care, €1830 per day[29]) and receive one HPA-matched platelet transfusion (€365 [personal communication]). In addition, brain imaging and platelet count measurements will take place. Health care related and travel costs that might be attributable to the father were not included in this analysis. No productivity costs were applied since postnatal treatment falls within the period of maternity leave.

Lifetime costs per health state

Additional lifetime costs related to FNAIT per health state are shown in Supplemental Table 4. Additional lifetime costs for the outcomes: healthy, not disabled or death were set at €0. Literature on the lifetime costs for FNAIT related disability is lacking, therefore we used reports on lifetime costs of cerebral palsy (CP). We used data from a study from Denmark[30] that reported on the lifetime costs for healthcare, productivity costs and societal costs for children with CP (€802,868 excluding informal costs). Productivity costs were subtracted from the lifetime costs using the friction cost approach.[27] According to this approach disabled children do not account for productivity costs since they never entered and therefore will never leave the labour market. Costs for informal caregiving (€341,000) were based on a study reporting on the mean hours of informal care per week for severe neurologic conditions[31] and the Dutch manual for healthcare costs.[27]

EFFECTS

In Supplemental Table 5 utility values, reflecting the quality of life within a particular health state, are shown. No data was available on health-related qualify of life related to FNAIT. One study systematically assessed the long-term outcome of children with FNAIT related ICH and reported that 70% had cerebral palsy, 40% had severe visual impairment and 40% was diagnosed with epilepsy.[14] Therefore, literature on the utility scores of children diagnosed with CP[32], visual impairment[33, 34] and epilepsy[35] was used. Based on the available literature, the utility score of FNAIT related disability was estimated at 0.55. A utility score of 0 was assigned to the 'death' as health state. For the healthy and not disabled health state the Dutch population norm score was used (0.910).[36] Life expectancy of disabled children was assumed to be 50 years[37] and 81.7 years for children not disabled.[38]

ASSUMPTIONS

Although the efficacy of IVIg treatment in immunised pregnancies identified via antenatal screening was never proven in a randomised controlled trial, in the base case we assumed no failure of antenatal treatment. Moreover, we assumed all cases with FNAIT-related ICH develop antibodies at 27th weeks or earlier, and that all cases with ICH had antibody quantitation > 3 IU/ml at one of the moments of screening.

ANALYSES

Base case analysis

A base case analysis was performed by using the values for the model parameters described above. We reported costs, QALYs, FNAIT related death and FNAIT related disability for the situation without screening and the situation with antenatal HPA-1a screening. To calculate the incremental cost effectiveness ratio (ICER), difference in mean costs between the situation with and without antenatal screening are divided by the difference in mean QALYs.

Sensitivity analyses

One-way sensitivity analysis (OWSA) and probabilistic sensitivity analysis (PSA) were performed to address the uncertainty of the model parameters and to quantify the impact on the costs and QALYs. To perform these analyses beta, gamma and Dirichlet distributions were used around the parameters: the beta distribution was applied to all parameters values that needed to stay within the 0-1 range, thus for the probabilities and utilities. A gamma distribution applies to parameters that are not allowed to drop below 0. This distribution has therefore been used for all costs, as well as the expression of an amount such as the length of stay in the hospital or the annual number of pregnant women. A Dirichlet distribution was chosen when a parameter consisted of more than two proportional parameters that had to add up to one every time. Ranges of these distributions were based on expert opinion (Thijs de Vos and Masja de Haas). For the beta and gamma distribution either a standard error has been assumed or values for alpha and beta were estimated in line with the assumed minimum and maximum value of the parameter. Assumptions about the standard error (SE) were made in collaboration with the experts, taking a percentage of the deterministic value depending on how much variation was considered likely. The more variation assumed, the higher the assumed SE. For the costs related to the disabled health state e.g., an SE of 50% was assumed because these costs are expected to show a lot of variation, given that lifetime costs depend greatly on the severity of the NDI.

OWSA included all probabilities except the parameters with Dirichlet distribution. The 15 parameters with the largest effect on the ICER were presented in a Tornado diagram. PSA was performed by random draws from the probability distribution for 1,000 simulations. Subsequently, costs and QALYs were calculated for each simulation. Results for this analysis were displayed in a cost-effectiveness (CE) plane and cost effectiveness acceptability curve (CEAC).

Scenario analysis 1 - Quality control after birth

In the first years after the introduction of HPA-1a screening quality control will be performed to verify if clinically relevant FNAIT cases will be left untreated. In this scenario analysis, platelet counts will be performed in all neonates of HPA-1a negative women to assess extra costs of this quality control.

Scenario analysis 2 – Improvement of risk stratification

In the base case analysis women are considered to have a high-risk pregnancy if antibody quantitation is > 3 IU/ml. Currently assays to identify pregnancies at high-risk with a higher sensitivity are being developed. To assess the cost reduction when these assays become available, we performed a scenario analysis in which we set the threshold at 10 IU/ml. At present, it is thought that increasing this threshold would lead to missing cases with ICH, but the number of ICH missed by increasing this threshold is currently unknown.

Scenario analysis 3 – Reduced sensitivity of risk stratification

In general, cases with ICH in prospective screening studies had high antibody levels.[10, 11, 39, 40] However, antibody quantitation is doubted as single predictor for disease severity because in retrospective studies cases were identified with ICH and low antibody levels.[41] To address this uncertainty, we performed a scenario analysis in which yearly one out of 194 pregnancies classified as low risk at 27 weeks gestational age, ended with the delivery of a child with ICH.

RESULTS

BASE-CASE ANALYSIS

Results of the base case analysis with an annual number of 171,713 pregnant women is shown in Table 1. Incorporating these annual expected numbers and the effect assumptions an expected yearly number of 2.5 children with FNAIT related disability and 3.8 cases of FNAIT related death was obtained for the Netherlands.

In the situation with antenatal screening, we expect to identify 64.7 high-risk pregnancies at 20th week of pregnancy and 10.7 high-risk pregnancies at 27th week of pregnancy. Due to the earlier HPA-antibody detection and antenatal treatment, we expect to prevent all FNAIT related disability and death: a gain of 226 QALYs was expected (discounted). Total costs increment of HPA-1a screening expected was €4,688,100. Dividing the difference in costs by the 226 QALYs gained resulted in a cost-utility ratio of €20,782 per QALY gained.

TABLE 1. Disaggregated results and increments compared to no screening situation for a cohort of 171.713 (€ 2022)

Category	No screening	HPA-1a screening	Increment
Annual number of dead children caused by FNAIT	3.83	0.00	- 3.83
Annual number of disabled children caused by FNAIT	2.48	0.00	- 2.48
Total QALYs attained (discounted)	7,208,369	7,208,595	+ 226
Diagnostic test costs	€26,200	€3,042,100	+ €3,015,900
Antenatal treatment costs	€252,400	€4,630,200	+ €4,377,800
Postnatal treatment costs	€66,800	€201,600	+ €134,800
Lifetime costs	€2,840,400	€0	- €2,840,400
Total costs	€3,185,800	€7,873,900	+ €4,688,100

Abbreviations: HPA, human platelet antigen; FNAIT, fetal and neonatal alloimmune thrombocytopenia, QALY, Quality-life adjusted years

SENSITIVITY ANALYSIS

Results of the OWSA are presented in Figure 2, in this analysis we changed the base case parameters to their minimum and maximum values (see Supplemental Tables 1 – 5). The uncertainty around the incidence of ICH in the group of unidentified FNAIT, lifetime costs of disabled children and the probability of having antibody quantitation > 3.0 IU/ml at 20 weeks of gestation had the highest impact on the ICER.

In addition, a probabilistic sensitivity analysis was performed, (Figure 3 and Figure 4), at a willingness to pay threshold of €20,000 per QALY the probability of the screening strategy being cost-effective compared to a situation without screening was 26%. At a willingness-to-pay threshold of €80,000 this percentage was 96%.

SCENARIO ANALYSES

Performing platelet count in all HPA-1a negative mothers as a quality control (scenario analysis 1) would lead to yearly additional costs of €26,387 and no additional effects. If diagnostic assays become available improving the selection of high-risk pregnancies equivalent to treating pregnancies only with antibody quantitation > 10 IU/ml, this would lead to considerable reduction in costs (scenario analysis 2). Costs increment will be €2,930,164 instead of €4,688,103. It is however currently uncertain to what extent this might lead to missing cases at risk for ICH. If yearly one case with ICH would be missed (scenario analysis 3), a gain of 192 QALYs was expected resulting in a cost-utility ratio of €26,559 per QALY gained.

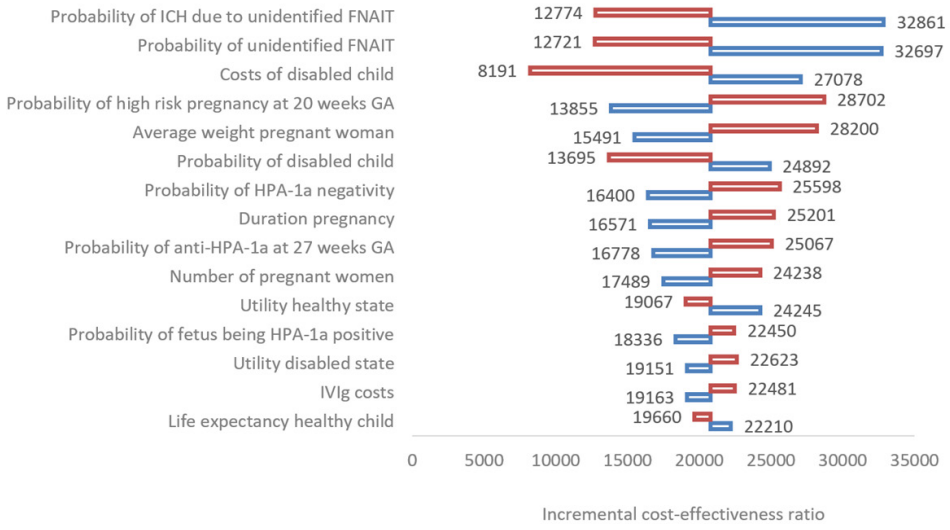

FIGURE 2. One way sensitivity analysis

Univariate sensitivity analysis: cost-effectiveness ratio (cost per QALY) for minimum (red bars) and maximum values (blue bars) of the input parameters. Base case ICER €20,782 per QALY (price level 2022).

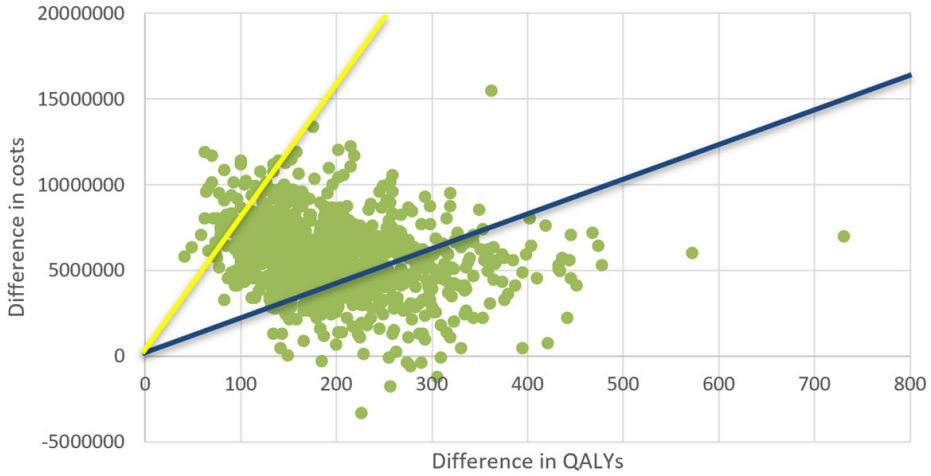

FIGURE 3. Probabilistic sensitivity analysis cost effectiveness plane

Cost effectiveness based on 1000 probabilistic simulations. The blue line represents the €20,000 per QALY threshold and the yellow line represents the €80,000 per QALY threshold.

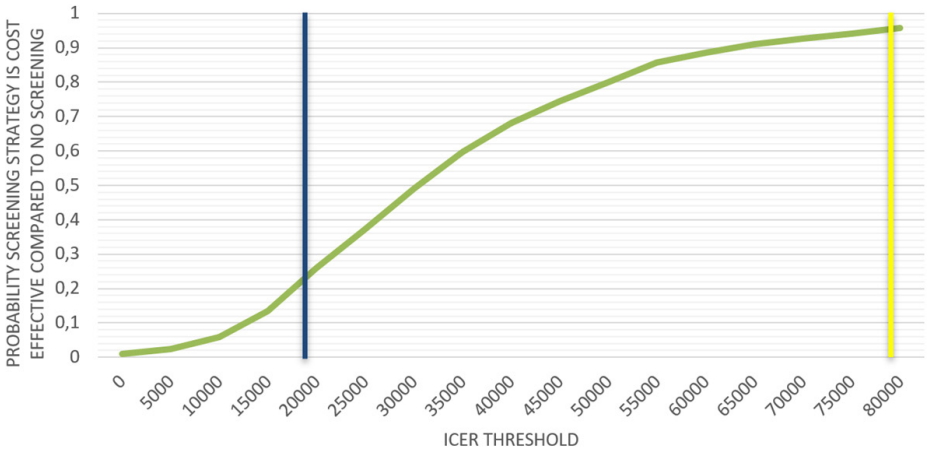

FIGURE 4. Probabilistic sensitivity analysis: cost effectiveness acceptability curve

Cost effectiveness based on 1000 probabilistic simulations. The blue line represents the €20,000 per QALY threshold and the yellow line represents the €80,000 per QALY threshold.

DISCUSSION

Based on our model we calculated that addition of HPA-antibody screening to the current antenatal screening program of the Netherlands will lead to additional cost of 4.7 million euro per year, and a gain of 226 QALY per year. Thus, the incremental cost-effectiveness ratio was €20,782 per QALY gained. This estimate was based on literature data and expert opinions. The one-way sensitivity analysis showed that the uncertainty around the incidence of ICH in the group of unidentified FNAIT, lifetime costs of disabled children and the probability of having antibody quantitation > 3.0 IU/ml at 20 weeks of gestation had the highest effect on the ICER.

Turner *et al.*[7] calculated $71,067 per QALY gained. This higher amount can be possibly explained by the fact that this study included only costs for diagnostic testing without taking the costs for treatment into account. Therefore, no effect on the reduction of life-time treatment costs was included, resulting in a higher cost-effectiveness ratio. Killie *et al*.[8] calculated that all screening strategies were cost-saving, based on the results of the largest screening study on FNAIT thus far.[11] In their screening, a near term caesarean section was considered to prevent adverse outcome in FNAIT. If this approach indeed would reduce FNAIT-related severe bleeding has however been questioned.[42] In the Norwegian study, it was estimated that screening of 100,000 women would lead to 210-230 gained QALYs (discounted rate). This was higher compared to the rate in our study (132 QALYs per 100,000 pregnant women). This difference can be explained by using a different probability of disability and death within the immunised population.

In line with the conclusions of Killie *et al*., cost-effectiveness ratio found in our study is possibly acceptable for European countries.[43] In addition, further cost reductions in future seems feasible. At present, maternal blood group typing (ABO, RhD, Rhc) is repeated in every pregnancy. When these test results including HPA-1 and HLA typing are stored in a central database, this information can be used for subsequent pregnancies also. This prevents unnecessarily retesting, and thus significantly reduce diagnostic test costs. In addition, when prophylaxis may become available in future, immunisation can be prevented and may further reduce the number of immunised and possibly high-risk pregnancies requiring (expensive) IVIg treatment.[44]

Our study has several limitations. Most importantly, our study assumed that IVIg treatment could prevent all FNAIT related ICH and that all immunisations leading to ICH will be detected in this screening strategy. It is not known whether IVIg also reduces the risk of bleeding in *first* HPA-1a immunised pregnancies. The impact of these assumptions can be explored in a scenario analysis in which the effectiveness of IVIg treatment in pregnancies identified by antenatal screening is assumed to be lower. The only way to finally obtain more

knowledge on this subject is to introduce population-based screening in a study setup with a control group. Given the low incidence of ICH, it would be preferable to conduct a national pilot screening. In addition to information about the effectiveness of IVIg treatment, this could also provide information about risk stratification within HPA-1a immunised pregnant women. Possibly, Fc core fucosylation of anti-HPA-1a[45] or the presence of certain subtypes of antibodies interfering with endothelial cell functioning[46] are antibody characteristics which could be used to improve risk stratification in FNAIT. It could be justifiable to start a pilot screening with an antibody threshold of 10 IU/ml instead of 3 IU/ml for discrimination of high-risk pregnancies with the start of IVIg. The threshold of 3 IU/ml was designed to detect cases with severe thrombocytopenia (platelet count < 50×10^9/L).[21] However, severe thrombocytopenia does not always lead to ICH. Most cases with ICH in prospective studies have antibody thresholds above 10 IU/ml.[10, 11, 39, 40] It could be that cases with ICH will be missed by using this threshold, however screening is not necessarily intended to find all cases, but to find as many as possible in a cost-effective way. In addition, improvements can be made with the knowledge gained in such a pilot program. Another limitation of our study is that knowledge about the long-term costs is limited, while our OWSA showed that the uncertainty around this value had the biggest impact on the ICER, this uncertainty should be addressed in future research.

Acknowledging the limitations of our study about the effect of IVIg treatment in first affected pregnancies and the uncertainty in estimating life-time costs of disabled children we think that HPA-1a screening in pregnancy has the potential to be cost-effective. For a screening program it is of the utmost importance to allow risk stratification within the group of HPA-1a immunised pregnant women, to restrict IVIg therapy to women with a high-risk of having a child with intracranial haemorrhage.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE 1. Probabilities situation without HPA-1a screening

Parameter	Probability	Distribution Beta (SE) or Dirichlet (n1, n2, n3,..)	Source
General			
Termination of pregnancy / fetal loss during pregnancy	0.033	Beta (0.002)†	Process Monitor PSIE [13]
Probabilities of pregnancies of women who were diagnosed with HPA-1a immunization in previous pregnancy			
Pregnant woman diagnosed with FNAIT in foregoing pregnancy	2.459×10^{-5}	Beta (4.918×10^{-6}) §	Nationwide FNAIT database [17]
Fetus HPA-1a positive if FNAIT was diagnosed in foregoing pregnancy	0.844	Beta (0.042) †	Calculated based on data from the HIP study [4]
False-negativity rate fetal HPA-1a typing	0.030	Beta (0.003) ‡	Assumed equal to fetal RHD typing. [47]
Fetal loss due to failure of antenatal treatment	0.000	Dirichlet (1,1700,1400,1000)	Expert opinion
PC > 100×10^9/L after antenatal treatment	0.415	Dirichlet (1,1700,1400,1000)	FNAIT registry 2020[18]
PC 25-100×10^9/L after antenatal treatment	0.341	Dirichlet (1,1700,1400,1000)	
PC < 25×10^9/L after antenatal treatment	0.244	Dirichlet (1,1700,1400,1000)	
Dead if PC > 100×10^9/L	0.000	Dirichlet (1,10,999989)	Expert opinion
Disabled if PC > 100×10^9/L	0.000	Dirichlet (1,10,999989)	
Not disabled if PC > 100×10^9/L	1.000	Dirichlet (1,10,999989)	
Dead if PC 25-100×10^9/L	0.000	Dirichlet (1,10,99989)	
Disabled if PC 25-100×10^9/L	0.000	Dirichlet (1,10,99989)	
Not disabled if 25-100×10^9/L	1.000	Dirichlet (1,10,99989)	
Dead if PC < 25×10^9/L	0.000	Dirichlet (1,5,94)	
Disabled if PC < 25×10^9/L	0.000	Dirichlet (1,5,94)	
Not disabled if PC < 25×10^9/L	1.000	Dirichlet (1,5,94)	
Probabilities if FNAIT is diagnosed in current pregnancy			
FNAIT detected during current pregnancy	6.022×10^{-6}	Beta (9.218×10^{-6})	Dutch nationwide FNAIT database [17]
Termination of pregnancy/IUFD due to FNAIT	0.800	Beta (0.160)‖	
Fetal loss due to failure of antenatal treatment	0.000	Dirichlet (2,1,1,100)	Expert opinion
PC > 100×10^9/L after antenatal treatment	0.000	Dirichlet (2,1,1,100)	
PC 25-100×10^9/L after antenatal treatment	0.000	Dirichlet (2,1,1,100)	
PC < 25×10^9/L after antenatal treatment	1.000	Dirichlet (2,1,1,100)	
Dead if PC < 25×10^9/L after antenatal treatment	0.000	Dirichlet (1,10,90)	
Disabled if PC < 25×10^9/L after antenatal treatment	0.100	Dirichlet (1,10,90)	
Not disabled if PC < 25×10^9/L after antenatal treatment	0.900	Dirichlet (1,10,90)	
Probabilities if FNAIT is diagnosed postnatally			
FNAIT detected after birth	5.601×10^{-5}	Beta (5.601×10^{-6}) ‡	Dutch nationwide FNAIT database [17]
PC > 100×10^9/L	0.000	Dirichlet (1,300,940)	
PC 25-100×10^9/L	0.242	Dirichlet (1,300,940)	
PC < 25×10^9/L	0.758	Dirichlet (1,300,940)	
Dead if PC 25-100×10^9/L	0.000	Dirichlet (1,10,99989)	Expert opinion
Disabled if PC 25-100×10^9/L	0.000	Dirichlet (1,10,99989)	
Not disabled if 25-100×10^9/L	1.000	Dirichlet (1,10,99989)	

SUPPLEMENTAL TABLE 1. Continued

Parameter	Probability	Distribution Beta (SE) or Dirichlet (n1, n2, n3,..)	Source
Death if PC < 25 × 10^9/L after postnatal diagnosis	0.021	Dirichlet (20,84,836)	Winkelhorst *et al.*[14] Tiller *et al.*[9]
Disabled if PC < 25 × 10^9/L after postnatal diagnosis	0.089	Dirichlet (20,84,836)	
Not disabled if PC < 25 × 10^9/L after postnatal diagnosis	0.889	Dirichlet (20,84,836)	
Probabilities concerning unidentified FNAIT			
Unidentified FNAIT	3.613 × 10^{-4}	Beta (7.227 × 10^{-5})||	HIP study[4] Kamphuis *et al.*[16]
ICH due to unidentified FNAIT	0.092	Beta (0.018)||	
Dead due to ICH	0.524	Dirichlet (11,7,3)	Winkelhorst *et al.*[14] Tiller *et al.*[9]
Disabled due to ICH	0.333	Dirichlet (11,7,3)	
Not disabled despite ICH	0.143	Dirichlet (11,7,3)	

† SE of 5%. ‡ SE of 10%. § SE of 15%. || SE of 20%. # SE of 50%.
Abbreviations: HPA, human platelet antigen; FNAIT, fetal and neonatal alloimmune thrombocytopenia; PC, Platelet count.

SUPPLEMENTAL TABLE 2. Probabilities situation with HPA-1a screening

Parameter	Probability	Distribution Beta (SE) or Dirichlet (n1, n2, n3,..)	Reference
General			
Termination of pregnancy / fetal loss during pregnancy	0.033	Beta (0.002)†	Proces Monitor PSIE[13]
Maternal typing first trimester			
HPA-1a negative pregnant women	0.024	Beta (0.002)‡	HIP study[4]
Women HLA DRB3*01:01 positive	0.330	Beta (0.017)†	Cohort from DISIII[23] and Bloodtyper study.[22]
Maternal HPA-1a typing false negative	0.035	Beta (0.003)‡	Winkelhorst *et al.*[48]
Antibody screening at 20 weeks' GA			
Anti-HPA-1a detected	0.232	Beta (0.023)‡	HIP study[4]
Fetus HPA-1a positive if mother is HPA-1a immunised (and DBR3*01:01 positive)	0.896	Beta (0.045)†	HIP study[4]
False-negative fetal HPA-1a typing	0.030	Beta (0.003)‡	Assumed equal to fetal RHD typing.[47]
Antibody quantitation > 3 IU/ml at 20 weeks GA. (High risk pregnancy)	0.242	Beta (0.048) #	HIP study[4] and Killie *et al.*[21]
Antibody screening at 27 weeks' GA			
Antibodies present at 27 weeks GA but < 3.0 IU/ml at 20 weeks GA.	1.000	Beta (N/A) alpha=40, beta=1	Killie *et al.*[21]
Pregnancy at high risk for FNAIT when antibodies are detected at 27 weeks GA when considered low risk at 20 weeks GA	0.040	Beta (0.008) #	HIP study [4] and Killie *et al.*[21]

SUPPLEMENTAL TABLE 2. Continued

Parameter	Probability	Distribution Beta (SE) or Dirichlet (n1, n2, n3,..)	Reference
Dead after being considered at low risk for FNAIT (no antenatal treatment)	0.000	Dirichlet (1,10,99989)	Expert opinion and Killie *et al*.[21]
Disabled after being considered at low risk for FNAIT (no antenatal treatment)	0.000	Dirichlet (1,10,99989)	
Not disabled after being considered at low risk for FNAIT (no antenatal treatment	1.000	Dirichlet (1,10,99989)	
PC > 100 × 10^9/L after being considered at low risk for FNAIT (no antenatal treatment)	1.000	Dirichlet (9989,10,1)	
PC 25-100 × 10^9/L after being considered at low risk for FNAIT (no antenatal treatment)	0.000	Dirichlet (9989,10,1)	
PC < 25 × 10^9/L after being considered at low risk for FNAIT (no antenatal treatment)	0.000	Dirichlet (9989,10,1)	
Dead after no antibodies were detected (no antenatal treatment)	0.000	Dirichlet (1,10,999989)	
Disabled after no antibodies were detected (no antenatal treatment)	0.000	Dirichlet (1,10,999989)	
Not disabled after no antibodies were detected (no antenatal treatment)	1.000	Dirichlet (1,10,999989)	
PC > 100 × 10^9/L if no antibodies were detected (no antenatal treatment)	1.000	Dirichlet (99989,10,1)	
PC 25-100 × 10^9/L if no antibodies were detected (no antenatal treatment)	0.000	Dirichlet (99989,10,1)	
PC < 25 × 10^9/L if no antibodies were detected (no antenatal treatment)	0.000	Dirichlet (99989,10,1)	
Fetus HPA-1a positive in HPA-1a negative mother in case antibodies are detected at 27 weeks' GA if were absent at 20 weeks' GA	1.000	N/A	
Antibodies present at 27 weeks' GA if were absent at 20 weeks' GA	0.020	Beta (0.002) ‡	HIP study [4] and Killie *et al*.[21]
Pregnancy at high risk for FNAIT when antibodies are detected at 27 weeks' GA if absent at 20 weeks' GA	0.132	Beta (0.026) #	
Outcome after antenatal treatment			
Fetal loss due to failure of antenatal treatment	0.000	Dirichlet (1,1700,1400,1000)	Expert opinion
PC > 100 × 10^9/L after antenatal treatment	0.415	Dirichlet (1,1700,1400,1000)	FNAIT registry 2020
PC 25-100 × 10^9/L after antenatal treatment	0.341	Dirichlet (1,1700,1400,1000)	
PC < 25 × 10^9/L after antenatal treatment	0.244	Dirichlet (1,1700,1400,1000)	
Dead if PC > 100 × 10^9/L	0.000	Dirichlet (1,10,999989)	Expert opinion
Disabled if PC > 100 × 10^9/L	0.000	Dirichlet (1,10,999989)	
Not disabled if PC > 100 × 10^9/L	1.000	Dirichlet (1,10,999989)	
Dead if PC 25-100 × 10^9/L	0.000	Dirichlet (1,10,99989)	
Disabled if PC 25-100 × 10^9/L	0.000	Dirichlet (1,10,99989)	
Not disabled if 25-100 × 10^9/L	1.000	Dirichlet (1,10,99989)	
Dead if PC < 25 × 10^9/L	0.000	Dirichlet (1,5,94)	
Disabled if PC < 25 × 10^9/L	0.000	Dirichlet (1,5,94)	
Not disabled if PC < 25 × 10^9/L	1.000	Dirichlet (1,5,94)	

† SE of 5%. ‡ SE of 10%. § SE of 15%. || SE of 20%. # SE of 50%.

Abbreviations: HPA, human platelet antigen; FNAIT, fetal and neonatal alloimmune thrombocytopenia; PC, Platelet count; IU, international units; ml, millilitre.

SUPPLEMENTAL TABLE 3. Diagnostic test costs

Parameter name	Value	Distribution Gamma (SE)	Source
Situation without HPA-1a screening			
Fetal HPA-1 typing	€1345.23	N/A	Sanquin Diagnostic Services [24]
Test to detect FNAIT in fetus or neonate	€1953.66	N/A	Sanquin Diagnostic Services [25]
Platelet count	€22.39	Gamma (2.24) ‡	Dutch rate decision[49]
Order rate	€9.01	N/A	Dutch rate decision [49]
With HPA-1a screening			
Maternal HPA-1 typing	€15.00	Gamma (0.75) †	Sanquin Diagnostics Services (calculated by LP and MdH)
Fetal HPA-1 typing	€43.00	Gamma (2.15) †	
HLA DRB3*01:01 test	€40.00	Gamma (8.00) ||	
HPA-1a antibody screening	€75.00	Gamma (3.75) †	
Risk typing (antibody titre)	€150.00	Gamma (7.50) †	
Platelet count	€22.39[∞]	Gamma (2.24) ‡	Dutch rate decision [49]
Order rate	€9.01[∞]	N/A	Dutch rate decision [49]

† SE of 5%. ‡ SE of 10%. § SE of 15%. || SE of 20%. # SE of 50%.
Abbreviations: SE, standard error; HPA, human platelet antigen; N/A, not applicable; HLA, human leukocyte antigen

SUPPLEMENTAL TABLE 4. COSTS

Parameter name	Value	Distribution Gamma (SE)	Source
Antenatal treatment			
NaCl 500 ml 0.9%	€2.13	Gamma (0.11) †	Medicijnkosten.nl[26]
IVIg 0.1g/ml, 25 ml vial	€223.45	Gamma (11.17) †	Medicijnkosten.nl[26]
IVIg administration in hospital	€304.46 per administration	Gamma (60.89) ||	Manual for cost research[27]
Sanquin home service	€200 per administration	Gamma (40.00) ||	Estimated by Sanquin, personal communication MdH
Advanced fetal ultrasound	€851.48	Gamma (42.57) †	Passer-by rate[28] assuming the highest rate; costs updated to 2022 using Dutch CPI
Standard fetal ultrasound	€166.66	Gamma (8.33) †	Passer-by rate[28] costs updated to 2022 using Dutch CPI
Consult gynaecologist	€185.87	Gamma (9.29) †	Manual for cost research.[27]
Consult midwife	€31.54	Gamma (3.17) ‡	Manual for cost research.[27]
Postnatal treatment			
HPA matched platelet transfusion	€365.37	Gamma (17.65) †	Sanquin, personal communication TWdV
Cranial ultrasound	€100.35	Gamma (5.02) †	Liem *et al*.[29]
Admission maternal ward (day)	€449.86	Gamma (44.99) ‡	Liem *et a* .[29]
Admission high care neonatology	€1830.87	Gamma (183.09) ‡	Liem *et al*.[29]
Lifetime costs per health state			
Healthy state	€0	NA	-
Not disabled state	€0	NA	-
Disabled state (excl. informal costs)	€802,868		Kruse *et al*.[30]
Lifetime informal care costs (disabled state)	€340,999		Mitchell *et al*.[31]
Total lifetime costs disabled health state	€1,143,867	Gamma (571,933.62)#	Liem *et al*.[29] and Kruse *et al*.[30]
Death state	€0		-

† SE of 5%. ‡ SE of 10%. § SE of 15%. || SE of 20%. # SE of 50%.
Abbreviations: SE, standard error; NaCl, natriumchloride [sodiumchloride in English]; IVIg, intravenous immune globulins; ml, millilitre; CPI, consumer price index; HPA, human platelet antigen, excl., excluding.

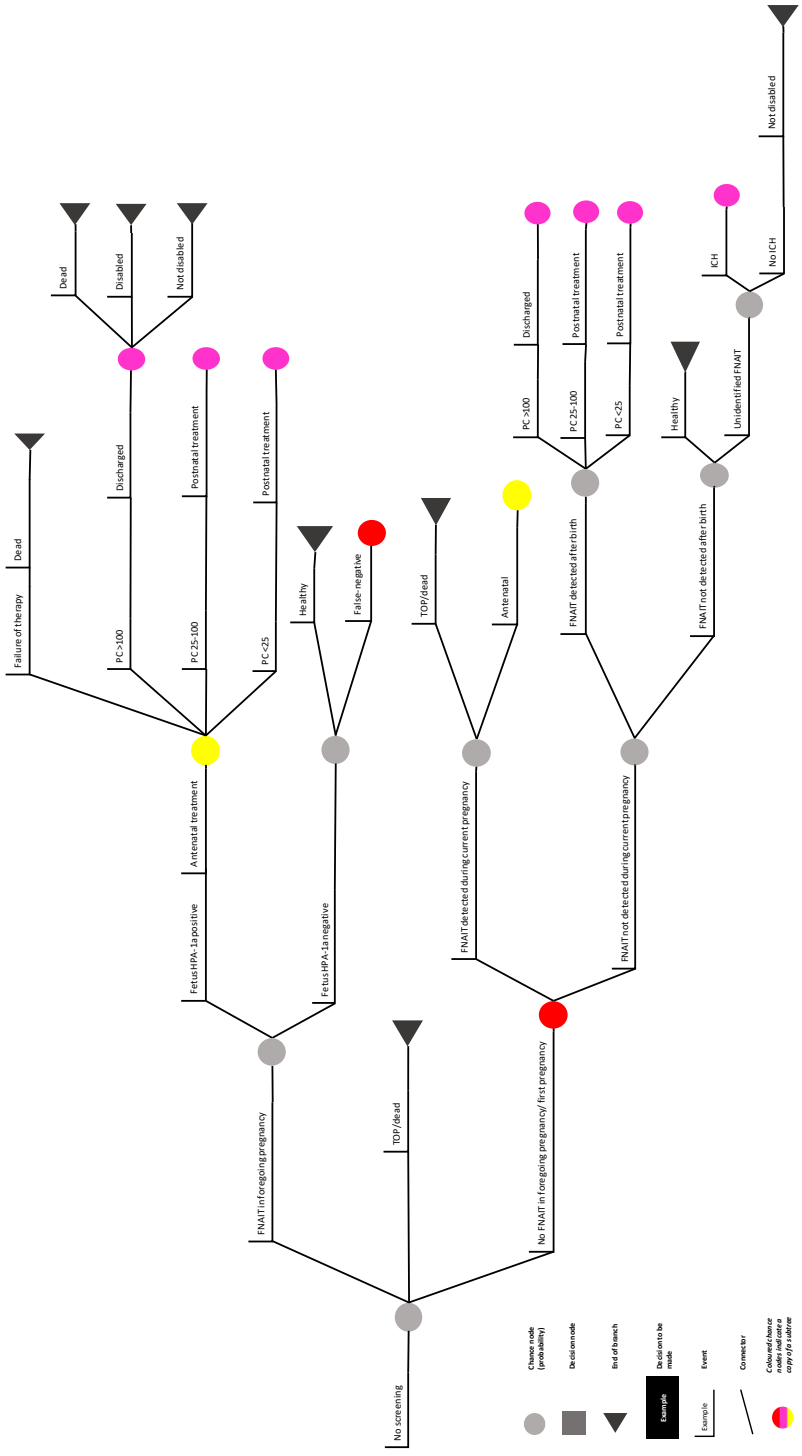
SUPPLEMENTAL TABLE 5. Utility, life expectancy and quality-life adjusted years

Parameter name	Value	Distribution Gamma (SE)	Source
		Utility per health state	
Dead	0	N/A	By definition
Disabled	0.550	Beta (0.110)||	Jarl *et al.*[32]; Macedo *et al.*[33]; Langelaan *et al.*[34]; Kirkham *et al.*[35]
Not disabled	0.910	Beta (0.046) †	Janssen *et al.*[36]
Healthy	0.910	Beta (0.046) †	Jansen *et al.*[36]
		Life expectancy per health state	
Dead	0	N/A	By definition
Disabled	50	Gamma (10)||	Strauss *et al.*[37]
Not disabled	81.66	Gamma (4.083) †	CBS[38]
Healthy	81.66	Gamma (4.083) †	CBS[38]

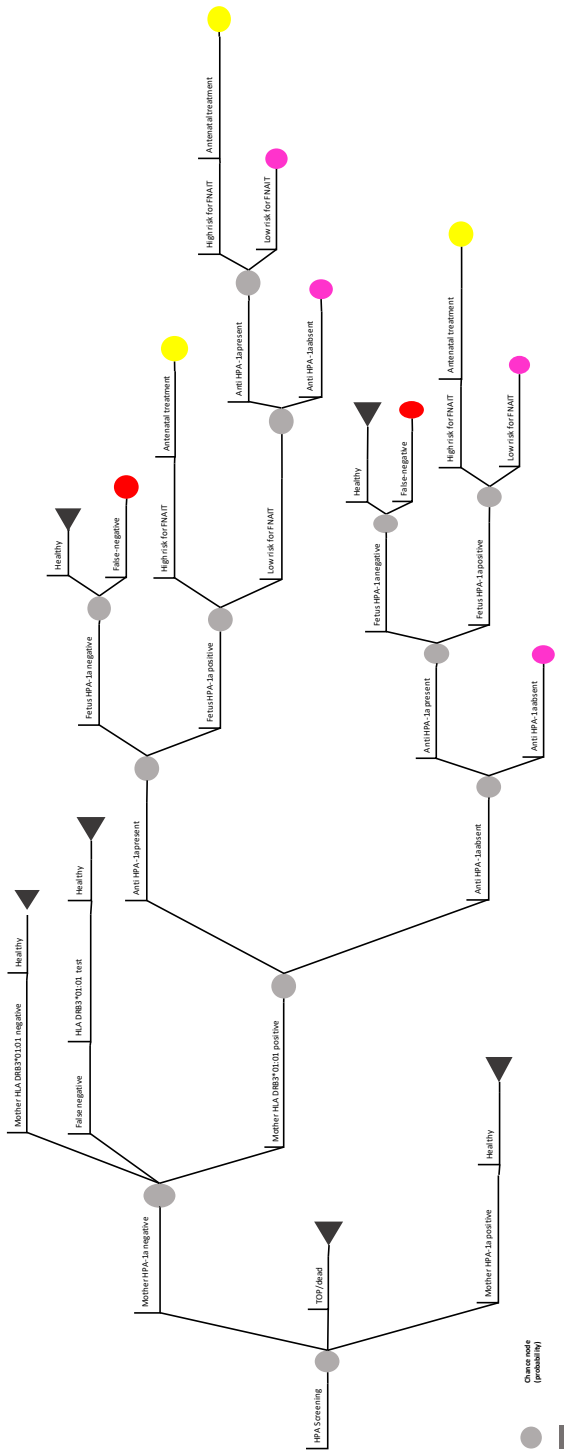
† SE of 5%. ‡ SE of 10%. § SE of 15%. || SE of 20%. # SE of 50%.
Abbrevation: SE, standard error.

SUPPLEMENTAL TABLE 6. Quality-adjusted life years per health state

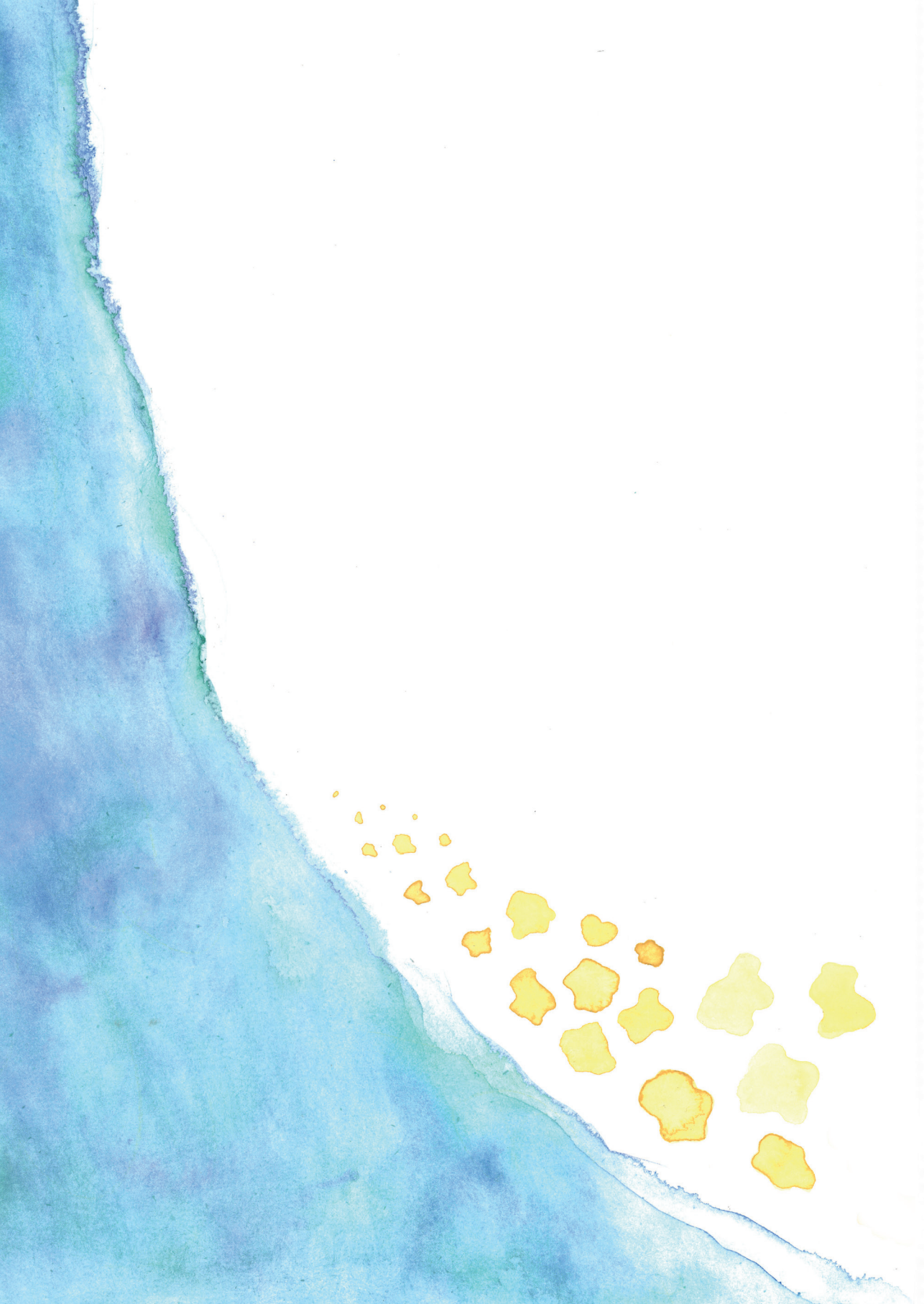
Health state	Value - undiscounted	Value - discounted
Dead	0	0
Disabled	27.5	19.54
Not disabled	74.31	43.41
Healthy	74.31	43.41



SUPPLEMENTAL FIGURE 1. Situation without screening - decision tree

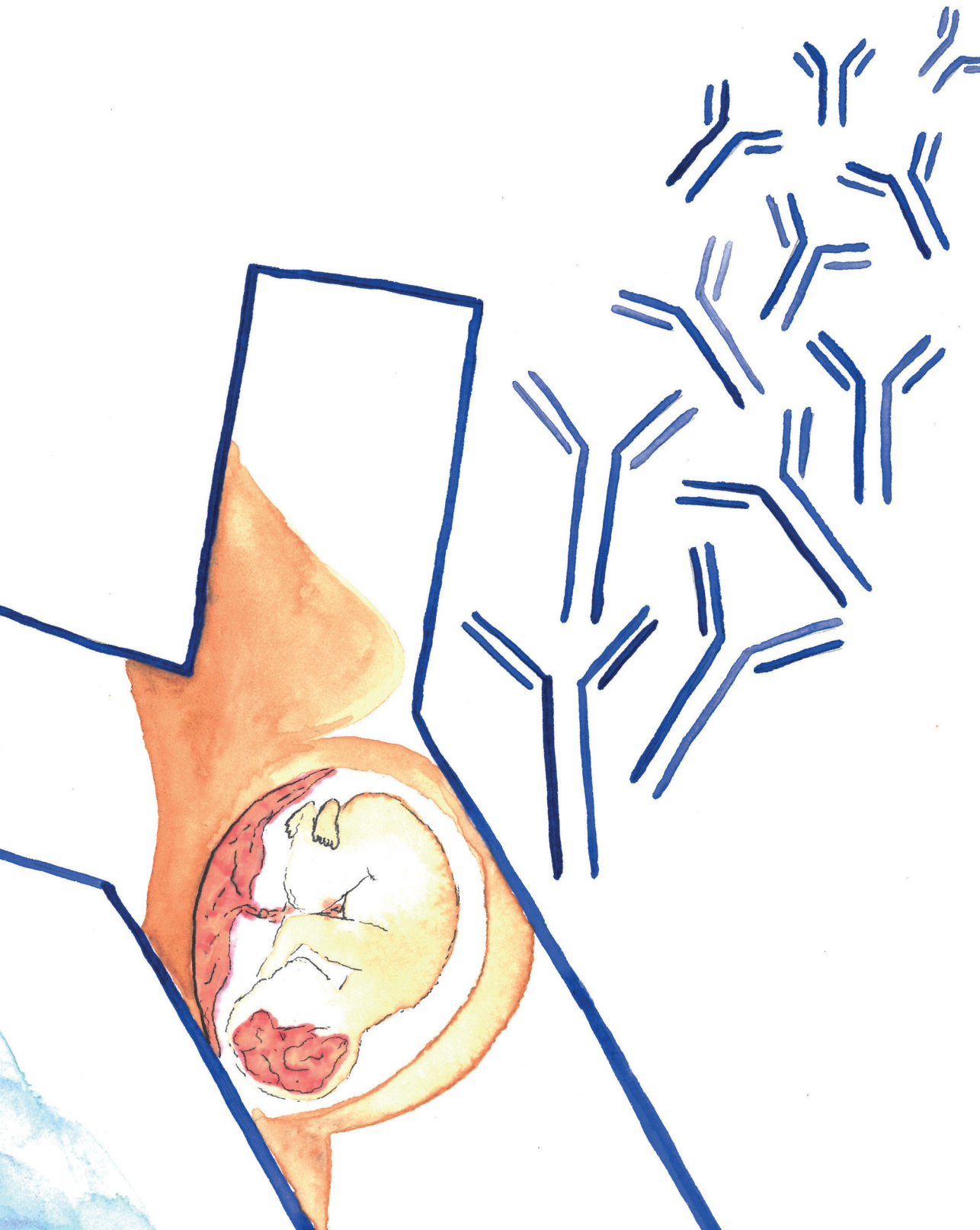


SUPPLEMENTAL FIGURE 2. HPA-1a screening - decision tree



PART SEVEN

Summary and discussion



CHAPTER 10

General discussion and future perspectives

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Already for decades maternal-fetal medicine specialists, neonatologists, hematologists and laboratory professionals are investigating the possibilities to prevent fetal and neonatal alloimmune thrombocytopenia (FNAIT) and its devastating sequalae. FNAIT occurs in approximately 1 in 1,500 pregnancies[1] and is the most common cause of thrombocytopenia in otherwise healthy term-born neonates.[2] FNAIT is characterized by alloantibody formation against paternally-derived fetal platelet antigens due to an incompatibility between the antigenic composition of the maternal and fetal platelets. In the white population, human platelet antigen (HPA)-1a is the most commonly involved antigen in FNAIT.[3] Fetal-maternal HPA-1a incompatibility can lead to alloimmunization and the production of HPA-1a directed antibodies by the mother. The immunoglobulin-G (IgG) fraction of these antibodies is transported across the placenta to the fetal circulation. In the fetus, the alloantibodies bind to HPA-1a carried by the β3 integrin (CD61). The β3 integrin is expressed on platelets,[4] endothelial cells,[5] activated leukocytes,[6] and the placenta.[7] Consequently, these alloantibodies can lead to platelet phagocytosis,[8] impairment of endothelial cell function and angiogenesis,[9-11] and likely to damage to the placenta.[12] In addition, the binding of HPA-1a antibodies to the fibrinogen receptor (GPIIbIIa, α2bβ3, CD41/CD61) can possibly lead to platelet dysfunction[13] and inhibition of megakaryopoiesis.[14] Presumably, the combination of severe fetal thrombocytopenia and endothelial cell dysfunction can then lead to fetal hemorrhage, (Figure 1) and in some cases neurological impairment and/or perinatal death.

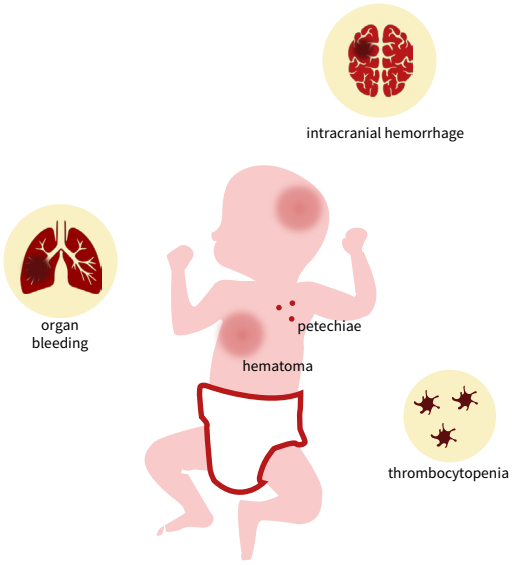


FIGURE 1. Clinical outcome in FNAIT

Although HPA-1a is most commonly involved in the white population, more than 41 other HPAs can cause FNAIT.[15] Limited evidence is available on the incidence of platelet antibodies in other populations. Due to differences in the allele frequency of platelet antigens, antibodies involved in FNAIT differ between ethnicities. For example, HPA-1a-negativity is very rare in the Asian and black population whereas FNAIT mediated by anti-HPA-4b is more common in Asians and very rare in whites.[16] Anti-CD36 (platelet glycoprotein IV) is found in FNAIT in Asian and black populations and not in whites.[17]

The most feared complication of FNAIT is intracranial hemorrhage (ICH), often leading to irreversible brain damage or death. Recurrence risk of ICH in a subsequent pregnancy again complicated by FNAIT is estimated between 29%[18] and 79%.[19] Currently, there is no screening program for platelet antibodies, and FNAIT is mostly diagnosed after birth in children with thrombocytopenia and/or bleeding symptoms. Therefore, prevention of fetal/neonatal bleeding in pregnancies of immunized women is only possible in subsequent pregnancies. In most high-income countries, administration of intravenous immunoglobulins (IVIg) to the pregnant women is recommended as a first-line treatment in HPA-immunized pregnancies, with the addition of corticosteroids in some centers.[20] The success of red cell alloimmunization screening programs inspired many to show that timely detection of HPA-1a-alloimmunized pregnancies could also lead to the successful prevention of fetal death or neurological impairment in FNAIT. This thesis aims to fill knowledge-gaps about several aspects of FNAIT to substantiate a decision on the introduction of a population-based screening program.

Most screening programs are introduced from a utilitarian approach which strives to achieve the best outcome (in this case neonatal outcome) by preventing the greatest amount of suffering. Of course, many other interests and issues also play a role and the weighing of a screening must be done carefully. Usually, this appraisal is performed based on the principles of screening posed by Wilson & Jungner (W&J) and published by the World Health Organization in 1968 (Figure 2). In this general discussion population-based screening for platelet antibodies is considered by evaluating the knowledge available from literature and studies presented in this thesis. This evaluation will be guided by the principles from Wilson and Junger.

	Knowledge of disease
1	The condition must be an important health problem. (W&J 1)
2	There should be a recognizable latent or early symptomatic stage. (W&J 4)
3	The natural history of the condition including development from latent to declared disease should be adequately understood. (W&J 7)
	Knowledge of test
4	There should be a suitable test or examination. (W&J 5)
5	The test should be acceptable to the population. (W&J 6)
	Treatment for disease
6	Agreed policy on whom to treat as patients. (W&J 8)
7	There should be an accepted treatment for patients with recognized disease. (W&J 2)
8	Facilities for diagnosis and treatment should be available. (W&J 3)
	Cost considerations
9	Costs of case finding (including diagnosis and treatment of patients diagnosed) economically balanced in relation to possible expenditure on medical care as a whole. (W&J 9)
10	Case-finding should be a continuing process and not a once and for all project. (W&J 10)

FIGURE 2. Principles of screening by Wilson & Jungner (W&J)

KNOWLEDGE OF DISEASE

1. THE CONDITION MUST BE AN IMPORTANT HEALTH PROBLEM (W&J 1)

There are several perspectives to regard a disease as an important health problem. It may be the incidence of the disease and/or the impact of the disease on the general wellbeing and long-term outcome of the affected person. It is generally accepted that FNAIT is a disease with a low incidence but with a serious risk of fetal/neonatal death and brain damage in affected surviving children.[1]

Neurodevelopmental outcome in children with newly diagnosed FNAIT

Several case series on the long-term neurodevelopmental outcome of children affected by FNAIT with intracranial hemorrhage (ICH) concluded that the long-term outcome of children with ICH due to FNAIT is poor.[21-23] Tiller *et al*.[22] reported on the short and long-term outcome of 43 cases with ICH related to FNAIT and found that five died in utero, one died during labor and nine died after birth. Of the 28 survivors, 23 children (82%) had severe neurological disabilities and only five were 'alive and well'. Winkelhorst *et al*.[24] assessed the long-term outcome of 21 children with FNAIT related ICH. In total, 11 (21%) children survived, in 10 children standardized cognitive and neurological tests were performed (one case lost to follow-up). Severe neurodevelopmental impairment (NDI) was diagnosed in six (60%) children and mild-to-moderate NDI in two (20%) children. These studies underline that FNAIT is a severe disease with a high risk of neurodevelopmental problems especially in the case of ICH. However, knowledge about the long-term neurodevelopmental outcome of children that were newly diagnosed with FNAIT with or without ICH was lacking and therefore part of the research performed and reported in this thesis.

In **chapter 6** we describe a study on the long-term outcome of 44 children that were newly diagnosed with FNAIT, of whom five (11%) had severe ICH and two had low-grade ICH. The results of this study are summarized in Figure 3. Prior to this study we hypothesized that HPA-antibodies could lead to subclinical cerebral damage resulting in developmental delay on the long-term.[25] In total, three (7%) children had severe NDI, two of those were diagnosed with severe ICH, the third one suffered from perinatal asphyxia and had a low-grade ICH. Mild-to-moderate NDI was detected in 11 (25%) children of which only one was diagnosed with severe ICH. Two other cases with severe ICH and one case with low-grade ICH had normal neurodevelopmental outcome. These results indicate that the risk of neurodevelopmental impairment in children with newly diagnosed FNAIT is high, also in children without ICH. However, the overrepresentation of boys (79%) in our study group could have biased the study since male sex is a risk factor for minor neurologic dysfunction.[26] Besides the unequal sex distribution, other risk factors for NDI (neonatal morbidity or low maternal education level) were present in 50% of the cases with NDI. Unfortunately, due to a limited sample size an independent risk factor analysis was not possible. Another limitation was the lack of a control group. Yet, we were the first to assess long-term neurodevelopment using standardized psychometric and neurological test. To better define the type, severity and impact of FNAIT-related NDI, longitudinal neurodevelopmental testing at the age of 2, 5 and 8 years should be included in the design of future prospective screening studies including a control group of children that were born after a non-HPA-immunized pregnancy.

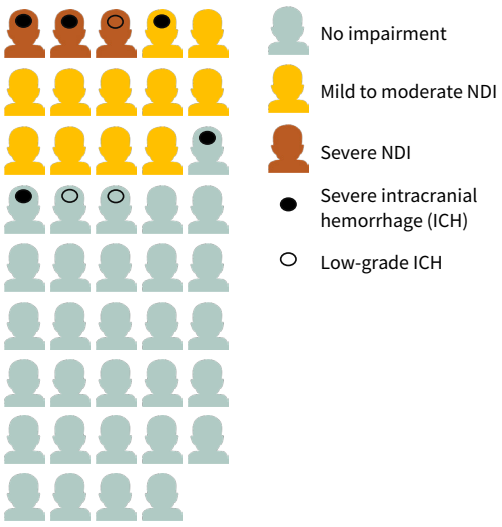


FIGURE 3. Neurodevelopmental impairment (NDI) in newly diagnosed FNAIT cases.

This figure shows the long-term neurodevelopmental outcome at school age in relation to the presence of intracranial hemorrhage of 44 individual cases that were newly diagnosed with FNAIT.
Abbreviations: NDI, neurodevelopmental impairment; FNAIT, fetal neonatal alloimmune thrombocytopenia; ICH, intracranial hemorrhage.

In conclusion, FNAIT is a severe health problem because it can lead to severe neurodevelopmental delay in children affected by ICH. Besides, the results of our study suggest that children with FNAIT without ICH are also affected by long-term neurodevelopmental problems, affecting the daily functioning of these children.

2. THERE SHOULD BE A RECOGNIZABLE LATENT OR EARLY SYMPTOMATIC STAGE (W&J 4).

Mechanism of HPA immunization

In order to start treatment to prevent the occurrence of severe bleeding, HPA-antibody screening should be performed between the moment of immunization and the occurrence of bleeding in the child. The time-interval between the first production of HPA-1a antibodies by the pregnant woman, and the moment the fetus develops a high bleeding tendency which lead to ICH can be regarded as the latent or very early symptomatic stage. Whether an early stage of bleeding may be amenable to treatment to prevent worsening is unknown. HPA-1a immunization occurs when an HPA-1a negative pregnant women is exposed to the HPA-1a antigen. HPA-1a is expressed by the fibrinogen receptor (αIIbβ3, CD41/CD61) on platelets and by the vitronectin receptor (αvβ3, CD51/CD61) on platelets, endothelial cells[5] and trophoblasts.[27] It was long thought that the main route of HPA-1a alloimmunization were fetal platelets gaining access to the maternal circulation, a common phenomenon during pregnancy and after delivery.[28] Another possible route of alloimmunization, described in 1998[29] is exposure to trophoblast cells from the placenta which express HPA-1a or upon cell decay resulting in the release of senescent trophoblast cells. Furthermore, the placenta continuously shed extracellular vesicles.[30] By unravelling the antibody specificity of the polyclonal IgG response, we can possibly learn more about the mechanism and timing of immunization. Previous studies have shown various subtypes of HPA antibodies in patient sera.[11, 31]

We can also learn more about the process of alloimmunization by comparing clinical characteristics of immunized subjects with controls. The HPA screening in pregnancy (HIP) study (**chapter 3**), an observational prospective screening study performed in the Netherlands including 913 HPA-1a negative women, offered the opportunity to assess clinical risk factors for HPA immunization. We found that 32% of the HPA-1a immunized women had blood group O compared with 45% of the non-immunized HPA-1a negative women and 43% of the HPA-1a positive women. It seems that the risk of HPA-1a immunization is lower in mothers with blood group O. For red blood cell immunization, it is known that ABO incompatibility reduces the risk of D alloimmunization.[32, 33] This protective effect was considered to be less likely in platelet-mediated HPA-1a alloimmunization since the expression of ABO antigens on platelets is lower compared with red blood cells. The ABO antigens are expressed only by fetal cells such as platelets, and not by placental cells.[34] However, similar as observed in RBC alloimmunization, in women with blood group O, regular anti-A and anti-B antibodies may bind directly to the fetal platelets. Subsequently, these platelets are lysed or efficiently

removed by macrophages before maternal immunization could have taken place, or binding might inhibit the B cell response via binding to the IgG-Fc receptor IIb of the B cell. These results suggests that fetal platelets are an important source of HPA-immunization. In the Norwegian screening study, the ABO distribution was similar for immunization compared with the general population, but it was noted that neonates from women with blood group O had higher platelet counts.[35] Probably variety in the study design of the screening studies may explain the different findings. First, in the Norwegian study no control group was included. Second, antibody screening was performed at multiple time points in pregnancy and with the MAIPA assay whereas we performed a single measurement at week 27 of pregnancy with the PAKLx assay. A difference in the sensitivity of these assays or the difference in timing of testing in pregnancy may have resulted in a different group being considered 'HPA-1a immunized'. Finally, in our study we were not able to collect platelet counts of the neonates. In conclusion, we do not fully understand the discrepancy between the study outcomes yet and more studies on this topic need to be performed.

Tolerization due to antigen exposure during pregnancy

During pregnancy, transplacental passage of cells occurs both from mother to fetus and from fetus to mother. It was described previously that these maternal and fetal cells can persist for decades in the circulation of the child and mother, respectively.[36, 37] It was observed in RhD immunization[38] that tolerization might occur if an RhD negative woman had an RhD positive mother and was thereby exposed in utero to the RhD antigen. Additionally, it was reported that exposure to non-inherited maternal allo-antigens reduces the risk of HLA immunization.[39] Whether HPA-immunization is also influenced by the HPA-1 status of mothers of HPA-1a negative women was investigated by Kjaer *et al.*[40]. These authors concluded that there was no evidence for toleration against HPA-1a in HPA-1a negative women who were exposed to HPA-1a. We also invited a cohort of mothers of HPA-1a immunized women, with children diagnosed with HPA-1a mediated FNAIT via the national reference laboratory for HPA-1 genotyping (L. Porcelijn, personal communication). If tolerization occurs after exposure to HPA-1a during fetal life, we expected more grandmothers typed as HPA-1bb compared to the general population. In total 22 mothers of women that were HPA-1a immunized were typed for HPA-1. Only three (14%) women were typed as HPA-1bb which did not significantly differ from the two (8%) as expected in the general population (see Table 1). Acknowledging that our study as well as the Norwegian study is hampered by a limited sample size, we conclude that our results at least show that non-inherited maternal alloantigen exposure does not abolish or greatly reduces the risk of HPA-1a immunization.

TABLE 1. Observed versus expected genotypes of mothers from HPA-1a immunized women

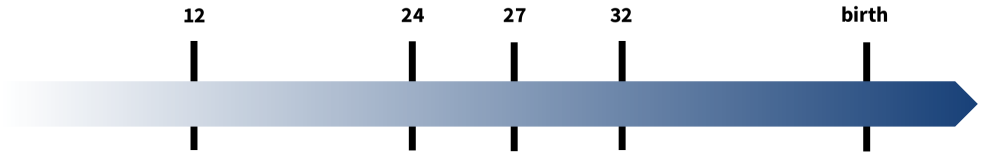
	Mother HPA-1ab	Mother HPA-1bb
Observed – n (%)	19 (86)	3 (14)
Expected – n (%)	20 (92)	2 (8)

We acknowledge Leendert Porcelijn and the laboratory of platelet and leukocyte serology for providing these data. [Unpublished data]

Timepoint of immunization

In the design of the HIP study (**chapter 2**) antibody screening was done only once in a sample drawn at 27 weeks of gestation. It was regarded as unethical to share HPA typing and antibody results with the care providers and therefore no cord blood platelet count measurement or repeated antibody testing was done. Screening studies from the United Kingdom (UK),[29] Norway,[41, 42] and Poland[43] performed antibody sampling during pregnancy with 4 – 6 week intervals. In Figure 4 we summarized data on the timepoint of the first antibody screening being positive stratified for primigravida and multigravida women. In primigravida the first detection of HPA-1a alloantibodies was observed throughout pregnancy, but not before week 14.[29] In 88% of the multigravida women, antibody samples were positive already in the first trimester of pregnancy. This suggests that in the majority of the cases immunization had occurred in the previous pregnancy, or around delivery. One would expect that also in multigravida women, new immunizations would take place throughout pregnancy irrespective whether they carried an HPA-1a positive child before, but this is not supported by these data. Based on the allele frequency we calculated that in 15% of the HPA-1a negative pregnant women the fetus is HPA-1a negative and therefore fetal-maternal incompatibility is absent in for example the first pregnancy. So, only in 7.5% of next pregnancies, a multigravida women will carry an HPA-1a positive child for the first time. However, this low number makes it difficult to detect late 'de novo' immunizations in this group. Nevertheless, these data suggest that most women who can form an anti-HPA-1a immune response do so upon their first encounter of the HPA-1a antigen.

In the Norwegian screening study[41] only 9% (14/154) of the HPA-1a antibodies were detected in women having their first pregnancy (primigravida), in the UK screening study in 14% (4/28) and in the Scottish screening study[44] only 4% (1/25). These low percentages suggest that a previous pregnancy is necessary for the formation of anti-HPA-1a.[45] In contrast to these earlier findings, in the HIP study (**chapter 3**) the percentage of primigravida women (32%) in the immunized group was similar to percentage in the control group of HPA-1a positive women (34%). A clear explanation for these different findings is not yet available. In both the Norwegian[46] and UK[29] screening studies antibodies disappeared in a relevant percentage of multigravida women (22% [32/147] and 25% [6/24]). Hence, in the HIP study these antibodies could have been missed by performing antibody screening only at 27 weeks of gestation. In addition, differences in study design (center-based screening/part of regional routine screening program, type of consent), study period (demographic differences in fertility rate/rate of (induced) miscarriages) and study duration may influence the study entry and characteristics of women eligible for the study. This is however difficult to substantiate because the previous screening studies do not report outcomes of the non-immunized women. Ultimately, our data underline that the risk of immunization and severe fetal disease in first pregnancies cannot be underestimated. ICH occur in a quarter[22] to three quarter[47] of cases in first pregnancies, which was confirmed by the data of our international cohort study (**chapter 6**) reporting that 59% of severe ICH had occurred in first pregnancies.



Author, year					
Williamson, 1998				**85% (*n* = 38)**	**15% (*n* = 7)**
Primigravida (12 HPA incompatible, in 2 cases timepoint first positive sample not determined)		2/8 between 14-22 weeks	2/8 at 26 weeks	4/8 between 30-37 weeks	2 after birth
Multigravida (28 HPA incompatible)	10/23 first trimester	13/23 between 14-22 weeks			5 after birth
Kjeldsen-Kragh, 2007/Killie, 2008				**75% (*n* = 172)**	**25% (*n* = 60 adjusted rate))**
Primigravida (13 HPA incompatible)		9/13 at 22 weeks	2/13 between 24-26 weeks	2/13 between 34-36 weeks	
Multigravida (147 HPA incompatible)	141/147 first trimester	4/147 between 14-22 weeks	1/147 at 24 weeks	1/147 at 38 weeks	
Debska, 2018				**68% (*n* = 22)**	**32% (*n* = 10)**
HIP study			(*n* = 81 at 27 weeks)		

FIGURE 4. Timing of first anti-HPA-1a detection during pregnancy and immunization detected after delivery

This figure shows the timepoint that anti-HPA-1a was detected for the first time in prospective antenatal screening studies stratified by primigravida and multigravida.
Abbreviations: HPA, human platelet antigen; HIP study, HPA screening in pregnancy study.

Timing of bleeding

Literature on the exact moment in pregnancy of occurrence of ICH is scarce. Tiller *et al.*[22] reported on the timepoint of bleeding in a cohort of 43 cases with ICH. In this study, more than half of the ICH (54%) occurred at 28 weeks of gestation or earlier and two third (67%) of the ICH occurred before 34 weeks. Jin *et al.*[47] described the timepoint of bleeding of 21 cases with ICH. In this study, 19% of the ICH were identified before 27 weeks (18, 22, 22 and 24 weeks).

Considering timepoint of screening

Preferably, antibody screening is done at a moment in pregnancy chosen *after* the majority of the pregnancies have become immunized and *before* the occurrence of ICH. We think antibody screening should be performed after the first trimester since in primigravida women antibodies were detected as early as 17 weeks. In multigravida women, antibodies detected before 20 weeks require confirmation because transient antibodies are not clinically relevant.[29] ICH were described in pregnancies from 20 weeks of gestation onwards,[22] therefore first antibody screening should be planned not later than 20 weeks of gestation. Based on the presently available knowledge on timepoint of immunization it is likely that if screening will be performed around 20 weeks of gestation only, one would miss an important proportion of primigravida women that did not show signs of HPA-1a alloimmunization yet (see Figure 4). As discussed, an important part of the ICH was found in primigravida women, it is therefore important to maximize the efforts to include high-risk pregnancies from this group.[22, 47] To include this group of women, a second antibody screening should be added to the antibody screening at the 20 weeks of gestation, for example around 27 weeks of gestation.

In conclusion, there is an interval between immunization and the onset of bleeding which can be seen as the early symptomatic or latent phase in which intervention can take place to decrease the risk of fetal bleeding. Results from the HIP study underline that immunization can occur in first pregnancies and based on data from cohort studies we know that immunization can also result in major bleeding in first pregnancies. Since it is reported that immunization in first pregnancies can occur throughout pregnancy, multiple screening timepoint are likely to be necessary. Data on the time of the onset of bleeding are limited, but it is clear that bleeding can occur early in pregnancy (beginning of the second trimester), which necessitates timely detection of HPA-1a antibodies.

3. THE NATURAL HISTORY OF THE CONDITION INCLUDING DEVELOPMENT FROM LATENT TO DECLARED DISEASE SHOULD BE ADEQUATELY UNDERSTOOD. (W&J 7)

Incidence of severe hemorrhage

To estimate the expected health gain from screening programs knowledge about the natural history of a disease is indispensable. The ultimate goal of a screening would be to prevent severe hemorrhage which is associated with perinatal death and neurodevelopmental impairment. In most previous screening studies on FNAIT, screening test results were reported

to the caregivers (**Table 2**). Only few studies did not intervene[48, 49] or only after birth with postnatal platelet transfusions[29, 44]. Other studies performed near term-cesarean section and had platelet transfusions available directly after delivery[41,50] or performed fetal blood sampling (FBS) either followed by intrauterine platelet transfusions (IUPT),[43, 51] administration of IVIg to the mother,[52, 53] or a combination of these therapies.[54] It was thought that the interventions in these studies could have reduced the risk of bleeding. In our screening study, both pregnant women and caregivers were blinded for maternal HPA-1a status and alloantibody detection allowing us to compare the outcome of HPA-1a immunized pregnancies without interference by medical interventions, observer or participant bias. Within a combined population of 278 HPA-1a immunized pregnant women from previous screening studies, we calculated that the incidence of severe FNAIT (defined as severe hemorrhage or perinatal death) in HPA-1a immunized and incompatible pregnancies was 2.2% (6/278). The incidence of severe ICH was 1.4% (4/278). The incidence of severe ICH in the HIP study (**chapter 3**) was 1 out of 81 (1.2% [95 CI 0 – 6.7%]), which was not higher compared to the incidence reported previous screening studies. Severe ICH were predominantly diagnosed during pregnancy in screening studies (at 29 weeks [HIP study] 34 weeks,[41] 37 weeks[29] and 48 hours after delivery[50] [timepoint of diagnosis was not reported in one study[54]]). Possibly the interventions (e.g., near-term caesarean section or readily available postnatal platelet transfusion) in other studies had only limited effect on the occurrence of antenatal ICH.

Placental damage in FNAIT

FNAIT generally manifests postnatally with signs of skin bleeding and thrombocytopenia. In addition, a Norwegian study suggested that HPA-1a immunization was associated with a reduced birthweight in male infants.[55] In line with this study, the proportion of cases born small for gestational age (SGA, birthweight below 10th percentile) was higher in a large international cohort study (2001-2010)[56] and the FNAIT registry (2010-2020) **(chapter 5).** A point of discussion raised is that the higher proportion of cases born SGA in retrospective studies may be due to selection bias. Additional diagnostic tests may have been performed in new-borns with low birthweight including full blood count which could have resulted into detection of neonatal thrombocytopenia as a chance finding. However, HIP study (**chapter 3**), which had a prospective study design, we also found that HPA-1a immunization was associated with a reduced birthweight. We found that birthweight of infants of HPA-1a immunized women was significantly lower compared to infants of HPA-1a positive women born at the same gestational age. Since the HIP study was prospectively performed, these findings could not have been influenced by selection bias. Interestingly, we found that birthweight percentile was significantly lower in primigravida but not in multigravida women (see Figure 5). This finding requires further investigation on aspects of the evolving immune response in first pregnancies and possibly also downregulation in subsequent pregnancies. It may be that antibody subtype in first pregnancies is different, for instance with a higher avidity to the epitope at the placenta, which affects the development and/or function of the placenta and thus fetal growth.

TABLE 2. Prospective antenatal screening studies

Author	No. pregnant women	HPA-1a negative women (%)	Immunized cases (antibodies detected during pregnancy) (%)	Severe thrombocytopenia (PC < 50) (%)	Mild bleeding	Severe bleeding	Death	Interventions
Mueller-Eckhardt, 1985 [48]	1,211	26 (2.1)	2/26 (8)	0	0/2	0	0	None
Reznikoff-Etievant, 1988 [49]	860	27 (3.1)	0	0	0	0	0	None
Blanchette 1990 [50]	5,000	81 (1.6)	3/50 (6)	1/3 (30)	0/3	1 severe ICH †	0	NTCS, PP
Doughty, 1995 [52]	3,473	74 (3.2)	1/68 (1)	1/2(50)	1/1 (100)	0	0	FBS, IVIg, PP
Durand-Zaleski, 1996 [53]	2,066	52 (2.5)	4/45 (9)	1/4 (50)	0/4	0	0	FBS, IVIg, steroids, PP
Williamson, 1998 [29]	24,417	618 (2.5)	36/385 (9.4)	8/38 (21)	7/36 (18)	1 severe ICH †	1‡	PP
Davoren, 2003 [51]	4,090	54 (1.7)	2/34 (6)	1/2 (50)	1/2 (50)	0	0	FBS, IUPT, PP
Maslanka, 2002 [54]	8,013	144 (1.8)	12/122 (10)	3/12 (25)	1/12 (8)	1 severe ICH †	0	IUPT, IVIg
Turner, 2005 [44]	26,506	546 (2.1)	25/318 (8)	5/25 (20)	3/25 (12)	0	0	PP
Kjeldsen-Kragh, 2007 [41]	100,488	2,111 (2.1)	171/1,990 (8.6)	55/161 (34)	17/117 (14)‖	1 severe ICH † 1 low-grade ICH	1§	NTSC, PP
Debska, 2018 [43]	15,204	373 (2.5)	22/373 (5.9)	3/14	NR	NR	NR	FBS, IUPT, PP
Total	**191,328**	**4,106 (2.2)**	**278/3411 (8.1)**	**78/263 (30)**	**30/205 (15)**	**4/278 (1)**	**-**	
HIP study, 2022	153,106	3,722 (2.4)	85/913 (9.3)	NR	3/81 (4)	1/81 (1)††	1††	None

† Antibody characteristics of the severe ICH cases were as follows; Blanchette *et al.* reported the presence of 'strong PL^{A1} antibodies', Williamson *et al.* reported 'Antibody titer 1 : 64' Maslanka *et al.* reported antibody titers '1 : 4 or higher', Kjeldsen-Kragh *et al.* reported '150 – 41 IU/L' in the case with severe ICH.

‡ IUFD after complications after FBS for investigation of unexplained fetal hydrops. No detectable red blood cell antibodies, platelet count 6×10^9/L. (Case was not counted as case with severe bleeding).

§ IUFD in a twin pregnancy, one child died, the other was born with platelet count of 45×10^9/L. (Case was not counted as case with severe bleeding.)

|| Unpublished results, personal communication Jens Kjeldsen-Kragh, data available from one of the participating centers.

†† Case diagnosed with ICH at 29 weeks gestational age. Termination of pregnancy at 34 weeks because of severe neurological damage. (Case was counted as severe bleeding and perinatal death).

Severe bleeding was defined as: intraventricular hemorrhage (IVH) grade III, intraventricular hemorrhage of any grade with parenchymal involvement, parenchymal hemorrhage, cerebellar hemorrhage, extra axial hemorrhage visible on cranial ultrasound. Any non-ICH was considered major if any therapy related to bleeding was given.

Mild bleeding was defined as: petechiae, hematoma, mucosal bleeding, IVH grade I or II or increased bleeding tendency as reported by the caregiver.

Interventions: NTSC, near term cesarean section; PP, postnatal platelet transfusions; IVIg, intravenous immune globulin; FBS fetal blood sampling

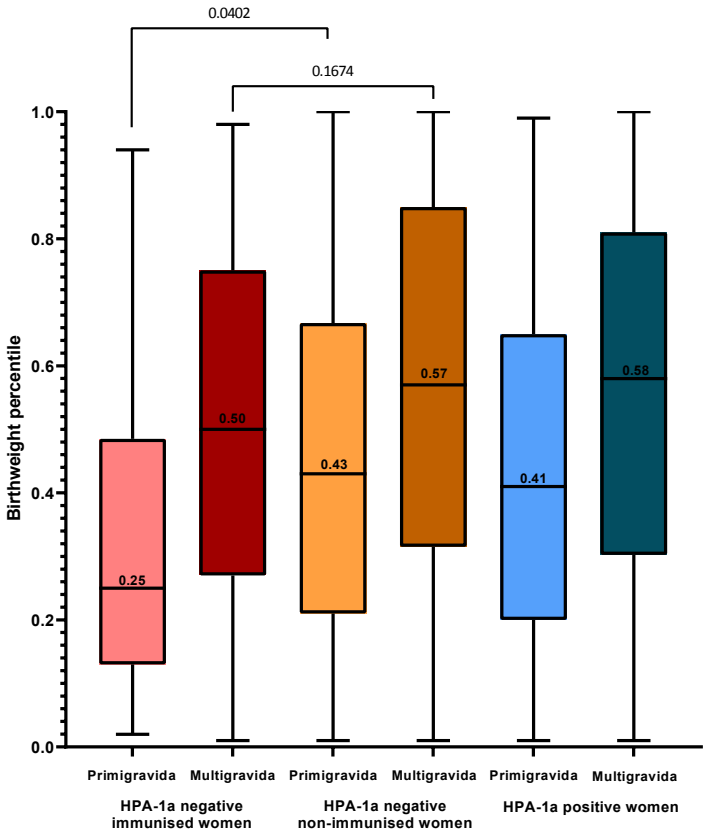


FIGURE 5. Birthweight percentile stratified for primigravida and primiparous women
This figure shows birthweight percentiles in the HIP study stratified by primigravida and multigravida pregnancies. Median percentiles were compared using the Kruskal-Wallis Test.
Abbreviation: HPA, human platelet antigen.

The pathological mechanism leading to reduced fetal growth in HPA-1a immunized pregnant women is not understood. In general, restricted fetal growth can be related to insufficient supply of oxygen and nutrients by the placenta.[57] The β3 integrin, which carries HPA-1a, is expressed together with integrin αIIb as the fibrinogen receptor with high expression levels on platelets. β3 integrin is also expressed together with integrin αV as the vitronectin receptor on trophoblast cells, fibroblasts and endothelial cells. Eksteen *et al.*[58] showed binding of the anti-HPA-1a antibody to the αVβ3 receptor on isolated trophoblasts and, that binding led to impaired functioning of the receptor with a reduced ability of the cultured trophoblast cells to migrate and adhere. In Figure 6 we visualize the binding of anti-HPA-1a to the syncytiotrophoblast, which represent a fetal-maternal interface. To our knowledge, we were the first to show the binding of anti-HPA-1a with immunohistochemistry in term placentas underlining that anti-HPA-1a can indeed bind to placenta tissue. Antibody binding

to fetal vessels was not found, which warrants further investigation. It is also still unknown from which gestational age HPA-1a is expressed on the placenta, with this method this could be examined in the future.

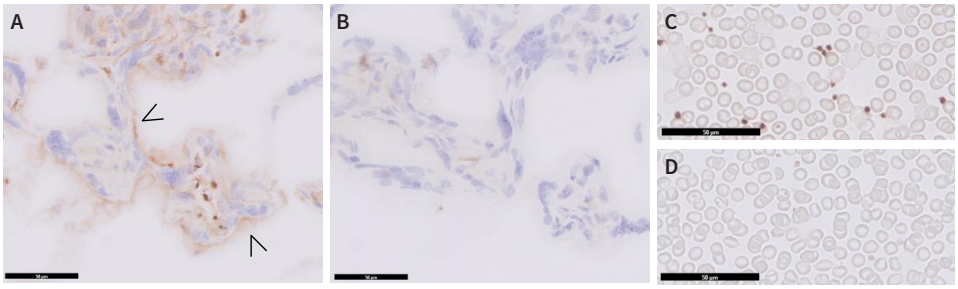


FIGURE 6. Binding of anti-HPA-1a to placenta and platelets

A. HPA-1a positive placenta to show binding of anti-HPA-1a (B2G1, MoAb, human IgG1) to the placenta, the syncytiotrophoblast (arrows) is positive. **B.** Placenta with negative control (anti-s (MNS:4), red blood cell specific antigen, polyclonal IgG antibody) that shows no binding at the syncytiotrophoblast. **C.** Full blood smear to show positive binding to platelets with anti-HPA-1a (B2G1, MoAb, human IgG1). **D.** Full blood smear with isotype control (anti-s (MNS:4), red blood cell specific antigen, polyclonal IgG antibody) to show specificity of anti-HPA-1a. [Unpublished data]

In **chapter 4** we report an explorative study on immunological damage in placentas of HPA-1a immunized pregnancies. We performed immunohistochemistry and histopathology of nine placentas from cases diagnosed with FNAIT after birth, 14 IVIg treated FNAIT cases and 20 controls. Complement deposition as C4d was observed at the syncytiotrophoblast in placentas in newly diagnosed HPA-1a immunized pregnancies and to a lesser extent in placentas of IVIg treated cases and controls. C4d is an accepted biomarker in antibody mediated transplant rejection and it is also acknowledged in antibody mediated pregnancy complications.[59, 60] The positive staining for C1q pointed in the direction of complement activation via the classical pathway. Histopathology of the FNAIT cases showed delayed placental maturation and low-grade. In contrast to other studies, we did not find cases with chronic histiocytic intervillositis [61], chronic villitis [62] or chronic intervillositis [63]. Nedberg *et al*.'s study[61] reported on CD8-positive lymphocytes that were observed around fetal endothelial cells possibly related to endothelial cell damage in FNAIT placentas. The observed heterogeneity of placental inflammation damage as observed in the histopathological studies calls for further investigation. Perhaps multicolor immunohistochemistry can be used for a greater understanding of the relationship between HPA-1a immunization and the presence and activation status of the wide variety of immune cells that are present in the placenta and may play a role in development of placental damage. On the other hand, it cannot be excluded that aberrant placental function with higher release of placental shedding increases the risk of alloimmunization making these observations more a cause of the alloimmunization and not a result.

In addition to a lower birthweight in children of HPA-1a immunized women, the HIP study (**chapter 3**) showed other clinical findings that could be related to placenta insufficiency. We found that HPA-1a immunization was associated with hypertensive disorder during pregnancy (11% in HPA-1a immunized pregnancies *vs* 4% in HPA-1a positive pregnant women) and premature delivery (15% in HPA-1a immunized pregnancies *vs* 5% in HPA-1a positive pregnant women). In a study using a murine model of FNAIT in which β3 integrin deficient mice were immunized with β3 integrin positive platelets it was shown that immunization leads to miscarriages and IUFD.[64] A limitation of this mouse model is that antibodies were directed against the β3 integrin instead of the HPA-1a epitope. As a result, the functional consequences of this broad immune response and its effects cannot be fully compared with HPA-1a immunization. In contrast to these findings in animal studies, the results of the HIP study did not show a relationship between HPA-1a immunization and miscarriages or fetal death in previous pregnancies. It would be interesting to perform HPA-1a antibody screening in a cohort of pregnancies complicated by recurrent miscarriages to assess the relationship between miscarriages and HPA-1a immunization. Additionally, it would be worthwhile to examine the histopathology and presence of complement activation in placentas in an alloantigen-specific animal model of FNAIT.[65]

In summary, the incidence of severe bleeding, which is associated with severe neurodevelopmental impairment, is 11 in 10,000 HPA-1a immunized pregnancies. Besides fetal bleeding and thrombocytopenia, signs of immunological damage in FNAIT placentas have been observed. Clinical observations in the HIP study confirm that HPA-1a immunization is associated with placenta related pathology.

KNOWLEDGE OF TEST

4. THERE SHOULD BE A SUITABLE TEST OR EXAMINATION. (W&J 5)

For population-based screening, it is important to identify only HPA-1a immunized pregnant women at high risk for severe adverse outcome in the most cost-effective and efficient way. These high-risk pregnancies have to be identified among the HPA-1a negative women (Figure 7).

Maternal HPA-1a typing

HPA-1a typing is the first step that already leads to focusing subsequent testing of only a small proportion of women, since it excludes 97.6% of the pregnant women because they are HPA-1a positive. Serological HPA-1a typing as designed for the HIP study (**chapter 2 and 3**) has the advantage that it is quick and suitable for testing large numbers of women at once.[66] However, with the emerging of new techniques to perform genotyping at large scale it is likely that molecular genotyping will gain broader application, perhaps also in antenatal screening programs.[67]

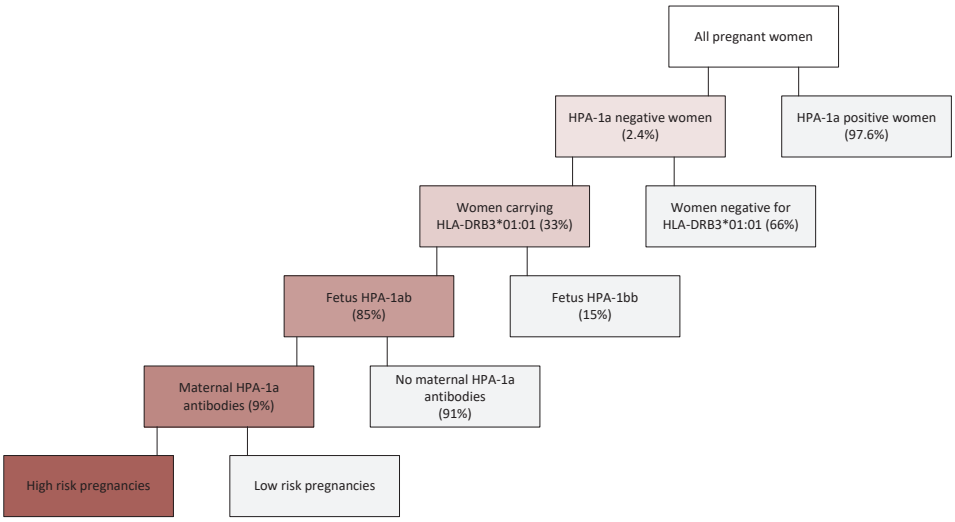


FIGURE 7. Flowchart selection of population at risk
Figure 7 shows a proposal in which women with a high-risk pregnancy are selected from the total pregnant population of pregnant women.
Abbreviations: HPA, human platelet antigen; HLA, human leukocyte antigen.

*Maternal HLA DRB3*01:01 carrier status*

One can argue that the next step in a screening program could be to assess maternal HLA-DRB3*01:01 carrier status. It is known that women positive for HLA DRB3*01:01, an HLA class II allele likely involved in HPA-1a antigen presentation,[68] have a 25 times higher risk of immunization[69] and likely also a higher risk of delivering a child with severe FNAIT.[70] By selecting women positive for this allele around 66% of the pregnant women can be excluded from further screening. Recently, new genotype arrays were validated to perform HLA typing in an efficient way at a large-scale making selection based on HLA DRB3*01:01 carrier status more feasible.[71] This array was also used for maternal HLA typing in the HIP study.

The association between maternal HLA DRB3*01:01 and HPA-1a immunization was first suggested by two retrospective studies[72, 73] and then confirmed in prospective screening studies. [29, 41] Presumably, antigen presentation via HLA DRB3*01:01 allele is very efficient due to the perfect fit of HPA-1a in the peptide binding cleft of the HLA class II molecule resulting in efficient T-cell and subsequent B-cell stimulation.[68, 74, 75] In HPA-1a negative women, that carry the HLA DRB3*01:01 allele the risk of immunization after delivery of an HPA-1a positive child is 12.7% (per pregnancy) compared to 0.5% in women lacking this HLA class II allele.[69] An important question is if excluding women that lack this HLA class II allele from antibody screening in a screening program would lead to missing pregnancies with high probability of adverse outcome. In a systematic review, prospective and retrospective studies were summarized that report on the clinical outcome of pregnancies with FNAIT and maternal

HLA DRB3*01:01 carrier status.[70] None of the 18 children born of HPA-1a immunized HLA-DRB3*01:01 negative women in the four prospective studies, were severely thrombocytopenic or suffered from ICH.[29, 41, 44, 54] Only one retrospective study reported on two neonates with ICH from HLA-DRB3*01:01 negative mothers, however no clinical characteristics of these cases were reported in this study.[76]

We found that the risk of immunization in HPA-1a negative/HLA DRB3*01:01 positive women carrying an HPA-1a positive child was 28.1% whereas the risk in HPA-1a negative/HLA DRB3*01:01 negative women carrying an HPA-1a positive child was 1.6% (**chapter 3**). These percentages were in line with pooled data[77] from previous screening studies[29, 44, 54] reporting percentages of HPA-1a immunization of 26.9% and 1.7% in HLA DRB3*01:01 positive and negative women, respectively. Interestingly the mother of the child that was diagnosed with severe fetal ICH was homozygous positive for HLA DRB3*01:01. This is in line with an earlier study that observed that there is a dose dependent impact of HLA DRB3*01:01, associated with the relative level of HPA-1a antibodies.[78]

Fetal HPA typing

Modern tests based on cell free fetal DNA isolated from maternal plasma such as the droplet digital PCR (ddPCR) make it possible to perform non-invasive fetal HPA typing.[79, 80] By performing this test after maternal HPA-1 typing, it is possible to reassure about 15% of the HPA-1a negative pregnant women that their current pregnancy is not at risk for FNAIT.

Antibody screening

The monoclonal antibody immobilization of platelet antigens (MAIPA) assay is still the golden standard for antibody screening in clinically suspected FNAIT cases. However, in a screening situation other antibody screening tests might be suitable. In our prospective screening study, we used the Luminex PAKLx assay.[66] This assay contains six beads with glycoprotein (GP) IIb/IIIa (either HPA-1a positive [3 beads aa, 1 bead ab] or negative [2 beads bb] isolated from platelets) and detection of anti-HPA-1a is based on the reaction pattern of these beads. Important advantage of this test is that it is easy to perform and only 10 µl of maternal plasma is needed. The disadvantage of this test is that reactions with the beads can show a weak anti-HPA-1a signal and the software algorithm does not pick up weak antibody signals, thus requiring additional manual assessment of the individual MFI values.[81, 82] At the same time it is questionable how clinically relevant the HPA-1a antibodies are that generate only weak signals. In the HIP study there were no cases with bleeding symptoms in the group with a low or borderline result. We conclude that it is possible to perform HPA-1a antibody detection on a large scale.

In conclusion, the results from the HIP study confirm previous studies that serological HPA typing, fetal HPA typing and antibody screening is feasible. Selection of HLA DRB3*01:01

positive women is recommended in a screening program, the risk of antibody formation leading to clinical severe disease is negligible in women lacking this allele.

5. THE TEST SHOULD BE ACCEPTABLE TO THE POPULATION. (W&J 6)

The attitude concerning prenatal screening for FNAIT was investigated by Winkelhorst *et al*.[83] making use of questionnaires. In total, 91% of the participants had a positive attitude towards screening. The willingness to participate in a screening program was 99%. Based on this study, we concluded that pregnant women had a positive attitude towards HPA antibody screening in pregnancy. At present, 99% of the pregnant women participate in the Dutch prenatal screening program for infectious diseases and erythrocyte immunization which is offered free-of-charge to all pregnant women in the Netherlands.[84]

In the HIP study, caregivers and patients were not asked about their experiences or maternal stress associated with HPA-1a status because test results were not reported to the caregivers or pregnant women. The Norwegian research group examined the experience of 40 immunized and 40 non-immunized women that participated in the nationwide screening study making use of questionnaires.[85] The response rates were 75%-85%. Between 71% and 80% of the pregnant women expressed that they were not appropriately informed. Half of the immunized women and 37% of the non-immunized women mentioned that they were probably more anxious than they would have been without screening during pregnancy. If in future studies maternal HPA status or antibody test will be reported to the pregnant women, it would be interesting to monitor their stress levels and anxiety related to the results of these tests in a prospective design. Another important subject for future studies is to assess the burden and side effects of maternal IVIg treatment. Serious maternal side effects are uncommon (~1%)[86] but other side effects as headache and fatigue are more commonly reported.[87]

KNOWLEDGE OF TREATMENT

6. AGREED POLICY ON WHOM TO TREAT AS PATIENTS. (W&J 8)

So far, no antenatal screening program has been introduced also because there is no consensus on the clinical management of HPA-1a immunized women identified through a screening program. Most pregnancies of women with HPA-1a alloantibodies are uneventful. Approximately a quarter of the HPA-1a immunized women will give birth to a severely thrombocytopenic child,[88] and within the group of severely thrombocytopenic children only ~10% of the children suffer from ICH or organ bleeding.[88] If all HPA-1a immunized women found through a screening program are antenatally treated this will lead to substantial overtreatment. Therefore, it is of great importance to design diagnostic assays that predict the risk of severe neonatal outcome. In the situation without screening, clinical management in subsequent pregnancies is mostly based on the outcome of the previous affected

pregnancy[89] guided by information on antibody levels in some centers.[90] In a screening situation, information about a non-treated previous pregnancy will be lacking and the decision to start treatment must be made based on other markers. With the collection of plasma samples of HPA-1a immunized pregnancies without severe neonatal outcome in the HIP study, we created a unique platform to test the predictive value of these markers in a screening setting, as these plasmas were not yet available.

Antibody quantitation

Prospective screening studies show that HPA-1a antibody levels in the mother correlates with lower platelet counts and a higher risk of bleeding in the child.[29, 46] In line with these results, Bertrand *et al*.[91, 92] showed that maternal anti-HPA-1a levels could be used to determine antenatal and perinatal policy.[91, 92] However, because in retrospective studies several cases were diagnosed with an ICH despite low maternal antibody levels, antibody quantitation is not generally acknowledged as a single marker to predict disease severity in FNAIT.[93, 94] It is likely that differences in the study design of these studies may explain these different findings. Cases within these retrospective studies are already diagnosed with FNAIT with more often high antibody levels due to the selection of symptomatic cases. Based on the results of screening studies[29, 41] including the HIP study (**chapter 3**), antibody quantitation seems to distinguish the ones who do develop severe thrombocytopenia from those who do not. An antibody threshold of 3.0 IU/mL at gestational age of 22 or 34 weeks was earlier suggested and had a diagnostic sensitivity and specificity of 93% and 63%, respectively for predicting severe neonatal thrombocytopenia (platelet count < 50×10^9/L).

During the HIP study, plasma samples of HPA-1a negative women with unknown consent were quarantined. When these women were diagnosed with HPA-antibodies due to neonatal bleeding/thrombocytopenia via the reference laboratory, antibody quantitation was performed in the antenatal sample. In total, 6 samples were available for antibody quantitation. Clinical data and antibody levels are shown in Table 3, all cases had antibody levels ≥ 2 IU/mL. Two out of three cases with ICH had antibody quantitation of 2 IU/mL, the third one had antibody level of 45 IU/mL. Bleeding disappeared spontaneously in one of these cases (case 2).

To determine an antibody threshold to predict for severe ICH, there is little data available. Anti-HPA-1a quantitation is technically challenging. Usage of international reference reagent for anti-HPA-1a is necessary to provide results which are comparable across laboratories.[95] Antibody quantitation was performed according to these methods in the Norwegian screening study and in the HIP study and we found that antibody levels 150-41 IU/mL in the Norwegian and 90 IU/mL in our case with severe ICH. These numbers would argue in favor of using a higher cut-off value in a pilot screening, (e.g., 10 IU/mL) if you only want to select the cases with severe ICH.

TABLE 3. Antibody quantification of cases with unknown consent in HIP study

#	G/P	GA at birth	Delivery mode	Sex	BW p	Signs of bleeding	Clinical course	Other risk factors for bleeding	HLA DRB3*01:01 alleles (mother)	Quant. (IU/mL)	Quant. (IU/mL) postpartum sample [timepoint sample days after delivery]
1	G2P1	40	Spontaneous vaginal delivery (at home)	M	p74	Petechiae and hematoma cUS: IVH grade II (PLT 10)	Admission NICU, PTx	-	1	45	49 [0]
2	G4P2	40	Spontaneous vaginal delivery	F	p35	Petechiae and hematoma cUS: possible geminal matrix bleeding grade I. cUS [7 days later]: no ICH (PLT 4)	Admission neonatology, PTx	-	1	2	19 [4]
3	G1P0	36	Emergency CS	M	p11	Petechiae cUS: no ICH (PLT 7)	Admission neonatology, PTx	Prematurity	1	71	66 [0]
4	G2P1	37	Spontaneous vaginal delivery after induction	M	p75	Petechiae and hematoma cUS: subcortical parenchymal bleeding in the left lobe, 12 mm. (PLT 7)	Admission neonatology, PTx	Anti-HPA-15a	1	2	18 [4]
5	G2P1	40	Spontaneous vaginal delivery	M	p48	Petechiae and hematoma cUS: no ICH (PLT 19)	Admission neonatology, PTx	-	1	11	29 [0]
6	G3P2	41	Spontaneous vaginal delivery	F	p38	Petechiae cUS: no ICH (PT 4)	Admission neonatology, PTx	-	1	17	77 [1]

This table shows the clinical characteristics and antibody levels in samples drawn at the 27th week of pregnancy of cases that were diagnosed with FNAIT postnatally via the national reference laboratory but not included in the HIP study because women did have initially unknown consent.

Abbreviations: G, gravidity; P, parity; GA, gestational age; BW, birthweight; p, percentile; IU/mL, international units/milliliter; M, male, cUS, cranial ultrasound; IVH, intraventricular hemorrhage; NICU, neonatal intensive care unit; PLT, platelet count in 10×10^9/L; PTx, platelet transfusion; F, female; CS, caesarean section; HPA, human platelet antigen

[Unpublished data]

Antibody Fc-glycosylation

For destruction of antibody-opsonized platelets, binding of the Fc-tail (effector part) of antibodies by the IgG-Fc receptors of phagocytes in the reticuloendothelial system is necessary. Different classes of IgG-Fc receptors exist. Macrophages of the spleen are thought to be important in phagocytosis of HPA-1a alloantibody opsonized platelets. Macrophages carry activating IgG Fc receptor class I, IIa and IIIa. Variation in composition of the N-linked glycan in the Fc domain of the antibody is important in the interaction with some IgG Fc receptors. It has been shown that a lower level of fucose in the sugar moiety leads to stronger FcγRIIIa binding.[96] In the context of FNAIT this is interesting because Fc fucosylation influences HPA-1a antibody opsonized phagocytosis. Sonneveld *et al.*[97] and Kapur *et al*.[98] showed a correlation between a decrease in, Fc fucosylation of isolated HPA-1a antibodies and severity of neonatal thrombocytopenia retrospective cohorts. In addition, it was shown that Fc-glycosylation profiles remained constant during pregnancy.[97]

Endothelial cell antibody binding

As mentioned previously the β3 integrin which carries HPA-1a is expressed not only by platelets but also by endothelial cells. It has been shown that anti-HPA-1a can reduce adhesion, spreading, and monolayer integrity of human umbilical vein endothelial cells (HUVECs).[10] This suggests that anti-HPA-1a can interfere in endothelial cell function in maintaining endothelial cell-layer integrity, perhaps especially in the situation of a growing fetal brain.[9] Whether antibody binding influences directly the function of the β3 integrin leading to functional impairment[99] or if antibody binding induces this is due to immunological cellular-mediated damage is currently unclear.[12] The idea that endothelial cell damage could be an important factor in the development of FNAIT-associated cerebral hemorrhage is supported by a study in which α2bβ3 (platelet fibrinogen receptor) directed antibodies were discriminated from αvβ3 (vitronectin receptor) directed antibodies.[11] Antibodies specifically reactive with αvβ3 were found and the presence was strongly associated with ICH.[11] Until now, this observation was not described in other cohorts. The above-mentioned study encompassed 18 cases with ICH and 18 cases without ICH.

HPA-1a antibodies with different β3, α2bβ3 or αvβ3 preferent binding may have different functional effects and antibody characteristics may be related to clinical outcome in the new-born. In the absence of screening, most maternal sera with anti-HPA-1a antibodies were identified because of clinical symptoms and in subsequent pregnancies mothers were treated to prevent ICH. The development of assays that can discriminate these antibodies with different type of specificity, preferent binding or functional effect is currently ongoing by several research groups (professor C.E. van der Schoot, dr. G. Vidarsson, drs. J. Oosterhoff and dr. C. Margadant). To see if HPA-1a antibody binding to endothelial cells was present we performed a pilot experiment. In this experiment maternal plasma was incubated with human umbilical vein endothelial cells (HUVECs, pool of 5 donors). Subsequently, we

assessed IgG binding in flowcytometry. In this experiment we included 25 samples from HPA-1a immunized women collected in the first year of the HIP study and 10 samples from HPA-1a immunized women who had children with ICH. Results of this experiment are shown in Figure 8. We observed that the degree of IgG binding was different between the three groups and that binding was in general higher in cases with severe bleeding, but also in the group without bleeding high binding was present. However, no adjustment has been made for antibody level and the presence of HLA class I antibodies. Future assays that will overcome these problems as HLA class I negative cell lines which express either α2bβ3 or αvβ3 are currently being developed (Oosterhoff *et al*. manuscript in preparation).

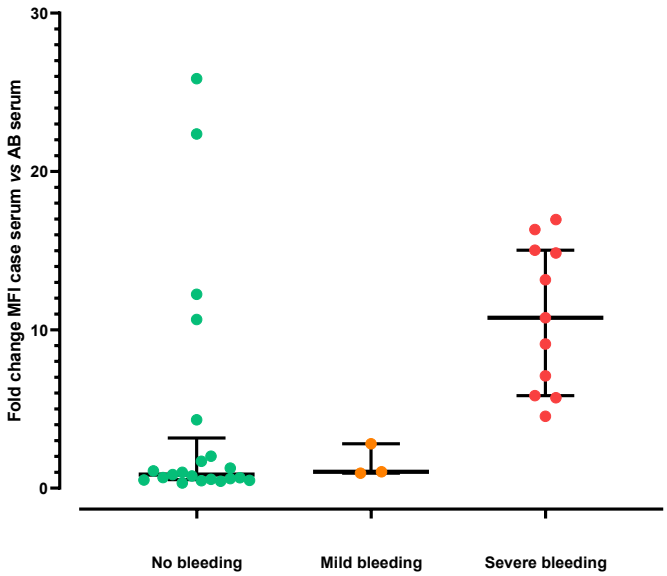


FIGURE 8. Endothelial reactivity and clinical outcome

This figure shows the IgG binding on human umbilical vein endothelial cells (HUVECs) as measured in flowcytometry. We calculated the ratio between HUVEC binding of samples versus healthy donor samples. In total samples of 25 HPA-1a immunized women (HIP study; 1 severe bleeding, 3 mild bleeding and 21 no bleeding) and 10 retrospectively collected samples (10 cases with severe bleeding) were included.
[Unpublished data]

In vivo prediction of fetal thrombocytopenia

At present, it is not possible to estimate fetal platelet count in a non-invasive way. Fetal platelet count can be determined by performing cordocentesis and fetal blood sampling. This procedure is however not recommended as it is associated with complications, most notably (especially in case of severe thrombocytopenia) bleeding and exsanguination from the puncture site, as well as other risks inherent to invasive procedures. It would be extremely valuable if fetal platelet count can be estimated by making use of imaging techniques similar

to Doppler blood flow velocity measurement for the prediction of fetal anemia.[100] Because the blood flow patterns in fetuses with thrombocytopenia are unaffected, other approaches will be necessary to estimate platelet count non-invasively. Perhaps if platelet-specific proteins can be measured with magnetic resonance spectroscopy,[101] this technique could be used to estimate the extent of thrombocytopenia. Little to no research has been done in this field, but the introduction of a pilot screening could make a study on this subject possible.

In the future, risk stratification will develop further, possibly involving endothelial cell assays or determination of Fc-glycosylation. The maternal plasma samples collected in the HIP study provided a unique collection of samples from mothers with HPA-1a immunization with and without neonatal bleeding symptoms. This collection allows evaluation of how HPA-1a antibody levels and Fc-tail glycosylation patterns correlate with different β3, α2bβ3 and αvβ3 preferential binding as well as studying their functional role in relation to clinical outcome in the new-born. It is complicated to determine a cut-off point for high and low risk pregnancies at the start of a screening. Although there is a risk of missing ICH with a restrictive policy in which only a few pregnancies are classified as high-risk, much can be said in favor of this. IVIg treatment is a burden for the pregnant women and expensive, and overtreatment is therefore undesirable.

HPA-5b antibodies and clinical FNAIT

The second most frequently involved antibody in FNAIT is anti-HPA-5b. Anti-HPA-5b is found in 1.96%[102] of unselected pregnancies and was detected in 1.7% (63/3605) of cases in the HIP study population. Given the high prevalence of anti-HPA-5b in pregnant women it is questioned whether these antibodies were causally associated with thrombocytopenia or merely a finding by chance in FNAIT suspected cases. In our retrospective cohort study (**chapter 5**), we detected anti-HPA-5b in 3.2% of the suspected FNAIT cases. In the HIP study, anti-HPA-5b was found in 1.7% of pregnancies meaning anti-HPA-5b was detected ~1.9 (3.2%/1.7%) times more often in FNAIT suspected cases. This suggest that anti-HPA-5b is associated with neonatal thrombocytopenia and bleeding. In a recent review,[102] the German research group reported that anti-HPA-5b was 1.73-fold higher prevalent in suspected FNAIT cases compared to unselected pregnancies. The authors suggested that the higher prevalence could be explained by (i) observer bias (interpretation of borderline results as positive), (ii) pregnancy related factors that caused neonatal thrombocytopenia or (iii) a higher number of pregnancies within the group of FNAIT suspected cases. The proposed explanations could be assessed by comparing the antibody levels and clinical characteristics between FNAIT suspected cases and unselected HPA-5b immunized pregnancies in the HIP study.

A second question is whether anti-HPA-5b actually causes serious bleeding. Although there is debate about whether severe thrombocytopenia can cause a cerebral hemorrhage, a study using a murine model show that severe thrombocytopenia is sufficient to cause

a cerebral hemorrhage.[103] It might be that there are children who are more vulnerable to developing brain hemorrhages. Coste *et al.*[104] recently published a cohort study in which genetic screening was performed in 194 fetuses diagnosed with ICH. Pathogenic variants of genes encoding for basement-membrane proteins were found in 19% of these cases, which underlines multicausality in the development of fetal ICH. This discussion indicates that it is urgently needed to have a better marker to predict pathogenicity of HPA-5b antibodies. Perhaps experimental studies like phagocytosis assays that assess whether anti-HPA-5b enhances phagocytosis of platelets *in vitro* may give more direction to the discussion whether anti-HPA-5b can indeed cause fetal/neonatal thrombocytopenia. However, not much is known on this subject and it will demand international collaboration to collect enough cases due to the rarity of severe ICH in HPA-5b associated FNAIT.

In conclusion, based on the current findings it cannot be excluded that anti-HPA-5b results in fetal thrombocytopenia in a minority of the cases. In very rare cases, this fetal thrombocytopenia could also contribute to the development of ICH. Given the high prevalence of antibodies and low incidence of adverse outcome in pregnancies with anti-HPA-5b, population-based screening for these antibodies is now strongly discouraged.

7. THERE SHOULD BE AN ACCEPTED TREATMENT FOR PATIENTS WITH RECOGNIZED DISEASE. (W&J 2)

Current antenatal treatment and outcome

Based on the successful treatment of pregnancies complicated by idiopathic thrombocytopenic purpura, IVIg administration during pregnancy was introduced as antenatal treatment for FNAIT in 1987.[105] The exact working mechanism of IVIg treatment during pregnancy is still not fully elucidated.[106] In pregnancies complicated by hemolytic disease of the fetus and neonate (HFDN) it was observed that it reduced the transport of IgG from the mother to the fetus by saturating the FcRn.[107] Another possible mechanism of action might be the direct binding of so called anti-idiotypic antibodies within the IVIg pool that bind and neutralize HPA antibodies. Nevertheless, antenatal IVIg treatment appears to prevent severe bleeding in 98.7% of pregnancies.[108] According to the Norwegian antenatal treatment guidelines, IVIg is offered only to women that delivered a child with ICH in a previous pregnancy. They argue that there is a lack of evidence to administer IVIg in all HPA-1a alloimmunized women.[90] At present, IVIg treatment is offered only to women in subsequent pregnancies after a previous child was diagnosed with FNAIT. Based on a cohort study including 71 untreated HPA-1a immunized pregnancies in a 20-year period they conclude that omitting antenatal IVIg treatment in low-risk pregnancies does not increase the risk of neonatal ICH.[18] Whether, IVIg treatment will be as effective in preventing bleeding complications in first affected pregnancies detected by screening has yet to be determined by future comparative trials.

Another issue here is what is the optimal time to start IVIg administration, which at present is based on expert opinion and timepoint of bleeding determined in retrospective cohort studies.

Because ICH can occur early in pregnancy[22] and IVIg is not working immediately after infusion, it is therefore proposed to start treatment in a screening setting around the 20th and 27th week.

Long-term outcome after treatment with intravenous immune globulins during pregnancy

Although IVIg administration to pregnant women to prevent severe bleeding is widely accepted, this treatment is currently still off-label. Severe maternal side effects of IVIg are rare, and include aseptic meningitis, pancytopenia[109] and hemolytic anemia.[110, 111] Milder side-effects such as headache and fatigue are common. It is unknown whether IVIg also affects fetal (neuro)development or the fetal immune system. In **chapter 8** we report the long-term neurodevelopmental outcome of children whose mothers were treated with IVIg during pregnancy for FNAIT. In total, 41 children were included for neurodevelopmental assessment at a median age of 9 years (see Figure 9). Mild-to-moderate neurodevelopmental impairment was observed in six (14%) children. Severe neurodevelopmental impairment was not detected. Cognitive scores, neurologic outcomes, behavioral scores and school results were not different from the Dutch norm groups. Two cases were diagnosed with severe ICH, for one of these (the most severe one) the bleeding had occurred one week before the start of IVIg treatment. Both cases had normal neurodevelopmental outcome. Based on this study we conclude that the neurodevelopmental outcome of children whose mothers were treated for FNAIT with antenatal IVIg is comparable to the general population.

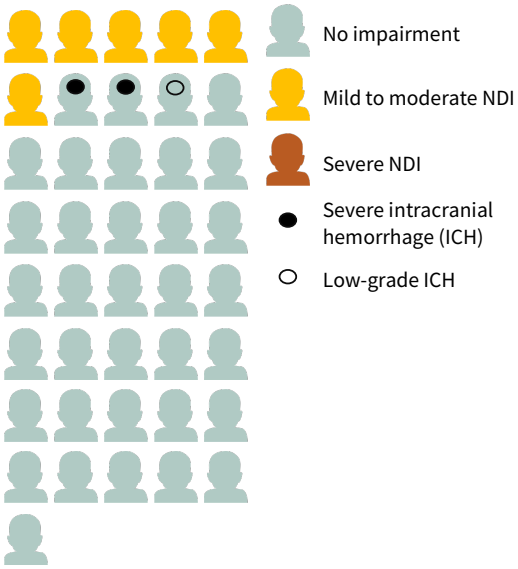


FIGURE 9. Neurodevelopmental impairment after antenatal IVIg treatment in FNAIT

This Figure shows the long-term neurodevelopmental outcome at school age from the individual cases of mothers who were treated with intravenous immune globulins (IVIg), during pregnancy.
Abbreviations: NDI, neurodevelopmental impairment; FNAIT, fetal neonatal alloimmune thrombocytopenia; ICH, intracranial hemorrhage.

Besides the neurodevelopmental outcome we assessed the presence of potential long-term immunological side effects in children whose mothers were treated with IVIg during pregnancy. Parents were asked to fill in questionnaires to determine the presence of allergies, eczema and asthma. Additionally, we assessed if there was an abnormal course or frequency of infections based on the 10 warning signs of primary immune deficiency[112] and a Dutch guideline for diagnostics in children with recurrent respiratory infections.[113] Outcomes of children whose mothers were treated with IVIg were compared with outcomes of children newly diagnosed with FNAIT. Results of the questionnaires filled in by the parents of these children are shown in Table 4. We acknowledge the limited sensitivity of parent-based questionnaires to assess the prevalence of eczema, asthma, allergies or abnormal history of infections and in addition our sample size was limited. Based on the results of the questionnaires, we found no difference in the proportion of children with eczema, asthma or allergies between the group of children antenatally treated with IVIg and children newly that were newly diagnosed with FNAIT. We found no higher percentage of children with an abnormal history of infectious diseases in the group of children whose mothers were treated with IVIg. In conclusion, we did not find evidence for long-term immunological side effects in children whose mothers were treated with IVIg during pregnancy.

TABLE 4. Immunological outcome of children antenatally treated with IVIg and children newly diagnosed with FNAIT.

	Children of HPA immunized mothers treated with IVIg during pregnancy n = 41	**Children newly diagnosed with FNAIT n = 42**
Male sex, n (%)	20 (49)	33 (79)
Age at follow-up, years and months, median (IQR)	9y8m (7y6m – 11y8m)	12y0m (9y6m – 14y11m)
Maternal parity (during pregnancy), median (IQR)	1 (1 – 2)	0 (0 – 1)
Parent reported eczema, n/N (%)	12/41 (29)	9/40 (23)
Parent reported asthma, n/N (%)		
No	36/41 (88)	31/40 (78)
History of asthma	3/41 (7)	5/40 (13)
Yes, medication on indication	1/41 (2)	3/40 (8)
Yes, daily medication	1/41 (2)	1/40 (3)
Parent reported allergy, n/N (%)	11/41 (27)	12/40 (30)
Abnormal history of infectious diseases, n/N (%)	2/41 (5)	4/40 (10)

† Two cases were excluded because their mothers were treated with IVIg after antenatal HPA-antibody detection upon the detection of intracranial hemorrhages at antenatal ultrasound.
Abbreviations: HPA, human platelet antigen; IVIg, intravenous immune globulin; FNAIT, fetal neonatal alloimmune thrombocytopenia; IQR, interquartile range, y, years; m, months;
[Unpublished data]

10

Future perspectives of antenatal treatment

Although IVIg treatment has been shown to vastly reduce the risk of bleeding in subsequent pregnancies with FNAIT, there are several disadvantages of this treatment. First, IVIg is costly

and second the production of this human blood product depends upon considerable donor commitments. It was estimated that standard dosage for IVIg treatment is equivalent to 1.4 kg of IgG (calculated based on 20 IVIg dosages of 1.0 gram/kg for a mother with body weight of 70 kg).[18] To produce this amount of medication, 310 L of donor-plasma is required which is equal to 500 plasma donations (calculated based on a yield of 620 mL per donation).[18]

Neonatal Fc receptor inhibitors (FcRn inhibitor)

One of the promising treatment modalities that could be applied in HPA-immunized pregnancies are neonatal Fc receptor (FcRn) inhibitors.[114] In physiological conditions IgG is taken up at the fetal maternal interface, the syncytiotrophoblast, by the random process of pinocytosis. In acidified intracellular components FcRn can bind to IgG and rescues it from destruction. Moreover, it subsequently shuttles the IgG to the basal cell side where the complex is moved to the cell surface. Because this eliminates the acidified environment, IgG is released into the fetal circulation. By blockage of the FcRn receptor one could envision that the passage of anti-HPA-1a from the mother to the fetus is reduced. In addition, FcRn expressed at the surface of endothelial cells allows recycling of the IgG complex in human serum resulting in maintenance of the half-life of IgG.[115] FcRn receptor blocking results in a significant lowering (around 20% of baseline) of the maternal IgG, thus also lowering the HPA-antibody concentration.[116]

FcRn inhibitors were designed for treatment of several IgG mediated diseases including ITP[117] and myasthenia gravis.[118] Possibly when these FcRn inhibitors are applied in alloimmunized pregnancies it would prevent the transport of IgG, including anti-HPA in FNAIT, across the placenta. Little is known about the fetal side effects of this drug. One of the potential drawbacks of FcRn inhibitors is hypogammaglobulinemia in neonates and mothers, resulting in an increased risk of perinatal infections.[119] To overcome this issue it is suggested to stop FcRn inhibition two weeks prior to delivery. Additionally, the FcRn receptor is important in maintaining albumin homeostasis and it is unknown whether this has also affected fetal development.[120] A first in-human trial in red cell alloimmunized pregnancies nears completion of inclusion (15 cases) (ClinicalTrials.gov Identifier: NCT03842189). When this (primarily safety-) study would prove to be successful, expanding its use to FNAIT seems a logical step.

Prophylaxis

Although current treatments appear effective in preventing fetal and neonatal bleeding, and future treatment strategies are on the rise, it would be better to prevent the occurrence of HPA-immunization instead of trying to minimize the consequences of HPA-immunization. In HDFN, prophylaxis using anti-D immunoglobulin was introduced 50 years ago and has been very effective in preventing immunization and thereby severe disease.[121] In analogy to anti-D prophylaxis, it is suggested to prevent HPA-1a immunization using hyperimmune anti-HPA-1a.[122] It is thought that anti-HPA-1a will opsonize the HPA-1a positive fetal platelets

in the maternal circulation after fetal-maternal hemorrhage before HPA-1a immunization could occur. However, the expression of the β3 integrin which carries the HPA-1a epitope by the placenta raises some concerns that administration of anti-HPA-1a could damage the placenta. Initially, you may only administer prophylaxis postpartum to assess the effect without adverse effects on the placenta. However, because clinically relevant immunization can already occur during a first pregnancy (as shown by the HIP study), the postpartum administration of prophylaxis may be too late to effectively reduce clinically significant FNAIT in subsequent neonates.

Postnatal treatment

Neonates with thrombocytopenia are thought to be at risk for severe bleeding and are consequently treated with platelet transfusions. However, a recent large, randomized trial in preterm neonates showed that a more liberal transfusion policy (using a lower transfusion threshold resulting in administration of more platelet transfusions) was associated with an increased risk of bleeding and death. Prophylactic platelet transfusions in thrombocytopenic preterm neonates may therefore, contra-intuitively, increase the risk of bleeding. Whether platelet transfusions in FNAIT could also have deleterious effects is unknown. A recent systematic review on postnatal treatment in FNAIT concluded that evidence on neonatal management is lacking.[123] In **chapter 6** we address the current postnatal treatment of cases affected by FNAIT. In total, 389 neonates were included from 7 countries. We observed a great variety in postnatal treatment strategies applied in children affected by FNAIT. Platelet counts were increasing within the first week of life in virtually all neonates despite the type of treatment. Because the timing of development of severe bleeding (ICH or organ bleeding) could not be established in the majority of the cases we could not assess the association between severe bleeding and type of treatment. 53% of the neonates received postnatal platelet transfusion of which 43% received random-donor platelet transfusions, 40% HPA-matched platelet transfusions and 17% both. We found that platelet count increment after the first HPA-matched transfusion was higher compared to the random-donor platelet transfusion. Whether the administration of HPA-matched platelets is superior in the prevention of bleeding compared to random-platelets remains undocumented.

The function of platelets in hemostasis is to react to bleeding by initiating plug formation. Recently, a murine model studied the relationship between bleeding and fetal thrombocytopenia by using platelet directed antibodies to induce thrombocytopenia during fetal life.[103] This animal study showed that severe thrombocytopenia is sufficient to cause ICH. In a follow-up study using the same model, this group demonstrated that the susceptibility to ICH was lost after the first week after delivery.[124] Whether this is also the case in humans is not known.

In summary, appropriate treatment currently appears to be available for the prevention of severe neonatal ICH. In addition, there are promising developments in the field of FcRn inhibitors and prophylaxis that may allow us to move away from intensive and expensive IVIg treatment in the future.

8. FACILITIES FOR DIAGNOSIS AND TREATMENT SHOULD BE AVAILABLE. (W&J 3)

A successful screening for red cell antibodies is currently implemented in the Netherlands which effectively prevents the occurrence of severe HDFN. The logistic structure on which a platelet antibody screening can be built is already in place.

COST CONSIDERATIONS

9. COSTS OF CASE FINDING (INCLUDING DIAGNOSIS AND TREATMENT OF PATIENTS DIAGNOSED) ECONOMICALLY BALANCED IN RELATION TO POSSIBLE EXPENDITURE ON MEDICAL CARE AS A WHOLE. (W&J 9)

Earlier studies on the cost-effectiveness of screening for FNAIT concluded that screening would be cost-effective or even cost-saving compared to a situation without screening.[44, 53, 125, 126] However, these studies had methodological limitations and screening strategies were different from the screening strategy as envisaged in the Netherlands. In one study, a hypothetical model was used in which prophylaxis was used as intervention to prevent the occurrence of FNAIT, but prophylaxis was hypothetical and is currently not (yet) available.[125] Two other studies were performed without the inclusion of (costs for) antenatal treatment.[44, 53] Killie *et al.*[126] performed a cost-effectiveness analysis on antenatal screening based on the results of the large prospective screening study from Norway. However, the beneficial effects of their screening were based on the assumption that all cases with ICH will be prevented with near-term cesarean section with HPA-matched platelets availably directly after birth. The question is to what extent these measures prevent ICH because more than half of ICH occur already before or around the 28th week of pregnancy.[22] Therefore in our opinion, in pregnancies at high-risk of severe outcome, antenatal IVIg should be offered. In **chapter 9** we describe a cost-effectiveness analysis applicable to the Dutch situation based on the numbers from the HIP study (**chapter 3**) and current situation without screening (**chapter 5**). We included the results of neurodevelopmental outcome of children whose HPA-immunized mothers were treated with IVIg during pregnancy (**chapter 8**). We calculated that, compared to the situation without screening, an increment of 226 QALYs is expected by implementing one of the three treatment strategies in the Dutch population of 171,713 pregnant women. The incremental cost-effectiveness ratio (ICER) was €20,782 per QALY compared to a situation without screening.

This study highlights important points for future research. First point is that it would be useful to perform a cost-effectiveness analysis to compare screening strategies with low-frequency antibody screening in a small subpopulation (e.g., HPA-1a negative and HLA DRB3*01:01 positive women with a first ongoing pregnancy, since those seem to have the highest risk on severe FNAIT) to strategies with high-frequency antibody screening in all pregnant women. Moreover, it could be included in the calculations to reduce diagnostic costs because women can be reliable typed once for HPA-1 and HLA DRB3*01:01 without necessity to repeat those tests in each pregnancy. Our cost effectiveness analysis study underlines that a diagnostic assay to distinguish HPA-1a-immunized women with high versus low risk of severe neonatal outcome is very important. In our strategy we used antibody quantitation as a diagnostic tool. New assays, as discussed previously, are being developed to improve high-risk pregnancy selection. Lastly, we assumed that antenatal treatment prevents severe bleeding in all pregnant women. However, no randomized studies have been performed assessing the effect of IVIg treatment in first immunized pregnancies.

In conclusion, acknowledging the limitations of this cost-utility analysis, we think that HPA-1a screening in pregnancy has the potential to be cost-effective.

10. CASE-FINDING SHOULD BE A CONTINUING PROCESS AND NOT A ONCE AND FOR ALL PROJECT. (W&J 10)

The evaluation of the Wilson and Junger principles shows that there are still two important points for attention in designing a screening program for FNAIT. First, it is unknown whether IVIg treatment also prevents bleeding in first HPA immunized pregnancies, which needs to be confirmed in a comparative trial. Second, risk stratification, can now only be based HPA antibody levels. New diagnostic assays should be developed narrowing of the group to be treated. Data on both subjects can be obtained if screening is started, for example in a study context. Effectiveness of antenatal IVIg treatment or the predictive value of new diagnostic tests can only be confirmed if outcomes can be compared with a control group. This group can be created by randomizing to give IVIg or not, or screening based on a geographical location or over a certain time period. While it is often argued that it is unethical to withhold IVIg treatment from HPA-1a immunized women, it is however also incorrect to not properly evaluate a screening program and thereby cause considerable overtreatment. By screening in a study design, evaluation also takes place and an enormous amount of knowledge can be gathered to ultimately decide on a definitive implementation.

CONCLUSION

The aim of this thesis was to provide knowledge on the natural history of FNAIT and scenarios of selection of high-risk pregnancies. The incidence of severe bleeding in FNAIT is 1 in 900 HPA-1a negative pregnancies with a high risk of severe neurodevelopmental impairment. A screening program can start with the use of HPA-1a antibody quantitation for risk stratification, which may be replaced in the future by tests with higher positive predictive value. The RhD screening program introduced in the 1960's to prevent hemolytic disease of the fetus and neonate may serve as an example. Also in this, currently regarded as very successful screening program, the decades after the introduction was continuously improved with new diagnostic assays to better identify pregnancies at risk for severe fetal outcome and to optimize treatment options.

International and national data is now available according to all Wilson & Jungner principles, therefore a nationwide screening program to identify pregnancies at risk for HPA-1a mediated FNAIT seems warranted. The benefits of a screening program will have to become apparent in practice after implementation.

The proof of the pudding is in the eating.

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SUMMARY

OVERVIEW AND AIM OF THIS THESIS

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most frequent cause of thrombocytopenia in otherwise healthy term-born neonates. Clinical presentation of FNAIT varies from asymptomatic thrombocytopenia to skin bleeding and, severe organ bleeding. The most feared complication is intracranial hemorrhage (ICH), often leading to irreversible brain damage or death. FNAIT results from fetal maternal incompatibility of the human platelet antigens (HPA). Maternal exposure to foreign, paternally inherited, fetal HPA can lead to the formation of alloantibodies of IgG type. These HPA alloantibodies are transported across the placenta and bind to fetal platelets resulting in platelet destruction by phagocytes. Some HPAs are also expressed by other type of cells than platelets such as endothelial cells and the syncytiotrophoblast cell layer of the placenta. Therefore, some HPA alloantibodies may also induce damage to endothelial cells and placental cells. Possibly the combination of fetal/neonatal thrombocytopenia and HPA alloantibody interference with endothelial cells leads to an increased bleeding risk during fetal development and in the first days after birth. In the white population, alloantibodies directed against HPA-1a are diagnosed in 78% of the FNAIT cases and in 9% of the FNAIT cases alloantibodies directed against HPA-5b are detected. At present, most children affected by FNAIT are diagnosed after birth when (skin) bleeding is observed or thrombocytopenia is detected as a finding by chance. In these children, postnatal platelet transfusions are often administered to reduce the risk of bleeding. However, because the risk of ICH is greatest during fetal development, it is preferable to start treatment already during pregnancy. If pregnancies at risk for FNAIT are identified upon antenatal screening, timely intervention could prevent the occurrence of fetal ICH.

Implementation of population-based screening to prevent FNAIT needs to be a carefully weighted. In 1968, Wilson and Jungner (W&J) published ten principles to guide the discussion on the introduction of population-based screening (Figure 1). Implementation of population based HPA-1a antibody screening is hampered by the lack of knowledge on six principles: W&J 1 important health problem, W&J 2 accepted treatment, W&J 5 suitable test W&J 7 natural history, W&J 8 whom to treat and W&J 9 costs of case finding.

Principles of screening on early disease detection by Wilson and Jungner (1968)

1. The condition should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic phase.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including a diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuous process and not a “once and for all” project.

FIGURE 1. Wilson and Jungner Criteria, World Health Organization, 1968.

THE NATURAL HISTORY OF ANTI-HPA-1a MEDIATED FNAIT

In **chapter 2** we described the study design of the HIP (HPA-screening in pregnancy) study aimed to obtain missing knowledge on the incidence and natural history of FNAIT and to identify pregnancies at high risk of severe outcome. Rh (Rhesus) D-negative or Rhc-negative pregnant women that participate in the nationwide prenatal screening program for erythrocyte immunization at the 27th week of pregnancy were eligible. We performed serological HPA-1a typing on platelets after regular testing and stored all left-over material for subsequent tests to be performed after due date. We included HPA-1a negative women as well as a control group of HPA-1a positive women (ratio 1:3). Pregnant women and caregivers remained blinded for the HPA-1a status and other test results. Researchers requested the caregivers to enter clinical data of mothers and the neonates in an electronic database. After clinical data collection and laboratory assessments, the clinical data were linked to the HPA-1a screening results. Pregnancy and neonatal outcomes of pregnancies from HPA-1a negative immunized women were outcomes of pregnancies of HPA-1a positive women. With this study design, the HIP study provides a unique possibility to assess the incidence of clinical detectable FNAIT and risk factors for HPA immunization or severe bleeding complications without interference of fetal medicine.

The results of the HIP study are described in **chapter 3**. In total, 2.43% (3,722/153,106) of the pregnant women were HPA-1a negative. Anti-HPA-1a was detected in 9.3% of the HPA-1a negative women (85/913 pregnancies, 32 women were included twice). Clinical information was collected of 81 HPA-1a immunised and incompatible pregnancies, 820 HPA-1a negative non-immunised pregnancies and 2704 HPA-1a positive pregnancies. In one (1.2%, 1/81) immunised pregnancy severe ICH was detected at antenatal ultrasound at 29 weeks of gestational age. Magnetic resonance imaging showed extensive damage to the fetal brain with large cysts and after multidisciplinary meeting, late termination of pregnancy was performed at 34 weeks of gestation. Three neonates (3.7%, 3/81) were diagnosed with mild bleeding after birth (two with hematomas and one with mucosal bleeding). In addition, we observed that neonates from HPA-immunized pregnancies were more often born premature (< 37 weeks) (15%, 12/81) compared to neonates of HPA-1a positive women (5%, 132/2749). Mean birthweight in neonates form immunized women was lower (3271 ±631) compared with neonates of HPA-1a positive women (3459 ±545). The proportion of primigravida was highly comparable between immunized, non-immunized and HPA-1a positive pregnancies (32%, 37% and 34% respectively). It was previously argued that the incidence of major bleeding might have been underestimated in other screening studies because of the interventions. However our data strongly suggests that this was not the case, as the proportion with major bleeding found in our study (1.2%) is lower but in line with combined figures from previous studies. We conclude that the incidence of major bleeding in FNAIT is 1 in 913 HPA-1a negative pregnancies, which translates into 11 in 10,000 HPA-1a negative pregnancies.

Besides the classical features of FNAIT such as bleeding complications and thrombocytopenia, it is hypothesized that HPA-1a antibodies can also bind to the placenta, causing placental damage. In **chapter 4** we describe a study that explored signs of placenta damage in FNAIT. We included 23 placentas of which 9 (14 samples) from newly diagnosed FNAIT cases and 14 (21 samples) from FNAIT cases after treatment with intravenous immune globulin (IVIg). As controls, 20 uncomplicated cases were included. Immunohistochemistry was performed, we stained for complement activation markers (C1q, SC5b-9 and mannose-binding lectin). Two blinded observers scored the presence of complement activation markers. Two experienced placenta pathologists scored the histopathology according to the Amsterdam criteria. A higher degree of C4d deposition was present at the syncytiotrophoblast of the newly diagnosed FNAIT cases (10/14 samples) compared to the IVIg treated FNAIT cases (2/21 samples) and healthy controls. Four (44%) placentas of the newly diagnosed FNAIT cases and five (36%) placentas of the IVIg-treated FNAIT cases showed delayed maturation compared to one in the controls. Both C4d deposition and low-grade villitis of unknown etiology was observed in three newly diagnosed FNAIT cases that were born with a birthweight below 10th percentile. In conclusion, we observed a higher rate of classical route complement activation in pregnancies of placentas complicated by HPA immunization that were not antenatally treated. This may impair placental development and fits the observation that HPA-1a immunisation is associated with a reduced birthweight.

CLINICAL RELEVANCE OF HPA-5b ANTIBODIES

Chapter 5 describes a retrospective cohort study aimed to describe the clinical outcome of HPA-1a and HPA-5b associated FNAIT cases. Due to the high prevalence of anti-HPA-5b in pregnant women (1.8%), the detection of anti-HPA-5b in FNAIT suspected cases may in some cases be an incidental finding. In total 1,864 cases were suspected for FNAIT and sent in for platelet antibody detection. In 161 (8.6%) cases, anti-HPA-1a was detected and in 60 (3.2%) anti-HPA-5b. Anti-HPA-5b was detected 1.8 (3.2%/1.8%) times more often in FNAIT suspected cases compared with unselected pregnant women. The percentage of cases affected by severe bleeding if anti-HPA-1a was present (11%, 4/126) was similar to the percentage of severe bleeding in cases with anti-HPA-5b (10%, 4/40). Based on the allele frequencies, we calculated the expected percentage of HPA incompatibility if HPA-1a or HPA-5b was not associated with bleeding: 86% and 52% were expected, respectively. All multigravida women (81/81) with anti-HPA-1a were HPA-1a incompatible with the neonate. In the HPA-5b group, 79% (38/48) of the multigravida women with anti-HPA-5b had an HPA-5b positive child, which is higher than the percentage expected by chance of 52%. Based on the higher proportion of HPA-5b antibodies in the FNAIT suspected population and the higher proportion of HPA-5b incompatibility, we could not exclude that anti-HPA-5b is associated with severe neonatal bleeding. Anti-HPA-5b-mediated FNAIT shows often a less severe clinical course with only moderate thrombocytopenia. However, based on our data, we conclude that anti-HPA-5b is not a coincidental finding in FNAIT suspected cases.

NEONATAL MANAGEMENT

Postnatal treatment aims to reduce the risk of bleeding in neonates affected by FNAIT. Treatment is based on limited evidence and it is currently unknown what the standard of care is in the international perspective. **Chapter 6** describes an international multicenter study on the postnatal management and outcome of liveborn FNAIT cases between 2010 and 2020. In total, 389 liveborn neonates were included from Australia (n = 74), Norway (n = 56), Slovenia (n = 19), Spain (n = 55), Sweden (n = 31), The Netherlands (n = 138), and United States (n = 16). A quarter of the neonates (24%, 92/380) was diagnosed with extreme thrombocytopenia (platelet count < 10×10^9/L). Severe ICH was diagnosed in 6% (22/389) of the FNAIT cases. Platelet transfusions were administered to 53% (207/389) of neonates, either as random donor platelets (43%, 88/207), human platelet antigen (HPA) matched platelets (41%, 85/207), or both (17%, 35/207). Median platelet increment after random and matched platelet transfusions was 59×10^9/L (IQR 35 - 94) and 98×10^9/L (IQR 67 – 134), respectively ($P < 0.0001$). Our data suggest that HPA-matched transfusions lead to higher platelet count increment. However, whether this is also associated with a reduced risk of bleeding remains unknown. The use of HPA-matched transfusions differed between centres from not being used at all to being first choice and used in 62% of cases. Additional postnatal IVIg treatment was given in 29% (110/389) of cases, varying between centres from 12% to 63%. We conclude that the postnatal management varied greatly between the centres highlighting the need of comparative trails.

LONG-TERM OUTCOME

Knowledge on the long-term outcome of children is crucial to provide adequate follow-up care for children affected by FNAIT and to judge the potential benefits of the introduction of an FNAIT screening program. In **chapter 7** we evaluated the neurodevelopmental outcome at school age in children newly diagnosed with FNAIT. Children were invited for cognitive and neurological testing. Behavioral questionnaires and school performance results were obtained. A composite outcome of neurodevelopmental impairment (NDI) was used, subdivided into mild-to-moderate and severe NDI. In total, 44 children were included at a median age of 12 years. Severe ICH was detected in 14% (5/36). Severe NDI was detected in 7% (3/44); in two children with severe ICH and one with low-grade ICH and perinatal asphyxia. Mild-to-moderate NDI was detected in 25% (11/44); in one child with ICH; eight without ICH and in two children neuroimaging was not performed. Adverse outcome (perinatal death or severe NDI) was 16% (8/46). Four children (9%) attended special needs education, of which three with severe NDI and one with mild-to-moderate NDI. Behavioral problem scores were comparable to Dutch norm scores. Based on the results of this study, we concluded that children who are newly diagnosed with FNAIT are at increased risk for long-term neurodevelopmental problems, even those without ICH.

In FNAIT, administration of IVIg to the mother during pregnancy is widely accepted for preventing the occurrence of antenatal or perinatal ICH in the child. However, knowledge about the long-term neurodevelopmental outcome of these children is lacking. The study in **chapter 8** evaluated the long-term neurodevelopmental outcome in children with FNAIT who were treated with IVIg antenatally, using the same methodology as in chapter 7. In total, 82% (41/50) of the eligible cases were included for neurodevelopmental assessment at a median age of 9 years. Severe NDI was not detected. The incidence of mild to moderate NDI was 14% (6/41, 95% confidence interval: 6%–29%). Severe ICH was diagnosed in two cases (5%), one antenatally before the start of IVIg and the other case 1 day after birth. Both cases had a normal neurodevelopmental outcome. The results in this chapter show that the long-term outcome of children whose mothers were treated for FNAIT with antenatal IVIg is comparable to that in the general population.

COST-EFFECTIVENESS

It is postulated that by screening for HPA-1a directed antibodies during pregnancy, timely intervention with antenatal treatment can prevent the occurrence of severe ICH. As the incidence of severe ICH due to FNAIT is low, assessing the cost-effectiveness of adding screening for anti-HPA-1a to the prenatal screening program is relevant for decision making. In **chapter 9**, lifetime costs and effects of antenatal platelet antibody screening were compared to the situation without screening in the Netherlands by developing a decision analysis model. Model parameters were based on literature and expert opinions. The results show that adding of screening for HPA-1a to the current antenatal screening program of the Netherlands will lead to additional costs of 4.7 million euro per year, and a gain of 226 QALY (Quality-adjusted life years) per year. The incremental cost-effectiveness ratio was €20,782 per QALY gained. Based on this model, we conclude that antenatal screening for anti-HPA-1a might be cost effective.

CONCLUSION

In **chapter 10** we consider population-based screening for platelet antibodies by evaluation of the knowledge gained in this thesis and the available literature, according to the principles of Wilson and Junger. Based on this evaluation, we conclude that knowledge is available to all principles and a nationwide screening for anti-HPA-1a during pregnancy seems warranted. FNAIT leads to severe bleeding in 11 in 10,000 HPA-1a negative pregnancies with a high risk of neurodevelopmental impairment. At this time, there are two issues on which additional evidence would be needed for final implementation of screening for HPA antibodies. First, the risk of severe disease can only be estimated with antibody quantification. New diagnostic tests should be developed in the future to narrow the group to be treated. Second, the effectiveness of IVIg in first immunized pregnancies must be proven. To gather knowledge on both topics, a pilot implementation of screening for HPA antibodies is needed. HPA antibody screening will have to prove itself in practice.

The proof of the pudding is in the eating.

NEDERLANDSE SAMENVATTING

OVERZICHT EN DOEL VAN DIT PROEFSCHRIFT

Foetale en neonatale alloimmuun trombocytopenie (FNAIT) is de meest voorkomende oorzaak van trombocytopenie bij aterm pasgeborenen. De klinische presentatie varieert van een asymptomatische trombocytopenie tot ernstige orgaanbloedingen. De meest gevreesde complicatie bij FNAIT is een hersenbloeding, omdat deze vaak leidt tot ernstige hersenschade en daarmee ontwikkelingsproblemen of perinatale sterfte. FNAIT ontstaat als er een verschil bestaat tussen het humaan bloedplaatjes antigeen (HPA) van moeder en kind en het immuunsysteem van de moeder dit verschil heeft herkend. Dit kan leiden tot een immuunrespons met vorming van antistoffen gericht tegen het HPA. Tijdens de zwangerschap worden IgG-antistoffen actief van de moeder naar het kind getransporteerd. Zo komen ook antistoffen gericht tegen de HPA op de bloedplaatjes van de ongeboren baby in de bloedsomloop van de baby terecht. Deze antistoffen binden vervolgens aan de bloedplaatjes van de ongeboren baby, waardoor deze worden afgebroken. Dan ontstaat er een tekort, trombocytopenie genoemd. Omdat sommige HPA's ook voorkomen op andere soorten cellen dan bloedplaatjes, zoals endotheelcellen en cellen van de placenta, kunnen HPA-antistoffen mogelijk ook leiden tot schade aan het endotheel (vaatwand) en de placenta. Wellicht leidt de combinatie van foetale/neonatale trombocytopenie en interactie van HPA-antistoffen met endotheelcellen tot een verhoogd bloedingsrisico bij de foetus en pasgeborene.

In de witte bevolking wordt in 78% van de gevallen FNAIT veroorzaakt door antistoffen gericht tegen HPA-1a en in 9% door antistoffen gericht tegen HPA-5b. Momenteel wordt bij de meeste kinderen met FNAIT deze diagnose pas na de geboorte gesteld, wanneer (huid)bloedingen worden waargenomen of trombocytopenie als toevalsbevinding wordt ontdekt. Bij deze kinderen worden vaak bloedplaatjestransfusies toegediend om het risico op bloedingen te verminderen. Het risico op het ontwikkelen van een hersenbloeding het grootste is tijdens de zwangerschap, daarom verdient het de voorkeur om al tijdens de zwangerschap een behandeling te starten. In Nederland krijgen HPA-geïmmuniseerde vrouwen die eerder een kind met FNAIT kregen, in de vervolgzwangerschap een behandeling met intraveneuze immunoglobulines (IVIg). Als met een prenatale screening zwangerschappen met een risico op FNAIT zouden worden geïdentificeerd, kan tijdig een antenatale behandeling worden gestart, wat het risico op een hersenbloeding vermindert.

De invoering van screening op bevolkingsniveau, zoals een prenatale screening, ter voorkoming van bloedingen bij FNAIT moet zorgvuldig worden afgewogen. In 1968 publiceerden Wilson en Jungner (W&J) tien criteria als leidraad voor de invoering van screening op bevolkingsniveau (Figuur 1). Wanneer screening op HPA antistoffen tijdens de zwangerschap wordt afgewogen op basis van deze tien criteria is voor vier criteria voldoende kennis aanwezig. Echter voordat kan worden besloten over de invoer van een

screening is meer gedegen onderzoek nodig op het gebied van zes criteria: belangrijk gezondheidsprobleem (W&J punt 1 in figuur 1), geaccepteerde behandeling (W&J punt 2), geschikte test (W&J punt 5), natuurlijk beloop (W&J punt 7), wie te behandelen (W&J punt 8) en de kosten-batenanalyse (W&J punt 9).

Het doel van het onderzoek beschreven in dit proefschrift is het verzamelen van kennis op het gebied van deze zes criteria, om zo een beslissing te kunnen maken over de invoer van een screening op HPA-1a-antistoffen tijdens de zwangerschap.

1. De op te sporen ziekte moet een belangrijk gezondheidsprobleem zijn.
2. Er moet een algemeen aanvaarde behandelingsmethode voor de ziekte zijn.
3. Er moeten voldoende voorzieningen voorhanden zijn voor diagnose en behandeling.
4. Er moet een herkenbaar latent of vroeg symptomatisch stadium van de ziekte zijn.
5. Er moet een betrouwbare opsporingsmethode bestaan.
6. De opsporingsmethode moet aanvaardbaar zijn voor de bevolking.
7. Het natuurlijke verloop van de op te sporen ziekte moet bekend zijn.
8. Er moet overeenstemming bestaan over de vraag wie behandeld moet worden.
9. De kosten van opsporing, diagnostiek en behandeling moeten in een acceptabele verhouding staan tot de kosten van de gezondheidszorg als geheel.
10. Het proces van opsporing moet een continu proces zijn en niet een eenmalig project.

FIGUUR 1. Criteria voor verantwoorde screening (Wilson and Jungner, 1968)

NATUURLIJK BELOOP VAN ANTI-HPA-1a-GEMEDIEERDE FNAIT

In **hoofdstuk 2** beschrijven we de onderzoeksopzet van de HIP-studie (HPA-screening In Pregnancy). Het doel van deze studie is om ontbrekende kennis te vergaren over het natuurlijk beloop van de ziekte en over de identificatie van zwangerschappen met een hoog risico op ernstige ziekte. Rhesus (Rh)D-negatieve en Rhc-negatieve zwangere vrouwen die deelnamen aan het landelijke prenatale screeningsprogramma voor erytrocytenimmunisatie in de 27e week van de zwangerschap, kwamen in aanmerking voor deelname aan ons onderzoek. Aan deze vrouwen werd toestemming gevraagd voor opslag van restmateriaal en voor opvragen van klinische gegevens bij hun zorgverleners. Na het verrichten van een HPA-1a-typering verricht werd restmateriaal opgeslagen. HPA-1a-negatieve vrouwen en een controlegroep van HPA-1a-positieve vrouwen (verhouding 1:3) werden geïncludeerd in ons onderzoek. De HPA-1a-status van de moeder werd niet teruggekoppeld aan zorgverleners en/of de onderzoekers. Na de uitgerekende datum werden de zorgverleners verzocht de klinische gegevens van de moeders en de pasgeborenen in te voeren in een elektronische databank. Tevens werd een antistofscreening verricht met het opgeslagen restmateriaal. Na het verzamelen van klinische gegevens en de uitkomsten van de laboratoriumbepalingen werden de klinische gegevens gekoppeld aan de HPA-1a-antistof screeningsresultaten en de uitkomsten van de HPA-1a geïmmuniseerde zwangerschappen vergeleken met een controlegroep van HPA-1a positieve zwangerschappen. Met deze studieopzet biedt de HIP-studie een unieke mogelijkheid om de incidentie van klinisch detecteerbare FNAIT en

risicofactoren voor HPA-1a-immunisatie of ernstige bloedingscomplicaties te beoordelen zonder tussenkomst van de foetale behandeling.

De resultaten van de HIP-studie worden beschreven in **hoofdstuk 3.** In totaal was 2,43% (3.722/153.106) van de zwangere vrouwen HPA-1a-negatief. HPA-1a antistoffen werden gedetecteerd in 9,3% van de zwangerschappen van de HPA-1a-negatieve vrouwen (85/913 zwangerschappen, 32 vrouwen werden tweemaal geïncludeerd). In totaal werden 81 HPA-1a-geïmmuniseerde en incompatibele zwangerschappen, 820 HPA-1a-negatieve niet-geïmmuniseerde zwangerschappen en 2.704 HPA-1a-positieve zwangerschappen meegenomen in de analyse. Bij één (1,2%, 1/81) geïmmuniseerde zwangerschap werd een ernstige hersenbloeding vastgesteld op de prenatale echo bij een zwangerschapsduur van 29 weken. Na aanvullende beeldvorming en multidisciplinair overleg werd een late zwangerschapsafbreking uitgevoerd bij 34 weken. Bij drie pasgeborenen (3,7%, 3/81) werd na de geboorte een milde bloeding vastgesteld (twee hadden hematomen, één had een slijmvliesbloeding). Bovendien vonden we dat neonaten van HPA-geïmmuniseerde zwangerschappen vaker te vroeg (< 37 weken) geboren werden (15%, 12/81) in vergelijking met neonaten van HPA-1a-positieve vrouwen (5%, 132/2749). Het gemiddelde geboortegewicht van neonaten van geïmmuniseerde vrouwen was lager (3271 ±631) in vergelijking met neonaten van HPA-1a-positieve vrouwen (3459 ±545). Het aandeel vrouwen die voor het eerst zwanger waren (primigravida) was vergelijkbaar tussen geïmmuniseerde, niet-geïmmuniseerde en HPA-1a-positieve zwangerschappen (respectievelijk 32%, 37% en 34%). Op basis hiervan concluderen we dat een eerdere zwangerschap geen risico is voor antistofvorming.

Eerder werd gedacht dat incidentie van grote bloedingen in andere screeningstudies werd onderschat omdat na terugkoppeling van resultaten van de antistofscreening aan vrouwen en zorgverleners een behandeling werd gegeven aan vrouwen en/of neonaten. Onze resultaten wijzen erop dat er geen sprake was van onderschatting, aangezien het percentage ernstige bloedingen (1,2%) in onze studie lager is, maar in lijn met de gecombineerde cijfers van eerdere studies (1,4%). Samengevat vonden wij dat de dat de incidentie van ernstige bloedingen bij FNAIT 1 op 913 HPA-1a-negatieve zwangerschappen is, wat neerkomt op 11 van de 10.000 HPA-1a-negatieve zwangerschappen.

Naast de klassieke symptomen van FNAIT, zoals bloedingscomplicaties en trombocytopenie, wordt verondersteld dat HPA-1a-antilichamen ook kunnen binden aan de placenta, waardoor schade aan de placenta ontstaat. In **hoofdstuk 4** beschrijven we een onderzoek naar tekenen van schade aan de placenta bij FNAIT. Wij includeerden 23 placenta's waarvan 9 (14 samples) van nieuw gediagnosticeerde FNAIT en 14 (21 samples) placenta's van zwangerschappen waarbij de moeder bekend was met FNAIT vanuit een eerdere zwangerschap en behandeling met intraveneuze immuunglobuline (IVIg) werd gegeven. Als controles werden 20 placenta's van ongecompliceerde zwangerschappen geïncludeerd. Met behulp van

immunohistochemie werd de aanwezigheid van complement-activeringsmarkers (C1q, C4d, SC5b-9 en mannose-bindend lectine) bekeken. Twee onderzoekers scoorden, geblindeerd voor mogelijke problematiek, de aanwezigheid van de complement-activatiemarkers. Twee ervaren placentapathologen scoorden, eveneens geblindeerd, de histopathologie van volgens de Amsterdamse criteria. Een hogere mate van C4d-depositie was aanwezig op de syncytiotrofoblast bij placenta's van nieuw gediagnosticeerde FNAIT (10/14 samples) in vergelijking met placenta's van antenaal met IVIg behandelde FNAIT (2/21 samples) en gezonde controles. Vier (44%) placenta's bij nieuw gediagnosticeerde FNAIT en vijf (36%) placenta's uit de antenataal met IVIg behandelde FNAIT groep vertoonden een vertraagde rijping van de placenta, vergeleken met één in de controlegroep. Bij drie placenta's uit de nieuw gediagnosticeerde FNAIT groepen een kind met geboortegewicht onder het tiende percentiel, zagen we zowel C4d-depositie als laaggradige villitis van onbekende oorzaak. Op basis van bovengenoemde resultaten concluderen we dat er meer klassieke complementactivatie is waar te nemen in placenta's van zwangerschappen gecompliceerd waren door HPA-1a immunisatie zonder antenatale behandeling. Deze complementactivatie kan de ontwikkeling van de placenta schaden en past bij de observatie dat HPA-1a-immunisatie gepaard kan gaan met een verlaagd geboortegewicht.

KLINISCHE RELEVANTIE VAN HPA-5b-ANTILICHAMEN

Hoofdstuk 5 betreft een retrospectieve cohortstudie waarin de klinische uitkomsten van HPA-1a- en HPA-5b-gemedieerde FNAIT worden beschreven. Vanwege de hoge prevalentie van anti-HPA-5b bij zwangere vrouwen (1,8%), kan de detectie van anti-HPA-5b bij moeders van kinderen die verdacht worden van FNAIT een toevalsbevinding zijn. Op basis van de prevalentie van HPA-5b antistoffen en HPA incompatibiliteit werd onderzocht anti-HPA-5b een toevalsbevinding zou kunnen zijn. Ons cohort bestond uit 1.864 vrouwen waarbij FNAIT-diagnostiek werd ingezet. In 161 gevallen werd anti-HPA-1a aangetoond (8,6%) en in 60 gevallen anti-HPA-5b (3,2%). Anti-HPA-5b werd 1,8 keer zo vaak gedetecteerd bij zwangerschappen die verdacht werden van FNAIT in vergelijking met de prevalentie bij niet geselecteerde zwangere vrouwen (3,2% versus 1,8%). Het percentage ernstige bloedingen bij aanwezigheid van anti-HPA-1a (11%, 14/126) was vergelijkbaar met het percentage ernstige bloedingen bij de aanwezigheid van anti-HPA-5b (10%, 4/40). In de groep zwangerschappen met een verdenking op FNAIT, was bij alle vrouwen met HPA-1a antistoffen het kind HPA-1a positief. Bij vrouwen met anti-HPA-5b had 79% (38/48) van de vrouwen een HPA-5b-positief kind, waarbij we in slechts 52% van de gevallen een HPA-5b incompatibiliteit zouden verwachten wanneer anti-HPA-5b een toevalsbevinding zou zijn. Omdat anti-HPA-5b 1,8 keer vaker vastgesteld werd dan in een niet geselecteerd zwangere populatie en het percentage HPA-5b incompatibiliteit hoger was dan verwacht zou worden op basis van toeval konden wij niet uitsluiten dat anti-HPA-5b geassocieerd is met FNAIT.

NEONATALE BEHANDELING

Postnatale behandeling is gericht op het verminderen van het risico op bloedingen bij pasgeborenen met FNAIT. De behandeling is gebaseerd op beperkt wetenschappelijk bewijs en het is momenteel niet bekend welke behandelingen in andere behandelcentra worden gegeven. In **hoofdstuk 6** wordt een internationale multicenterstudie beschreven naar de postnatale behandeling en uitkomst van levend geboren kinderen met FNAIT (2010-2020). In totaal werden 389 neonaten geïncludeerd uit Australië (n = 74), Noorwegen (n = 56), Slovenië (n = 19), Spanje (n = 55), Zweden (n = 31), Nederland (n = 138) en de Verenigde Staten (n = 16). Bij een kwart van de pasgeborenen (24%, 92/380) werd extreme trombocytopenie vastgesteld (trombocytengetal < 10×10^9/L). Ernstige hersenbloedingen werden vastgesteld bij 6% van de neonaten met FNAIT (22/389). Bij 53% van de neonaten werden bloedplaatjestransfusies toegediend (207/389), hetzij een willekeurige bloedplaatjestranfusie (43%, 88/207), een bloedplaatjestransfusie waarbij werd geselecteerd op het humaan plaatjes antigeen (HPA) (41%, 85/207), of beide soorten transfusies (17%, 35/207). De mediane toename van bloedplaatjes na willekeurige en speciaal geselecteerde bloedplaatjestransfusies was respectievelijk 59×10^9/L (IQR 35 - 94) en 98×10^9/L (IQR 67 - 134) ($P < 0{,}0001$). Onze gegevens suggereren dat speciaal geselecteerde transfusies leiden tot een hogere toename van het aantal bloedplaatjesgetallen. Of deze behandeling ook leidt tot een verminderd risico op bloedingen is onbekend. Het gebruik van speciaal geselecteerde transfusies verschilde tussen centra, in sommige centra werden geen geselecteerde transfusies toegedient en in sommige centra in 62% van de gevallen. Aanvullende postnatale IVIg-behandeling werd gegeven in 29% van de gevallen (110/389), variërend tussen centra van in 12% van de gevallen tot in 63% van de gevallen. Wij concluderen dat de postnatale behandeling tussen de centra sterk varieert. Onze studie benadrukt de noodzaak van toekomstige onderzoek waarin de effecten van de postnatale behandelingen bij FNAIT worden vergeleken.

UITKOMSTEN OP LANGE TERMIJN

Kennis over de langetermijnuitkomsten van kinderen met FNAIT is cruciaal om adequate nazorg te kunnen bieden en om de mogelijke voor- en nadelen van de invoering van een FNAIT-screeningsprogramma en antenatale en postnatale behandeling te kunnen beoordelen. In **hoofdstuk 7** evalueerden we de langetermijnontwikkeling van kinderen met FNAIT. Kinderen werden uitgenodigd voor cognitief en neurologisch onderzoek. Gedragsvragenlijsten werden ingevuld en schoolrapporten werden aangeleverd door ouders. Een samengestelde uitkomstmaat werd gebruikt, onderverdeeld in mild tot matige en ernstige ontwikkelingsproblemen. In totaal werden 44 kinderen geïncludeerd op een mediane leeftijd van 12 jaar. Bij 14% (5/36) van de kinderen werd een ernstige hersenbloeding vastgesteld. 7% (3/44) van de kinderen hadden ernstige ontwikkelingsproblemen, twee van deze kinderen hadden een ernstige hersenbloeding en één kind werd gediagnostiseerd met een laaggradige hersenbloeding en perinatale asfyxie. Bij 25% (11/44) van de kinderen werden milde-tot-matige ontwikkelingsproblemen vastgesteld. Eén kind had een ernstige

hersenbloeding, acht kinderen hadden geen hersenbloeding en bij twee kinderen werd geen beeldvorming van de hersenen verricht. In totaal was 16% (8/46) van de kinderen overleden of had ernstige problemen. Vier kinderen (9%) volgden speciaal onderwijs, waarvan drie met ernstige ontwikkelingsproblemen en één met milde tot matige ontwikkelingsproblemen. De gedragsprobleemscores waren vergelijkbaar met de Nederlandse normscores. Op basis van de resultaten van deze studie concluderen wij dat kinderen die nieuw gediagnosticeerd worden met FNAIT een verhoogd risico lopen op neurologische ontwikkelingsproblemen op lange termijn, ook kinderen zonder hersenbloeding.

Om bij vervolgzwangerschappen hersenbloedingen bij het (ongeboren) kind te voorkomen wordt tijdens de zwangerschap IVIg toegediend aan de zwangere vrouw. Kennis over de langetermijnontwikkeling van kinderen waarvan de moeder tijdens de zwangerschap behandeld werd met IVIg ontbreekt. In **hoofdstuk 8** evalueren we de langetermijnuitkomsten van deze kinderen. Er werd gebruik gemaakt van dezelfde onderzoeksopzet als in hoofdstuk 7. In totaal werd 82% (41/50) van de kinderen die in aanmerking kwam voor ons onderzoek geïncludeerd. De mediane leeftijd was 9 jaar. Geen van de kinderen had ernstige ontwikkelingsproblemen. De incidentie van milde tot matige ontwikkelingsproblemen was 14% (6/41, 95% betrouwbaarheidsinterval: 6%-29%). Bij twee kinderen (5%) werd een ernstige hersenbloeding vastgesteld: bij één antenataal voor de start van IVIg en bij de ander één dag na de geboorte. Bij beide kinderen werden geen ontwikkelingsproblemen vastgesteld. De resultaten in dit hoofdstuk laten zien dat de langetermijnuitkomst van kinderen van wie de moeders voor FNAIT werden behandeld met IVIg vergelijkbaar is met de ontwikkeling van kinderen in de algemene bevolking.

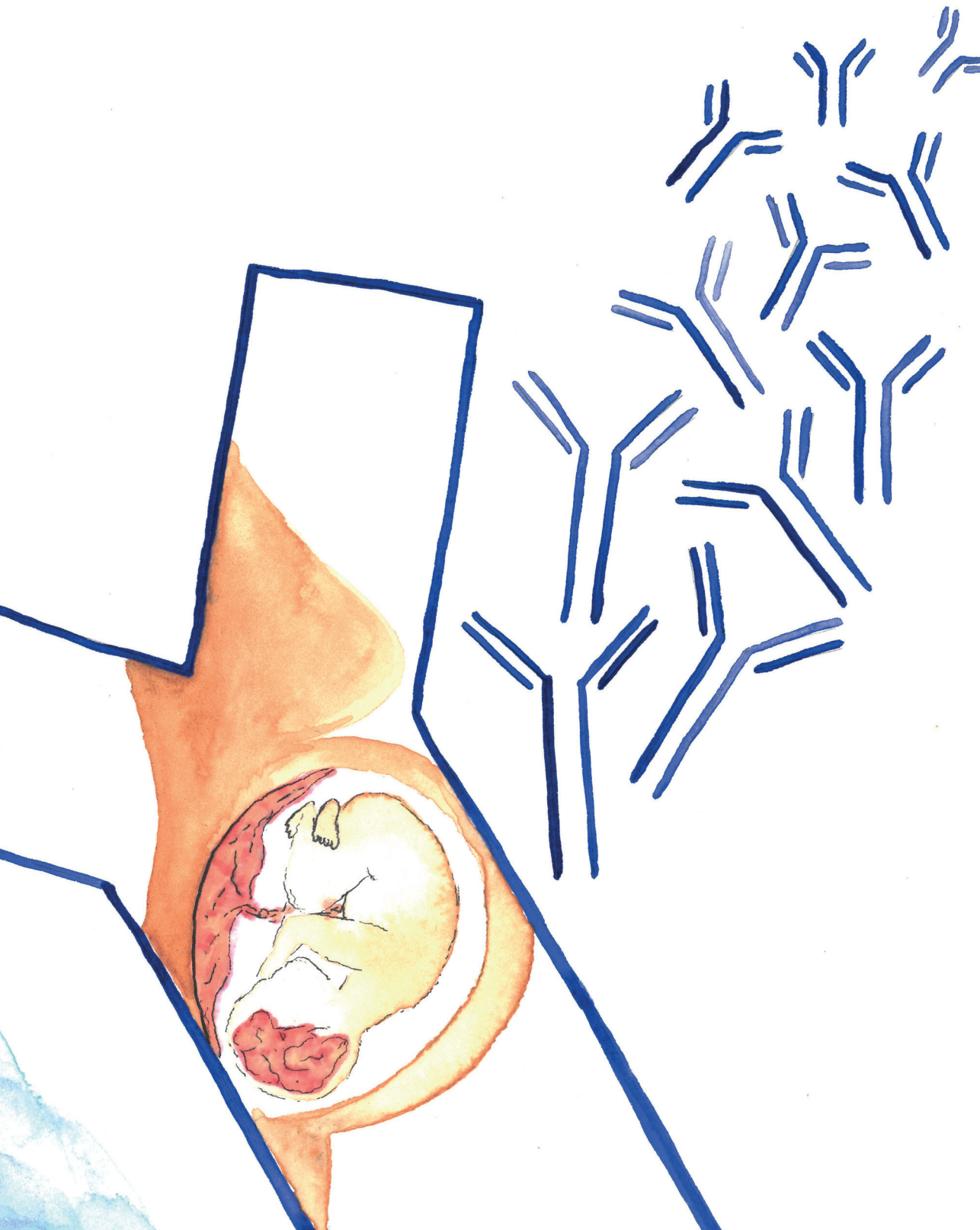
KOSTENEFFECTIVITEIT

Gesteld wordt dat screening op HPA-1a gerichte antistoffen tijdens de zwangerschap tijdige interventie met antenatale behandeling mogelijk maakt, wat de ontwikkeling van een ernstige hersenbloeding kan voorkomen. Aangezien de incidentie van ernstige hersenbloedingen door FNAIT laag is, is de beoordeling van de kosteneffectiviteit van het toevoegen van screening op anti-HPA-1a aan het bestaande prenatale screeningsprogramma relevant voor de besluitvorming. In **hoofdstuk 9** werden de levenslange kosten en effecten van prenatale screening op bloedplaatjesantistoffen vergeleken met de situatie zonder screening in Nederland. Dit werd gedaan door een beslissingsanalysemodel te ontwikkelen. De modelparameters werden gebaseerd op literatuur en advies van medici, gezondheidseconomen en epidemiologen. De resultaten laten zien dat toevoeging van screening op HPA-1a aan het huidige prenatale screeningsprogramma in Nederland zal leiden tot extra kosten van 4,7 miljoen euro per jaar, tegenover een winst van 226 *quality-adjusted life years* (QALY) per jaar. De incrementele kosteneffectiviteitsratio bedroeg 20.782 euro per gewonnen QALY. Op basis van dit model concluderen wij dat prenatale screening op anti-HPA-1a kosteneffectief kan zijn.

CONCLUSIE

FNAIT leidt tot ernstige bloedingen bij ongeboren kinderen in 11 op 10.000 HPA-1a-negatieve zwangerschappen, met een hoog risico op ontwikkelingsstoornissen. In **hoofdstuk 10** toetsen we invoer van een landelijke screening op HPA-1a-bloedplaatjesantistoffen tijdens de zwangerschap aan de hand van de eerder genoemde criteria van Wilson en Junger (Figuur 1), waarvoor we gebruik maken van de in dit proefschrift beschreven nieuw opgedane kennis. Op basis van deze evaluatie concluderen wij dat een landelijke screening op anti-HPA-1a tijdens de zwangerschap gerechtvaardigd lijkt. Op dit ogenblik zijn er twee punten waarvoor aanvullend bewijs of onderzoek nodig is voordat een screening op HPA-1a-bloedplaatjesantistoffen tijdens de zwangerschap ter preventie van FNAIT definitief kan worden ingevoerd. Ten eerste kan het risico op ernstige ziekte momenteel alleen worden ingeschat op basis van een kwantificering van de antistoffen. Er zouden nieuwe diagnostische tests moeten worden ontwikkeld om de te behandelen groep te verkleinen. Ten tweede moet de effectiviteit van IVIg in het voorkomen van bloedingen bij eerste geïmmuniseerde zwangerschappen worden aangetoond. Met een proefimplementatie van screening op HPA-1a-antistoffen kan de benodigde kennis over deze twee vraagstukken worden verkregen; zo kan de praktijk de antwoorden uitwijzen.

The proof of the pudding is in the eating.



APPENDICES

List of abbreviations
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LIST OF ABBREVIATIONS

ADHD	attention deficit hyperactivity disorder
BSA	bovine serum albumin
BW	birthweight
CBCL	Child Behavior Checklist
CD	cluster of differentiation
CE	cost effecitveness
CEAC	cost effectiveness acceptability curve
cffDNA	Cell-free fetal deoxyribonucleic acid
CI	confidence interval
CMV	cytomegalovirus
CP	cerebral palsy
CPI	consumer price index
CRF	case report form
CRP	C-reactive protein
CS	cesarean section
CST	antenatal corticosteroids
cUS	cranial ultrasound
ddPCR	droplet digital PCR
DNA	Deoxyribonucleic acid
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immuno sorbent assay
excl.	excluding
F	female
FBS	fetal blood sampling
FcRn	neonatal Fc-receptor
FFPE	formalin fixed paraffin-embedded
FGR	fetal growth restriction
FNAIT	Fetal and neoantal alloimmune thrombocytopenia
FSIQ	Full Scale Intelligence Quotient
g	gram
G	gravidity
GA	gestational age
GD	gestational diabetes
GMFCS	Gross Motor Functioning Classification Scale
GP	glycoprotein
H&E	hematoxylin and eosin
HDFN	hemolytic disease of the fetus and newborn
HIP study	HPA-screening in pregnancy study
HLA	Human leukocyte antigen
HPA	human platelet antigen

HRP	Horseradish peroxidase
HUVEC	human umbilical vein endothelial cells
ICER	incremental cost-effectiveness ratio
ICH	intracranial hemorrhage
IgG	Immuno globulin G
IQ	intelligence quotient
IQR	interquartile ranges
ITP	idiopathic thrombocytopenic purpura (
IU	international units
IU/ml	international units/mililiter
IUDF	intrauterine fetal demise
IUGR	intrauterine growth restriction
IUPT	intrauterine platelet transfusion
IVH	intraventricular hemorrhage
IVIg	intravenous immunoglobulin
kg	kilogram
L	Liter
LUMC	Leiden University Medical Center
M	male
MAC	membrane attack complex
MAIPA	monoclonal antibody immobilization of platelet antigens
MBL	mannose binding lectin
MD	medical doctor
METC LDD	Medical Ethical Committee Leiden-The Hague-Delft
MFI	mean fluorescence index
MHC	major histocompatibility complex
mL	mililiter
MND	minor neurologic dysfunction
MoAb	Monoclonal antibody
MRI	magnetic resonance imaging
NaCl	natriumchloride [sodiumchloride in English]
NDI	neurodevelopmental impairment
NEC	necrotizing enterocilits
NICU	neonatal intensive care unit
NR	not reported
NT	not tested
NTSC	near-term cesarean section
OD	optic density
OSWA	one-way sensitivity analysis
p	percentile
P	parity
PBS	phosphate buffered saline
PC	platelet count

PCR	polymerase chain reaction
PE	pre-eclamspia
PhD	Doctor or Philosophy
PIFT	platelet immunofluoresence test
PIH	pregnancy induced hypertension
PLT	platelet count
PP	postnatal platelets available for transfusion
PSA	probabilistic sensitivity analysis
PSIE	Prenatale Screening Infectieziekten en Erytrocytenimmunisatie
PTx	platelet transfusion
PVHI	periventricular hemorrhagic infarction
QALY	Quality-Adjusted Life Years
RBC	red blood cell
Rhc	Rhesus c
RhD	Rhesus D
RIVM	Rijksinstituut van Veiligheid en Milieu
RR	relative risk
SD	standard deviation
SE	standard error
SGA	small for gestational age
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphisms
SON	Snijders-Oomen nonverbal intelligence test
SVD	spontaneous vaginal delivery
TOP	termination of pregnancy
TRIS	tris(hydroxymethyl)aminomethane
USA	United States of America
VP	ventriculoperitoneal
VWS	Volksgezondheid Welzijn en Sport
W&J	Wilson and Jungner
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children
wk	week
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

LIST OF PUBLICATIONS

THIS THESIS

The natural history of human platelet antigen (HPA)-1a alloimmunised pregnancies: a prospective observational cohort study
T.W. de Vos*, D. Winkelhorst*, L. Porcelijn, M.Beaufort, G. Oldert, J.G. van der Bom, E. Lopriore, D. Oepkes, M. de Haas# C.E. van der Schoot#
** both authors contributed equally*
 # both authors contributed equally
Submitted

Cost-utility analysis of screening of pregnant women for fetal neonatal alloimmune thrombocytopenia
T.W. de Vos, I. Tersteeg, E. Lopriore, D. Oepkes, C.E. van der Schoot, E.J.T. Verweij, D. Winkelhorst, M. de Haas*, M.E. van den Akker-van Marle*
** both authors contributed equally*
Submitted

Children newly diagnosed with fetal and neonatal alloimmune thrombocytopenia: neurodevelopmental outcome at school age
T.W. de Vos, M. van Zagten, M. de Haas, D. Oepkes, R.N.G.B Tan, C.E. van der Schoot, S.J. Steggerda, L.S. de Vries, E. Lopriore, J.M.M. van Klink
Under revision in The Journal of Pediatrics. Dec 2022

Postnatal treatment for children with fetal and neonatal alloimmune thrombocytopenia: a multicentre, retrospective cohort study
T.W. de Vos, D. Winkelhorst, V. Árnadóttir, J.G. van der Bom, C. Canals-Suris, C. Caram-Deelder, E. Deschmann, H.E. Haysom, H.B.C. Hverven, J. Lozar-Krivec, Z.K. McQuilten, E. Muñiz Diaz, N. Nogués, D. Oepkes, L. Porcelijn, C.E. van der Schoot, M. Saxonhouse, M. Sola-Visner, E. Tiblad, H. Tiller, E.M. Wood, V. Young, M. Železnik, M. de Haas, E. Lopriore
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Long-term neurodevelopmental outcome in children after antenatal intravenous immune globulin treatment in fetal and neonatal alloimmune thrombocytopenia.
Am J Obstet Gynecol. 2022 Jun 6;S0002-9378(22)00438-0

T.W. de Vos, L. Porcelijn, S. Hofstede-van Egmond, E. Pajkrt, D. Oepkes, E. Lopriore, C.E. van der Schoot, D. Winkelhorst, M. de Haas
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T.W. de Vos, D. Winkelhorst, H.J Baelde, K.L. Dijkstra, R.D.M. van Bergen, L.E. van der Meeren, P.G.J. Nikkels, L. Porcelijn, C.E. van der Schoot, G. Vidarsson, M. Eikmans, R. Kapur, C. van der Keur, L.A. Trouw, D. Oepkes, E. Lopriore, M.P. van der Hoorn, M. Bos, M. de Haas
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Allergisch voor schapenmelk maar niet voor koemelk
Nederlands Tijdschift van Allergologie, 2017

CURRICULUM VITAE

Thijs de Vos was born in The Hague, the Netherlands on April 13 1990. In 2002 he started pre-university education at the Ashram College in Alphen aan den Rijn. From 2006 to 2008 he also attended courses at Leiden University as part of the pre-university college. In 2008 he finished secondary school and started Medical School at the Leiden University. In the academic year 2010-2011, he was a board member of the Medical Faculty of Leiden Students. At the beginning of his masters he did a research internship at the Department of Gastroenterology at the Leiden University Medical Center (LUMC) on the interaction between colorectal carcinomas and the stromal tissue surrounding the tumor. In addition to his regular rotations in the Netherlands, he went to Paramaribo, Suriname and Blantyre, Malawi. In 2015, he was awarded his master's degree cum laude. Right after his studies, he first gained experience as a clinical resident, working in the Pediatrics Department of the Alrijne Ziekenhuis in Leiderdorp, Reinier de Graaf Gasthuis in Delft and the Willem Alexander Children's Hospital, LUMC in Leiden. In 2018, he was offered a PhD position, to gain knowledge about fetal neonatal alloimmune thrombocytopenia (FNAIT) in order to consider screening during pregnancy. This research was performed in collaboration with Sanquin Research (Sanquin, Amsterdam), Sanquin Diagnostic Services (Sanquin, Amsterdam), Willem Alexander Children's Hospital (LUMC, Leiden) and the Department of Obstetrics and Gynecology (LUMC, Leiden). He was supervised by prof. dr. Masja de Haas (Sanquin, LUMC), prof. dr. Enrico Lopriore (LUMC), dr. Jeanine van Klink (LUMC). Prof. Ellen van der Schoot (Sanquin, University of Amsterdam) and Dick Oepkes (LUMC) were also involved in his research. In 2023, he will start his training as a pediatrician training at the Willem Alexander Children's Hospital, LUMC in Leiden (dr. Robert Bredius and drs. Lieke Rozendaal).

Thijs and his wife Kimberley have a son Olivier (2021). They live together in Leiden.

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Aan de HIP studie droegen 450 verloskundigen praktijken, 60 vakgroepen verloskunde en gynaecologie, tientallen artsenlaboratoria, klinisch chemici, vakgroepen kindergeneeskunde, medewerkers van het Centrum voor Bevolkingsonderzoek en vele anderen bij, iedereen bedankt.

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