



Universiteit
Leiden
The Netherlands

Fetal and Neonatal Alloimmune Thrombocytopenia: evidence based screening

Winkelhorst, D.

Citation

Winkelhorst, D. (2019, November 26). *Fetal and Neonatal Alloimmune Thrombocytopenia: evidence based screening*. Retrieved from <https://hdl.handle.net/1887/81084>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/81084>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/81084> holds various files of this Leiden University dissertation.

Author: Winkelhorst, D.

Title: Fetal and Neonatal Alloimmune Thrombocytopenia: evidence based screening

Issue Date: 2019-11-26



Chapter 10

General discussion

Parts of this discussion have been submitted for publication as:

Winkelhorst D, Oepkes D. Fetal and neonatal alloimmune thrombocytopenia: a 2020 update. *Prenatal Diagnosis* (invited review)

General discussion

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) can be a highly unexpected and dramatic complication in pregnancies. This form of alloimmunization can lead to devastating bleeding problems that might have been prevented if the alloimmunization would have been known. In current practice, all known alloimmunized pregnancies are treated with highly effective, weekly, intravenous immunoglobulin infusions (IVIg). Without population-based screening, these pregnancies are virtually solely in focus after a previously affected pregnancy or newborn. Affected cases that might have been prevented if the alloimmunization would have been detected prior to the occurrence of bleeding. Identifying these pregnancies before they lead to clinical damage would mean a major improvement in the management of FNAIT, but will only be achievable by implementing population-based screening. Implementing screening for detection of several diseases, such as erythrocyte immunization, congenital infections, breast and cervical cancer, and a number of diseases identified by neonatal heel-prick screening, has drastically decreased associated morbidity and mortality.¹⁻⁴ New and promising population-based screening programs cannot be implemented without carefully considering the pros and cons. There should be an acceptable balance between offering treatment to those in need with undetected disease, while avoiding harm and overtreatment to those not in need. To guide this careful consideration and weighing of benefits, burdens and costs, the World Health Organization published ten screening criteria, posed by Wilson & Jungner (W&J, figure 10.I).⁵

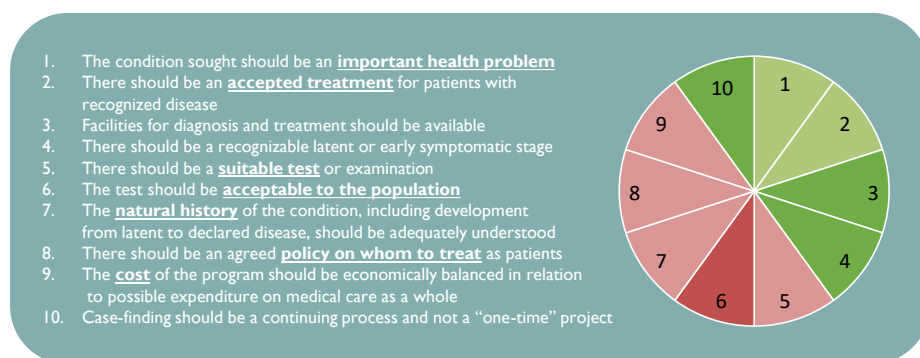


Figure 10.1 – Evidence for screening - Wilson & Jungner criteria⁵

Dark green, criterion fulfilled; light green, some extra information needed for fulfillment; light red, little information available but insufficient for fulfillment; dark red, no information available.

In this thesis, we aimed to gather and evaluate the evidence that is necessary to answer the highly-debated question whether it is feasible and sensible to implement prenatal screening to prevent the devastating consequences of FNAIT.

Important health problem

The importance of a health problem can be defined by both the severity of the disease and its incidence. A rare disease with major individual and social impact can be as important as diseases with milder outcome but higher incidence.⁶ FNAIT fits the first category, a rare disease with major consequences. One of the most-feared consequences is a fetal or neonatal intracranial hemorrhage (ICH), due to its high mortality rates and possibly even worse, a high risk of lifelong handicaps and neurological sequelae in survivors.⁷ This complication is generally well-known and described in most guidelines and literature. And whereas most case series and articles do report on highly unfavorable short-term consequences, no detailed and structured long-term follow-up data of children suffering ICH caused by FNAIT have been reported. Information that is indispensable in the screening debate. Indispensable, because the information is necessary for assessment of consequences and costs to be prevented, as well as for the counseling of parents. In *chapter 3* we describe ICHs that were managed at our center, which is the national referral center for neonatal alloimmune disease. The identification of 31 cases in 21 year is probably not an adequate representation of the true prevalence of ICH in our country, which has a birth rate of 170,000 per year. Current underdiagnosing of FNAIT in absence of screening might be a strong contributor to this small number of 1-2 cases per year. This was previously suggested by Davoren *et al.*⁸, who reported a discrepancy between retrospectively collected cases and expected cases based on prospective screening studies.

Neurodevelopmental outcome of the surviving children in our cohort was unfavorable. More than half were severely neurodevelopmental impaired (NDI). This is in line with previously described case-series. Within a series of nine ICHs due to FNAIT, three infants died, and severe impairment was reported in all surviving children.⁹ Another series reported three ICHs caused by FNAIT, of which one died and the two surviving children suffered severe NDI.¹⁰ Both series are limited by the small numbers, absence of long-term follow-up and lack of standardized testing. Also, both might have been strongly biased by the selection of only severe cases. This selection bias might have occurred in our cohort as well. The fact that our study was single center at a referral hospital and data were retrospectively collected, together with considerable missing clinical data, might have led to an overrepresentation of the more severe cases. This is supported by the relatively high rate of perinatal mortality. Compared with the largest cohort of retrospectively collected ICHs, described by Tiller *et al.*¹¹, our mortality rate is somewhat higher (48% versus 35%). However, the proportion of first-born children affect by FNAIT is similar. Over half of the described severe cases occur in first-born children.

Prospectively collected data from non-intervention studies might tackle the risk of selection bias to more severe ICHs, but these numbers are too small to extract useful data from (Table 10.1).^{17,21} Further, a prospective study that identifies all clinically overt cases of ICH that are symptomatic in the first day of life, might still miss cases that are initially asymptomatic. Intracranial bleeding,

causing discrete brain damage that is not big enough to be symptomatic (e.g. leading to epilepsy, reduced consciousness or other clinical problems), will remain undetected but might still lead to behavioral or cognitive problems in later life. Whether this milder, initially subclinical, phenotype of ICH exists as well, is something to be assessed in future prospective research with long-term follow-up.

Table 10.1 – Prospective cohort studies assessing incidence and natural history

Author, year	HPA-1a negative	Antenatal anti-HPA-1a	PLT <50 x 10 ⁹ /L	Mild bleeding	Severe bleeding	Intervention
Mueller-Eckhardt, 1985 ¹²	26/1,211 (2.1)	2/26 (7.7)	0	0	0	None
Reznikoff-Etievant, 1988 ¹³	27/860 (3.1)	0/27 (0)	0	0	0	None
Blanchette 1990 ¹⁴	81/5,000 (1.6)	3/50 (6.0)	1	0	1	NTCS, PP
Doughty, 1995 ¹⁵	74/3,473 (3.2)	1/71 (1.4)	0*	0*	0	FBS/IUPT, IVIg, PP
Durand-Zaleski, 1996 ¹⁶	52/2,066 (2.5)	4/45 (8.9)	1	0	0	FBS, IVIg, CST
Williamson, 1998 ¹⁷	618/24,417 (2.5)	37/385 (9.6)**	8	7	1	PP
Davoren, 2003 ¹⁸	54/4,090 (1.3)	2/34 (5.9)	1	1	0	FBS, IUPT, PP
Maslanka, 2002 ¹⁹	144/8,013 (1.8)	12/122 (9.8)	3	1	0	IUPT, IVIg
Turner, 2005 ²⁰	546/26,506 (2.1)	25/318 (7.9)	5	3	0	PP
Kjeldsen-Kragh, 2007 ²¹	2,111/100,448 (2.1)	144/1,990 (7.2)	48	NR	2	NTCS, PP
Debska, 2018 ²²	373/15,204 (2.5)	22/373 (5.9)	3	NR	NR	IUPT, IVIg
Winkelhorst, (HIP-study) unpublished results	986/40,945 (2.4)	24/262 (9.2)	NT	3	1	None

Numbers are n/N (%). 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 10⁹/L. Mild bleeding is defined as only skin or mucosal bleeding. Severe bleeding is defined as internal organ hemorrhage or ICH.

* 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.

A fetal or neonatal ICH in an otherwise healthy, but thrombocytopenic infant would in most cases be strongly associated with FNAIT, especially when other causes for thrombocytopenia are excluded. Though seldom described in (international) guidelines or reports on FNAIT, the site of bleeding can conceptually be in every organ in the body. These other hemorrhages are therefore likely to be underdiagnosed, which might not only lead to inadequate diagnoses and treatment in the affected infants, but also lead to inadequate follow-up and management in potential subsequent pregnancies. In *chapter 4*, we described a case series and literature review

of these under-reported outcomes of FNAIT that were detected and diagnosed at our tertiary center. Two of the three described hemorrhages were fatal. Besides these gastrointestinal and pulmonary bleedings at our center, in literature we found cases of ocular, spinal cord, renal, subgaleal and genitourinary bleeding as well.^{8,23-37} In absence of population based screening, to avert denying these infants and women of adequate care, a wider scope when dealing with bleeding problems in full-term newborns is necessary. Awareness is key!

Broadening the clinical spectrum of FNAIT

Classical features of FNAIT are a thrombocytopenia with or without mild to severe bleeding complications. These features have long been the only described clinical consequences. However, more and more indicative evidence implies that this might just be the tip of the iceberg. The cornerstone of this suggested broadening of the clinical spectrum of FNAIT is the placenta. The HPA-1a epitope, which is targeted in the vast majority of (severe) FNAIT cases, is expressed on the $\beta 3$ integrin. Interestingly, this $\beta 3$ integrin, besides being present on platelet membranes, complexed to $\alpha 2b$ ($\alpha 2b\beta 3$ or glycoprotein IIb/IIIa) as the fibrinogen receptor, is present on syncytiotrophoblast cells as well. Here, $\beta 3$ is complexed to αV ($\alpha V\beta 3$) as the vitronectin receptor.^{38,39} Syncytiotrophoblast is the outer layer of the trophoblast that is predominantly involved in invasion of the endometrium and implementation in early pregnancy. After penetration of the endometrium, the syncytiotrophoblast cells eventually reach the maternal circulation and form chorionic villi, which is the start of placental development. Fetal $\beta 3$ is expressed on these cells in blastocysts and early, first-trimester placentas.³⁸

This early fetal $\beta 3$ exposure to the maternal circulation might explain the increased occurrence of FNAIT in first pregnancies or first-born children, when compared to HDFN. Further, once alloimmunized, the possible binding and interaction of maternal anti-HPA-1a to syncytiotrophoblast might interfere with placental function as well. Generally, placental insufficiency can lead to various clinical problems, such as oligohydramnios, (asymmetric) intrauterine growth restriction (IUGR), premature birth, pre-eclampsia, miscarriage, fetal distress and intrauterine fetal demise (IUFD).⁴⁰⁻⁴² A recent study with a murine FNAIT model showed poor placental perfusion and abnormal placental vascularization in placentae of immune mice.⁴³ Further, they describe a decrease in fetal weight and increase in miscarriages and fetal death. A reduced birth weight has been reported in human studies as well, Tiller *et al* reported a reduced birth weight only in boys in their prospective study and a rate of 23% small for gestational age infants in their retrospective cohort of ICHs.^{11,44} We have found similar results. For *chapter 6*, we analyzed neonatal treatment of all newly detected cases of FNAIT, born between 2006 and 2017, diagnosed at the national reference laboratory and therefore a complete presentation of all cases diagnosed in the Netherlands. Within this cohort, an increased number of small for gestational age (SGA) children, boys and girls, could be identified as well (Table 10.2).

Table 10.2 – Retrospective cohort (unpublished data)

	Newly detected FNAIT (n = 102)	General population* (n = 779)
GA at birth	38 ⁺² (36 ⁺⁶ – 40 ⁺⁰)	39 ⁺⁶ (38 ⁺⁵ – 40 ⁺⁴)
Premature delivery (< 37wk)	22 (22)	42 (5)
Birth weight	3020 (2491 - 3414)	3510 (3130 – 3845)
Male sex	68 (67)	396 (51)
SGA	22 (22)	88 (11)
Obstetric history		
Miscarriage**	29 (41)	196 (39)
IUFD***	4 (7)	6 (1)

Numbers are n (%) or median (IQR). CS, cesarean section; GA, gestational age; FNAIT, fetal and neonatal alloimmune thrombocytopenia; IUFD, intrauterine fetal demise; SGA, small for gestational age.

* General population, numbers from HIP-study control group.

** primigravidae excluded (n = 70 in newly detected FNAIT, n = 508 in control group).

*** nulliparae excluded (n = 57 in newly detected FNAIT; n = 435 in control group).

Also, compared to numbers reported for the general pregnant population in the Netherlands, an increased number of IUFDs and miscarriages in obstetric history and cases of pre-eclampsia were detected. An increased number of premature deliveries was also reported. Naturally, besides an interesting new insight, no conclusions regarding causal relationship between anti-HPA-1a alloantibodies and these outcomes can be drawn from these data. Besides an important bias of selection in this retrospective cohort, we should also take into account that confounding might play a role. First, the reason for assessing platelet counts in these infants might be the prematurity or birthweight itself, which introduces a considerable selection bias. Second, the thrombocytopenia that is detected can be caused by the prematurity or SGA as well. Although platelet counts in these cases are not usually below $50 \times 10^9/L$.⁴⁵ In our prospective cohort, described in *chapter 9*, this increase in the number of infants that were SGA was not observed. So this difference displayed in table 10.2 might very well be due to confounding and bias in selection. However, an increased number of miscarriages and IUFD in obstetric history in both cohorts as well as a higher proportion of premature deliveries was also observed in our prospective cohort. Whether the increased number of miscarriages and IUFD was a result or the cause of the immunization remains undetermined.

To investigate the interaction and role between anti-HPA-1a and placenta, we collected placental tissue of cases of newly detected FNAIT, placentae of FNAIT cases that were treated with IVIg and control placentae of uncomplicated pregnancies. Cases were identified retrospectively through our cohort described in *chapter 6*. At the moment of writing this thesis, our group is working on examining macroscopic and histopathologic characteristics of these placentae. Also, immunohistochemical staining assays will be performed to assess IgG deposition and possible placental damage and dysfunction through complement activation via the classical pathway.

Natural history

Without knowledge on natural history of the disease it is merely impossible to determine the potential health gain from screening programs. Assessing natural history should start with a complete and representative population and then monitoring subgroups at risk.⁶ For FNAIT, this would mean starting with a large number of unselected pregnant women, then determining which women are HPA-1a negative and subsequently evaluating the alloimmunization rate.

Furthermore, conclusions on natural history of FNAIT can only be adequately drawn from prospective observational (thus non-interventional) studies. Aiming to obtain knowledge on natural history, there have been several large prospective screening studies performed (Table 10.1). Non-interventional studies, however, are ethically challenging. Testing for alloimmunization and subsequently observing its natural course would mean withdrawing antenatal treatment from pregnancies that would have been treated in current practice. Therefore, merely all studies applied some kind of intervention. Either fetal blood sampling (FBS), intrauterine platelet transfusion (IUT), near-term cesarean section (NTCS), or a directly postnatal platelet transfusion. This prevents drawing conclusions on natural history of FNAIT. In contrast, alloimmunized pregnancies detected in current practice because of a (previous) affected child, which are now antenatally treated with IVIg, are arguably different from alloimmunized pregnancies detected through screening.

The HIP-study was designed as a prospective and observational cohort study to collect these missing data on incidence and natural history (*chapter 2*). To overcome the ethical dilemma even further, we decided to postpone screening maternal sera for anti-HPA-1a antibodies until after birth. Although the study, at the time of writing this thesis, is not yet completed, we performed an interim-analysis after the first 10 months (*chapter 9*) to carefully estimate preliminary natural history of FNAIT in the Netherlands. Within the 24 detected immunized cases, one severe case of FNAIT and three cases with only minor bleeding problems were identified. These numbers are comparable to those extracted from prospective studies without antenatal intervention and only postnatal platelets available for transfusion. Williamson *et al.* detected one severe bleeding in 37 immunizations and Turner *et al.* did not identify any severe bleeding complications in 25 immunisations.^{17,20} The immunization rate of 9.2% and a HPA-1a negativity rate of 2.4%, in the HIP-study, the detected 24 immunizations would represent the screening of 9,958 unselected pregnant women. Assuming a total number of pregnancies of 170,000 per year in the Netherlands, this is approximately 1/17 of the total pregnant population in the Netherlands.⁴⁶ Because of the small numbers, extrapolation should be interpreted with caution. Extrapolation would mean identifying 4,080 HPA-1a negative pregnant women each year and 375 HPA-1a immunizations. Further deducing the findings from the HIP-study would indicate 17 severe bleeding complications and 51 minor cases per year.

To reflect on whether this incidence of FNAIT might justify prenatal screening, we can take a look at implemented perinatal screening programs that focus on identifying pregnancies and newborns at risk and initiating prenatal or postnatal treatment to preventing severe complications for newborns. In the Netherlands, five of such perinatal screening programs have been instated: 1) Prenatal screening of infectious diseases and red blood cell immunization, 2) Prenatal screening for Down's, Edwards' and Patau's syndrome, 3) Structural Ultrasound Scan; and 4) Neonatal blood spot screening and 5) Neonatal hearing screening (Table 10.3). With those numbers varying from 1 – 200 identified or prevented cases each year, the incidence of FNAIT is definitely comparable to other implemented perinatal screening programs.

Table 10.3 – Incidences of identified or prevented cases per year in the Netherlands

Prenatal screening for infectious diseases and erythrocyte immunization (PSIE)	
Severe RhD mediated HDFN*	320**
Severe Rhc-mediated HDFN*	6-7 ¹
Congenital HIV	5
Active syphilis during pregnancy	100
Prenatal screening for Down's, Edwards' and Patau's syndrome	
Down syndrome	195
Edwards' and Patau's syndrome	17
Structural ultrasound scan	
Spina bifida or anencephaly	55
Neonatal blood spot screening ³	
CAH, PKU	10-20
Galactosemia, metabolic diseases (e.g. MSUD, MCD, LCAD, OCTN2, 3-MCC)	<1
Alpha/beta-thalassemia, metabolic diseases (e.g. TYR-1 VLCAD)	1-5
Sickle cell disease	17
Cystic Fibrosis	23
Neonatal hearing screening	
Double sided hearing loss	119

3-MCC, 3-Methylcrotonyl-CoA carboxylase deficiency; CAH, congenital adrenal hyperplasia; HDFN, hemolytic disease of the fetus and newborn; HIV, human immunodeficiency virus; LCAD, Long-chain hydroxyacyl-CoA dehydrogenase deficiency; MCD, multiple CoA carboxylase deficiency; MSUD, maple syrup urine disease; OCTN2, Carnitine transporter deficiency; PKU, phenylketonuria; PSIE, prenatal screening infectious diseases and erythrocyte immunization; TYR-1, Tyrosinemia type 1; VLCAD, very-long-chain acyl-CoA dehydrogenase deficiency.

*Severe HDFN defined as perinatal death, the need for intrauterine transfusion (IUT) or exchange transfusion in the first week of life.

**Severe HDFN before 1969 $n = 350$ ⁴⁷ – severe HDFN 2008 $n = 30$ ⁴⁸.

A part from the difficulty of extrapolating due to small numbers, the numbers extracted from the HIP-study are likely still an underestimation. First, the antibody screening was performed only once in every pregnancy, at 27 weeks' gestation. Alloimmunization might occur throughout the whole pregnancy and especially during delivery. Previous studies have shown that in pregnancies with negative antibody screening at 28-34 weeks' gestation, anti-HPA-1a antibodies can be detected after delivery.^{17,21} Although this might lead to a possible underestimation, alloantibodies formed only during and detected after delivery could not have resulted into severe FNAIT in that pregnancy, a case that would have been missed by screening. Second, because of our timing of screening, at 27 weeks' gestation, we miss alloimmunizations that have resulted in IUFD earlier in pregnancy. Third, one of the limitations of our study is the lack of routine neonatal platelet count measurements. This way we are unable to detect cases with an asymptomatic thrombocytopenia. Nonetheless, the goal of screening is not to prevent specific laboratory values but identifying cases at risk of symptomatic disease that can be prevented. Fourth, by obtaining clinical information from obstetric care givers, we will miss cases that were asymptomatic in the first eight days of life but developed complications thereafter. Lastly, besides absence of routine laboratory evaluation our study does not include routine ultrasound examination of the neonatal brain. In conclusion, taken all these limitations into account, the results and numbers that will be extracted from the HIP-study will not be a perfect reflection of the natural history of FNAIT. Yet, they will likely be an underestimation rather than an overestimation, which is preferable in evaluating feasibility and efficacy of implementing screening.

Accepted treatment

At present, the diagnosis of FNAIT is most often made postnatally, in case of an unexplained chance finding of an isolated neonatal thrombocytopenia or in case of a newborn with an unexpected bleeding and corresponding thrombocytopenia in the first days of life. In these cases, postnatal treatment that is aimed at reducing the (risk of) bleeding by increasing platelet counts, can be administered. Commonly, treatment is applied when platelet counts drop below a certain threshold or in case of major bleeding, mostly $20 \times 10^9/L$ or $30 \times 10^9/L$.⁴⁹⁻⁵¹ In clinical guidelines for the treatment of neonatal thrombocytopenia in FNAIT, various treatment strategies exist. In *chapter 6* we analyzed different neonatal treatment strategies applied in over one hundred cases of newly detected FNAIT. Strategies were observation/no treatment, transfusion with HPA-compatible and/or random platelets, IVIg or a combination. A favorable outcome of all included cases, regardless of applied treatment strategy, was observed. All infants reached a platelet count $> 50 \times 10^9/L$ within four days after birth without the occurrence of new hemorrhages.

Obviously, the choice of treatment is strongly determined by the outcome that measures the effect of the treatment as well, namely platelet count. Therefore, we should be careful with posing strong statements on effectiveness. However, with comparable platelet counts at diagnosis and clinical disease, newborns that are treated with transfusions with random-donor

platelets do seem to have similar increases in platelet counts over time, compared to those treated with compatible platelet transfusions. Additionally, receiving a first transfusion did not seem to increase the need for another transfusion in these cases, when considering a threshold of a platelet count below $20 - 30 \times 10^9/\text{L}$ for transfusion. Therefore, one might argue that these random-donor platelet transfusions can be safely used as first line therapy when encountering an unexpected case of neonatal thrombocytopenia, suspicious for FNAIT.

In a potential future screening program, however, these cases of neonatal thrombocytopenia will not be unexpected, but anticipated. Compatible platelets for transfusion is then logistically less challenging and preferable. In line, a recently published systematic review that included 14 studies with 754 infants, concluded that although compatible transfusions are more effective in increasing platelet counts, random-donor platelets are often effective enough to achieve clinical goals.⁵² An appropriate threshold for transfusion should be a platelet count of $30 - 35 \times 10^9/\text{L}$, according to available evidence.^{21,53} Although studies on transfusion thresholds in FNAIT are lacking, we would advise to lower this threshold to $25 \times 10^9/\text{L}$ in newly detected as well as anticipated cases of FNAIT without major bleeding, as is currently used in our national transfusion protocol.⁴⁹ First, it seems that no new hemorrhages occur after the diagnosis of FNAIT, regardless of platelet count or initiation of therapy. Second, a recent study on thrombocytopenia in premature infants shows that a lower transfusion threshold ($25 \times 10^9/\text{L}$ instead of $50 \times 10^9/\text{L}$) actually leads to better outcome.⁵⁴

In contrast to the treatment of unanticipated cases, which is administered postnatally, pregnancies with known alloimmunization can be treated antenatally in order to prevent the occurrence of bleeding complications. In the vast majority of these cases the immunization is known because of a previous affected sibling. In rare cases, antenatal treatment is applied because of antenatal diagnosis of FNAIT, either due to the detection of fetal bleeding on ultrasound or due to diagnostic work-up because of a family member (a sister) with FNAIT. Antenatal treatment is aimed at prevention of bleeding complications. Due to the similarity to HDFN, which for decades is treated with intrauterine blood transfusions, the first available antenatal treatment for this indication was serial intrauterine intravascular platelet transfusions. An invasive treatment that obviously introduces an additional and substantial bleeding risk. Puncturing the umbilical cord for fetal blood sampling, determining fetal platelet count and if necessary transfusion of platelets is much more risky in fetuses with low platelet counts than in 'just' anemic fetuses.⁵⁵ Quite remarkably, now that a safe and effective non-invasive antenatal treatment using IVIg is available, there are still specialized centers performing this procedure, either as antenatal treatment itself or as pre-delivery diagnostics to determine fetal platelet counts and decide on the mode of delivery.^{22,56,57} In *chapter 5* we evaluated all available evidence on antenatal management strategies. Next to three published, randomized controlled trials we also critically appraised and analyzed all published cohort studies. This systematic review

did clearly show that non-invasive treatment with weekly IVIg infusions was as effective in preventing severe bleeding complication, as the invasive treatment using fetal blood sampling (FBS) with or without intrauterine platelet transfusion (IUPT). In 11% of pregnancies that were treated with FBS/IUPT complications occurred, of which a quarter resulted in fetal or neonatal death. More than half of the reported perinatal mortalities were associated, and likely caused by the invasive treatment with FBS/IUPT.

Non-invasive treatment consists of different dosages of IVIg with or without the addition of corticosteroids. No clear benefit of adding corticosteroids has been shown. The only study that did report a significant improvement in platelet counts when adding steroids used a somewhat questionable, not predefined, outcome measure for this effect.^{58,59} With regards to both start and dose of IVIg therapy, pregnancies can be stratified into a high-risk and a standard-risk group, based on whether or not a sibling had an ICH.^{60,61} The most commonly used dose is 1 g/kg maternal weight/week, and is adapted from treatment in immune thrombocytopenic purpura (ITP). However, our group has shown that for standard-risk pregnancies a lower dose of 0.5 g/kg/week, was not inferior to 1g/kg/week. Therefore, we advise to treat standard-risk pregnancies with 0.5 g/kg/week and high-risk pregnancies with 1g/kg/week. Because many ICHs occur before 30 weeks of pregnancy, therapy should start before.^{11,62} Given the 79% recurrence rate of ICH we would advise in high-risk pregnancies to start treatment earlier in pregnancy, at 16 weeks' gestation, and in standard-risk pregnancies at 20-24 weeks' gestation.⁷

Although antenatal weekly IVIg treatment is considered to be the safest and most effective management for pregnancies at risk for bleeding complications, this has only been investigated in subsequent pregnancies. No data are available about the treatment of pregnancies at risk identified through screening. Therefore, whether weekly IVIg treatment is effective in a screening setting and can prevent the occurrence of bleeding complications in first cases as will remain unanswered until screening will actually be implemented.

A novel drug to be used in the treatment of fetal and neonatal alloimmune disease is M281, an anti-FcRn antibody. Blockade of FcRn and therefore preventing the interaction of IgG with FcRn, might inhibit fetal exposure to pathogenic IgG by preventing or minimizing transplacental transfer of pathogenic IgG as well as inhibiting IgG recycling.⁶³ The phase I human clinical study illustrated that M281 was safe and well tolerated in healthy human volunteers.⁶⁴ A clinical phase II studies in early onset severe HDFN is currently being set up (NCT03842189). If that study is successful, a study in FNAIT would be one of the logical next steps to consider.

Suitable test

In outlining population-based screening for FNAIT, one of the most challenging factors, financial as well as logistical, is identifying the 2% HPA-1a negative pregnant women. Because the number of women in need of follow-up testing decreases 50-fold after this, the first line of screening is strongly contributing to the total costs, and is therefore very important for the cost-effectiveness of a potential screening program.^{65,66} In terms of costs, serological testing has benefits over genotyping. Different serological HPA-1a typing assays have been used in performed prospective screening studies, flow-cytometry and enzyme-linked immunosorbent assay (ELISA).^{17,20,21} For the applicability in population-based screening the assay needs to be quick and suitable for high-throughput testing. Both serological assays, depending on the material used, are quick. They were both previously validated, but unfortunately only in a small number samples and merely in random donors instead of pregnant women. The ELISA used in Scottish screening study was reported to cost £3.01 per sample.²⁰ The largest screening study to date, performed in Norway, used both ELISA and flow-cytometry and reported €1.72 per sample for flow cytometry and €21.28 for genotyping.^{21,65} Both assays are quick, but flow-cytometry needs considerable more analyzing time at a rather expensive flow-cytometer, which increases costs, therefore we used an ELISA assay for HPA-1a typing in our prospective study. Aiming to further reduce costs and time, to increase validation in pregnant samples and to increase applicability in future screening, the previously described ELISA was optimized and described in *chapter 7*. We used material of moderate quality, 3-6 days-old tubes without spinning or swirling them, to increase the applicability for high-throughput screening. Assay costs, excluding labor, were only €0.25 per sample. The goal of screening, obviously, is to identify all HPA-1a negative women, so the test needs to reach a 100% sensitivity (i.e. a zero false-HPA-1a positive rate). Achieving this comes at the cost of an increased the rate cases falsely identified as HPA-1a negative to 0.03. For future population-based screening, one might consider skipping the costly step of genotyping and perform follow-up antibody screening of all serologically HPA-1a negative cases.

Acceptability of screening for FNAIT

The success and effectiveness of any screening program will be determined by the uptake and willingness to participate to the program. Increasing consumerism in our current health care system leads to an important role of patient's attitude towards the screening in determining this participation rate. Thus, when evaluating the sixth W&J criterion, the acceptance of pregnant women to potential future prenatal screening for FNAIT, we need to assess women's attitude towards such a screening program. Obviously, we prefer women to take the decision to participate in a screening program to be based on knowledge and understanding of the disease, the test and the consequences of (a positive) outcome of testing. Informed decisions are determined by adequate knowledge and should be in concordance with their attitude towards the screening program. These three factors (uptake, knowledge and attitude), leading to informed choice, can be measured by using the validated Multidimensional Measurement

of Informed Choice (MMIC) model that is developed and described by Marteau and colleagues (Figure 10.2).⁶⁸ In *chapter 8* we report the first study that assessed women’s attitude towards potential population-based screening for the prevention of morbidity and mortality caused by FNAIT. Overall, the attitude of women towards potential future HPA-screening in order to prevent FNAIT was very positive and the willingness to participate in a future screening was almost 99%. The (small) groups that were less likely to express a positive attitude were highly educated women and women that intended to deliver at home. Without any difference in knowledge or uptake, this led to a lower rate of value-consistent decisions in these groups. Within our participants, we had an underrepresentation of non-European women and a slight overrepresentation of highly educated women (60% compared to 48% reported for the general population of 25-45-year-old Dutch women). The first group had a higher rate of uninformed decisions. The latter had no significant differences in knowledge or attitudes scores. Based on these findings we would expect a high rate of informed uptake when implementing HPA-screening in pregnancy. Further, our data confirm that it is important to adapt the provision of information to women’s ethnicity and to pay attention to its potential to cause anxiety and uncertainty.

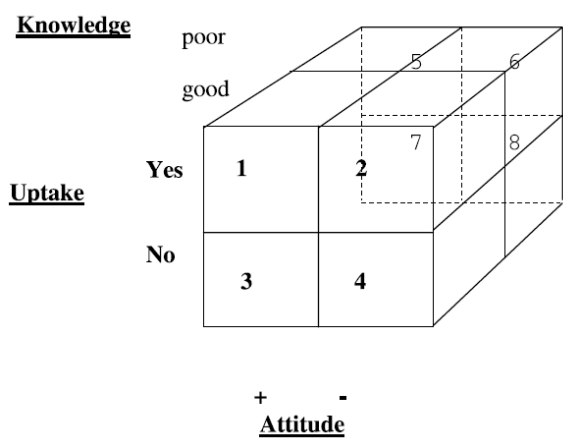


Figure 10.2 – Multidimensional Measurement of Informed Choice⁶⁷
 Box 1 and 4 represent informed choices. Box 3 and 2 represent value inconsistent choices, despite sufficient knowledge.
 Box 5, 6, 7 and 8 represent uninformed choices based on insufficient knowledge.

Risk assessment

The greatest challenge still ahead is the identification of those alloimmunized pregnancies that are truly at high risk of developing fetal bleeding complications. The eighth W&J criterion demands an agreed policy who to treat as patients. In current practice, all known platelet alloimmunized pregnancies are antenatally treated. If this policy would be applied to a screening

program, there would be a considerable overtreatment whereas it is estimated that only around 5% of alloimmunized pregnant women will have a fetus or neonate with a significant bleeding complication (Table 10.2, *chapter 9*).^{14,17,21} Therefore, there is a need of non-invasive tests to better predict fetal risk and outcome. Ideally, we would like to have maternal serum markers that reliably determine the biological effect of the alloantibodies and thereby predict disease severity. For this purpose, a couple of markers and assays have been suggested.

Antibody level - In some centers, antibody levels are monitored by titration and quantification. While high titers are significantly correlated with lower platelet counts and more severe disease, this does not seem to be a consistent relationship.⁶⁹ There are cases of severe hemorrhages with barely detectable antibody levels.⁷⁰⁻⁷² When trying to predict disease severity, antibody titer might be a useful contributor but not a reliable measurement to solely guide follow-up testing and management.

*HLA-DRB3*0101* – The majority of HPA-1a incompatible pregnancies, in which alloimmunization will occur, HLA-DRB3*0101 positive.^{21,73-75} Only 0.5% of the HLA-DRB3*0101 negative incompatible pregnancies will result in the formation of anti-HPA-1a antibodies.⁷⁶ Recently, the presence of 1 or 2 HLA-DRB*0101 alleles was suggested to be significantly correlated to anti-HPA-1a levels in alloimmunized samples from a prospective study.⁷⁷ These authors reported this dose of the HLA-DRB*0101 allele to be significantly correlated to neonatal platelet counts as well. Whether this effect was through anti-HPA-1a levels and whether this has clinical implications independently of anti-HPA-1a levels, is unclear.

Fc-glycosylation of anti-HPA-1a antibodies – Immunoglobulin G (IgG) antibodies can vary in glycosylation pattern of the Fc-part, which influences the affinity to Fc-receptors and thus antibody effector activity.⁷⁸ This pattern is determined by the composition of a glycan attached to the Asn297 residue on the Fc-part and can vary in amount of, for example, galactose and core fucose. A lower level of this core fucose has been demonstrated to result in stronger binding to the Fc-receptors (FcγRIIIa and FcγRIIIb).⁷⁹ In archived samples of 48 cases of anti-HPA-1a mediated FNAIT, low levels of fucosylation on anti-HPA-1a specific antibodies led to an enhanced phagocytosis of platelets.⁸⁰ The same significant decrease in fucosylation was observed by Sonneveld *et al.*⁸¹ in a cohort of 80 anti-HPA-1a immunized women, showing a significant correlation with bleeding severity. Unfortunately, despite the large numbers of samples in both studies, the lack of prospectively collected samples and the lack of an optimal control group of immunized cases without disease, hamper the current use of these markers for disease prediction.

Endothelial damage - As discussed previously in *chapter 1*, binding and interaction with endothelial cells have been proposed to be correlated to the occurrence of ICH. *In vitro* studies have illuminated the direct interaction between anti-HPA-1a and human umbilical vein endothelial cells (HUVECs),

demonstrated by a decreased HUVEC spreading as well as a decreased integrity of their monolayer in electric cell-substrate impedance sensing (ECIS) assays.⁸² In addition, *in vivo* murine studies showed that mice without circulating platelets and/or fibrinogen do not show any bleeding problems in utero. This supports the hypothesis that, instead of just the thrombocytopenia, another mechanism might play a key role in causing bleeding complications. Recently, a large study with both active and passive murine models of anti-HPA-1a mediated FNAIT and showed that ICHs in these mice occurred regardless of platelet count.⁴³ Also, HPA-1a antibodies inhibited angiogenic signaling, induced endothelial cell apoptosis and decreased vessel density in affected brains as well as retinas. The first analysis with a small number of human sera containing HPA-1a antibodies suggested that three subtypes of anti-HPA-1a alloantibodies exist.⁸³ The first type, that only interacts with HPA-1a on $\beta 3$ when in complex with $\alpha 2b$, predominantly on platelets. A second type, interacting with HPA-1a on $\beta 3$ regardless of the complex, so with platelets and endothelial cells as well. And a third type, that binds specific to HPA-1a on $\beta 3$, when in complex with αV . This type of anti-HPA-1A alloantibodies might be $\alpha V\beta 3$ -specific, might therefore be responsible for and possibly predict the occurrence of ICH.⁸³

To evaluate this interaction and its clinical consequence even further we selected a series of 55 serum samples of anti-HPA-1a mediated FNAIT cases, 5 samples of anti-HPA-5b mediated FNAIT and 13 negative controls of HPA-1a positive pregnant women without HLA antibodies. Our aim was to demonstrate binding of the anti-HPA-1a from the maternal sera to HUVECs as well as a functional effect on endothelial cells, decreased angiogenesis. For both assays we used purified IgG fractions from the sera.

Flow-cytometry showed increased binding to HUVECs in the ICH cases (Figure 10.3). Binding of anti-HPA-1a cases without ICH, anti-HPA-5b mediated cases and negative controls were comparable. Although the differences in median between the groups were significant, the ranges in delta MFI within the groups were quite large. Still, cases with ICH did not show any binding in this assay, and vice versa. Also, there is a clear increase in binding when comparing the cases with and without anti-HLA Class-I alloantibodies. To eliminate this effect of anti-HLA on HUVEC and therefore overestimation of the binding of anti-HPA-1a in the sera to HUVEC, we plan to repeat these experiments with an immortalized HUVECs that does not express HLA Class I, through a HLA-ABC heavy chain knock-out. Alternatives might be the use of beads that express either $\alpha V\beta 3$ or $\alpha 2b\beta 3$ or to use HEK-cells that express either $\alpha V\beta 3$ or $\alpha 2b\beta 3$. This is currently work in progress.

Further, we used the same IgG fractions from our retrospectively collected FNAIT sera to evaluate functional effect of this binding of anti-HPA-1a to endothelial cells. Sprouting angiogenesis is fundamental for the expansion of blood vessels during embryonic development.⁸⁴ To mimic sprouting angiogenesis *in vitro* we performed a 3D bead-based sprouting assay (in collaboration with Coert Margadant) based on previous protocols with some modifications.^{85,86} At the time of writing this thesis, the first ten samples have been imaged, but not yet analyzed (Figure 10.4).

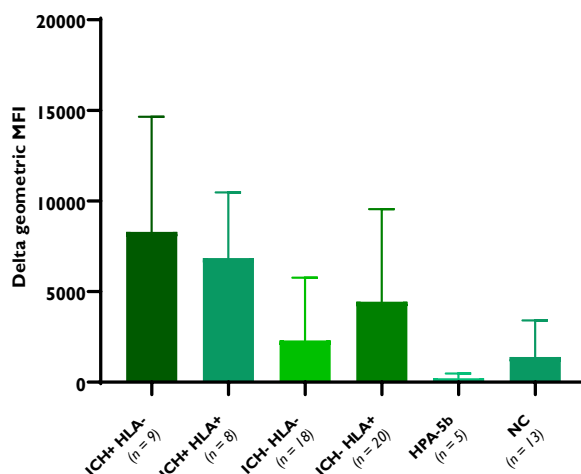


Figure 10.3 – Flow-cytometry assay: purified IgG from FNAIT sera and binding to HUVECs

HLA, human leukocyte antibody; HPA, human platelet antigen; HUVEC, human umbilical vein endothelial cell; ICH, intracranial hemorrhage; MFI, mean fluorescence index; NC, negative control.

Although it would be highly preferable, the chance of detecting that one marker that is perfectly correlated with disease severity and can be used for selecting pregnancies at high risk for adverse perinatal outcome is probably small. More likely, a set of the above-mentioned markers and/or assays is able to select the high-risk population that benefits from antenatal preventive treatment without the hazard of enormous overtreatment. An important pitfall with these identified markers is that they have never been tested on large series of samples and, even more important, they have never been compared to an adequate control group. For optimal identification of high-risk pregnancies, it is very important to compare immunized pregnancies with disease to immunized pregnancies without disease. The absence of this group in all previously described studies assessing potential predictors for disease severity hampered the identification of reliable and clinically applicable predictors. The HIP-study is collecting a large number of these cases, to be used for an adequate and specific risk assessment model. At this time it is uncertain if we can include the desired number of cases. Substituting this group with severe cases of ICH that were retrospectively identified, is complicated by the different time points at sampling (after birth in the retrospective cases and at 27 weeks' gestation in the prospective cases). Overall, whilst collecting a great and unique control group for the development of a specific risk-assessment model, still the hurdle of determining and optimizing sensitivity of such a model needs to be overcome.

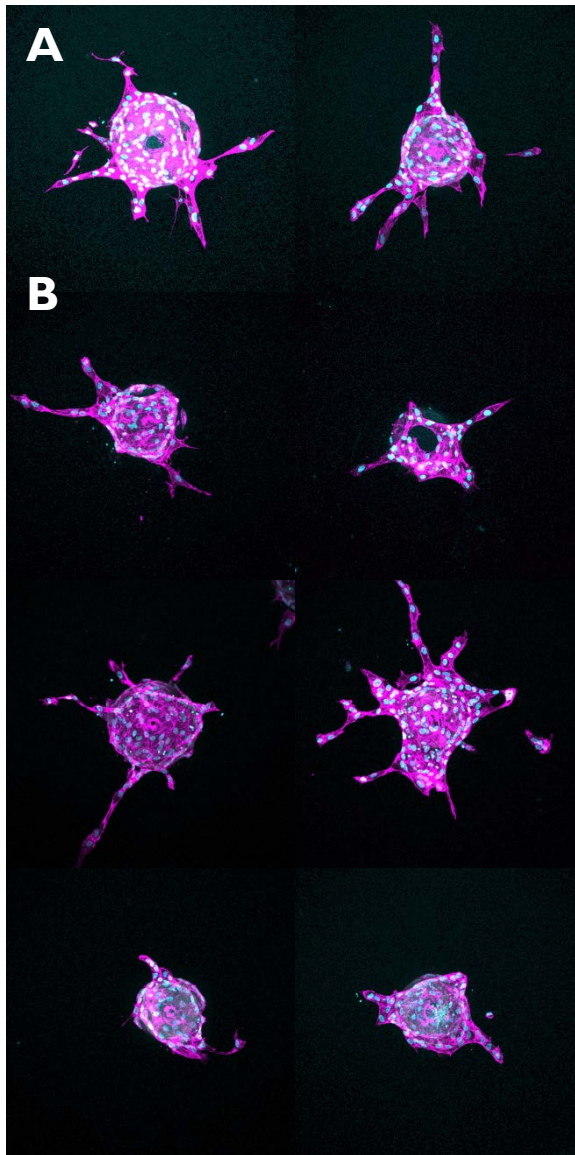


Figure 10.4 – Confocal images of sprouting assay

A. Two examples of beads without the added IgG fractions from FNAIT sera, positive control. **B.** Six examples of beads with the added IgG fractions from FNAIT sera.

Screening scenario

Timing of screening. The first decision to be made is whether screening should take place during pregnancy or postnatally. Killie and colleagues analyzed anti-HPA-1a immunizations and antibody levels from the Norwegian screening study and concluded that primary immunization occurs mainly during or after delivery and that FNAIT is very unlikely to occur in a first-time pregnancy.^{87,88} In contrast, retrospective data show a high proportion of primigravidae or first-born children suffering from severe FNAIT. So, there might be a difference between the moment of immunization in prospectively identified cases, of which the majority does not lead to disease, and the immunizations that do result in severe FNAIT. The goal of screening would be to prevent the latter. So, when designing a screening program, it would make sense to do so during pregnancy. Additionally, since severe cases of ICH can already occur in second trimester, we would suggest starting screening early in pregnancy. To optimize logistics, it could be easily added to the already implemented screening program for red blood cell immunization in the 12th week of gestation (Figure 10.5). First, from the complete pregnant population the group of HPA-1a negative women will need to be identified. In case of suggested HPA-1a negativity, it might be cost-saving to skip supportive genotyping, as performed in the HIP-study, and perform anti-HPA-1a antibody screening in all cases. The optimal timing of antibody screening needs to be determined. Logistically easiest, and similar to the red blood cell immunization screening, this could be done at 12 weeks' gestation, possibly within the same sample. This could be useful in all pregnancies but the first one: in a first pregnancy, antibodies may not yet be detectable. A (second) antibody screening between 18 and 20 weeks may be more useful. Not later, because bleeding may have already occurred.¹¹ An option for easy logistics would be to combine this with the 20 week-anomaly scan, which has an uptake of around 99%.⁴⁹

Non-immunized HPA-1a negative women. In case of negative antibody screening, adequate follow-up of HPA-1a negative women would be advised, at least a repeated antibody screening around 28-30 weeks and 6 weeks postpartum. These women might be the target population that would qualify for potential prophylaxis (NAITgam, developed by PROFNAIT project, described in chapter 1).⁸⁹

Immunized HPA-1a negative women. When anti-HPA-1a is detected, ideally a combination of risk-assessment markers should separate a low-risk from a high-risk group. IVIg treatment can then be given only to the remaining small high-risk group (estimated 50-100 women per year). Whereas the low-risk group still consists of HPA-1a alloimmunized pregnancies and no prospective screening and follow-up data on this groups exists yet, careful monitoring, at least for the first period after implementation of population-based screening, seems justified.

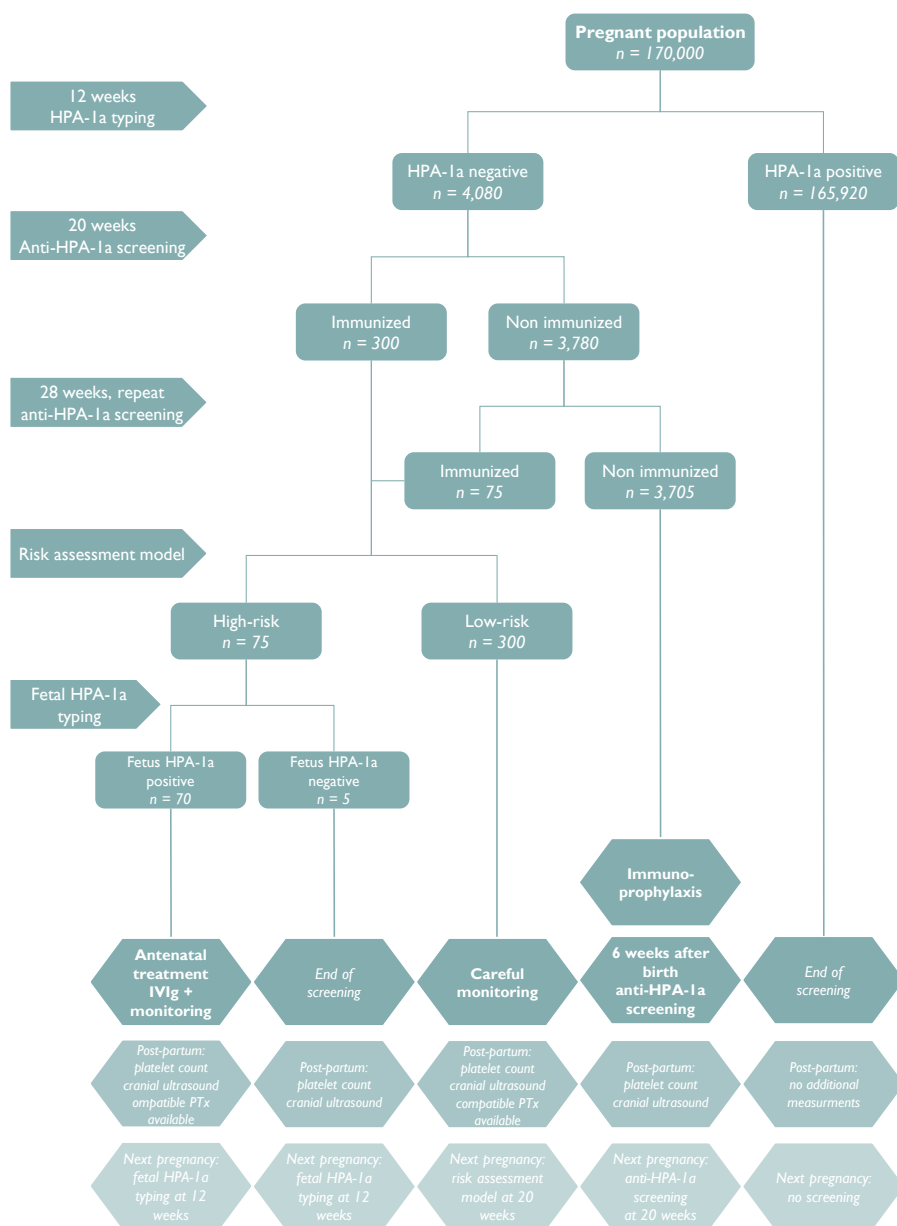


Figure 10.5 – Flow-chart of hypothetical screening scenario

With number of estimated cases per year in the Netherlands, based on a birth rate of 170,000⁴⁶.

Further, it might be considered to perform non-invasive fetal HPA-1a typing in either all identified HPA-1a negative pregnant women, to refrain from follow-up testing, or in (high-risk) immunized HPA-1a negative to refrain from unnecessary treatment if the fetus turns out to be HPA-1a negative as well.

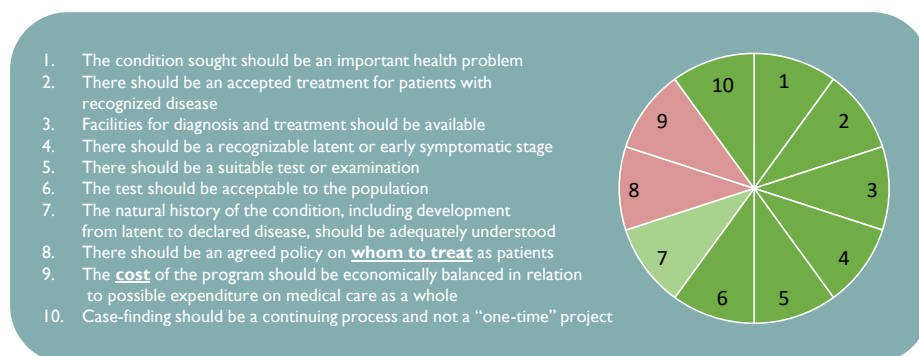


Figure 10.6 – Principles of early disease detection, Wilson & Jungner criteria⁵

Dark green, criterion fulfilled; light green, some extra information needed for fulfillment; light red, little information available but insufficient for fulfillment; dark red, no information available.

In conclusion - Evidence based screening

With this thesis, we have gathered a large amount of information for fulfillment of the W&J criteria (Figure 10.6). Furthermore, we describe the design and interim results of a promising prospective non-interventional study that is the base for collecting the remaining missing knowledge and for enabling a verdict on the feasibility and efficacy of potential population-based screening to prevent FNAIT. Thus far, we have established that FNAIT is an important health problem, for which acceptable and affordable diagnostic and treatment options are available. Further, we have developed a suitable test, we have reported on the acceptability of a potential screening program and presented the first preliminary results on natural history of the disease. These can be further confirmed when our prospective study (HIP-study) is completed in 2020. Still important knowledge, regarding the 8th and 9th criteria of W&J is missing (Figure 10.6). For this, we will need the prospectively collected samples of the HIP-study. Our unique, prospectively collected control group of immunized pregnancies without clinical disease will enable the development of a risk-assessment model to determine whom to treat as patients. Consequently, when we have more details of this policy, overall costs of the program can be calculated. We do expect that a national screening and prevention program for FNAIT will be feasible and cost-effective, and that implementation in the coming years will lead to a significant reduction in fetal and neonatal bleeding complications due to this disease.

References

1. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. *Transfusion* 2008; **48**(5): 941-952.
2. McBain RD, Crowther CA, Middleton P. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. *Cochrane Database Syst Rev* 2015; (9): Cd000020.
3. van der Ploeg K, Wins, S., Olthof, R., Eekhout, I., Verkerk, P.H. The newborn blood spot screening in the Netherlands - Monitor 2017: TNO - Innovation for life, 2018.
4. Verkerk PH, van Trotsenburg AS, Hoorweg-Nijman JJ, Oostdijk W, van Tijn DA, Kempers MJ, *et al.* [Neonatal screening for congenital hypothyroidism: more than 30 years of experience in the Netherlands]. *Ned Tijdschr Geneesk* 2014; **158**: A6564.
5. Wilson JM, Jungner YG. [Principles and practice of mass screening for disease]. *Bol Oficina Sanit Panam* 1968; **65**(4): 281-393.
6. Dudok de Wit C, Borst-Eilers E, Weerd CM, Kloosterman GJ. Prevention of rhesus immunization. A controlled clinical trial with a comparatively low dose of anti-D immunoglobulin. *Br Med J* 1968; **4**(5629): 477-479.
7. Radder CM, Brand A, Kanhai HH. Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? *Vox Sang* 2003; **84**(4): 318-325.
8. Davoren A, McParland P, Barnes CA, Murphy WG. Neonatal alloimmune thrombocytopenia in the Irish population: a discrepancy between observed and expected cases. *J Clin Pathol* 2002; **55**(4): 289-292.
9. Mao C, Guo J, Chituwo BM. Intraventricular haemorrhage and its prognosis, prevention and treatment in term infants. *J Trop Pediatr* 1999; **45**(4): 237-240.
10. Jocelyn LJ, Casiro OG. Neurodevelopmental outcome of term infants with intraventricular hemorrhage. *Am J Dis Child* 1992; **146**(2): 194-197.
11. Tiller H, Kamphuis MM, Flodmark O, Papadogiannakis N, David AL, Sainio S, *et al.* Fetal intracranial haemorrhages caused by fetal and neonatal alloimmune thrombocytopenia: an observational cohort study of 43 cases from an international multicentre registry. *BMJ Open* 2013; **3**(3).
12. Mueller-Eckhardt C, Mueller-Eckhardt G, Willen-Ohff H, Horz A, Kuenzlen E, O'Neill GJ, *et al.* Immunogenicity of and immune response to the human platelet antigen Zwa is strongly associated with HLA-B8 and DR3. *Tissue Antigens* 1985; **26**(1): 71-76.
13. Reznikoff-Etievant MF, Kaplan C, Muller JY, Daffos F, Forestier F. Allo-immune thrombocytopenias, definition of a group at risk; a prospective study. *Curr Stud Hematol Blood Transfus* 1988; (55): 119-124.
14. Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Decary F. Alloimmunization to the PIA1 platelet antigen: results of a prospective study. *Br J Haematol* 1990; **74**(2): 209-215.
15. Doughty HA, Murphy MF, Metcalfe P, Waters AH. Antenatal screening for fetal alloimmune thrombocytopenia: the results of a pilot study. *Br J Haematol* 1995; **90**(2): 321-325.
16. Durand-Zaleski I, Schlegel N, Blum-Boisgard C, Uzan S, Dreyfus M, Kaplan C. Screening primiparous women and newborns for fetal/neonatal alloimmune thrombocytopenia: a prospective comparison of effectiveness and costs. Immune Thrombocytopenia Working Group. *Am J Perinatol* 1996; **13**(7): 423-431.
17. Williamson LM, Hackett G, Rennie J, Palmer CR, Maciver C, Hadfield R, *et al.* The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PIA1, Zwa) as determined by antenatal screening. *Blood* 1998; **92**(7): 2280-2287.
18. Davoren A, McParland P, Crowley J, Barnes A, Kelly G, Murphy WG. Antenatal screening for human platelet antigen-1a: results of a prospective study at a large maternity hospital in Ireland. *BJOG* 2003; **110**(5): 492-496.

19. Maslanka K, Guz K, Zupanska B. Antenatal screening of unselected pregnant women for HPA-1a antigen, antibody and alloimmune thrombocytopenia. *Vox Sang* 2003; **85**(4): 326-327.
20. Turner ML, Bessos H, Fagge T, Harkness M, Rentoul F, Seymour J, *et al.* Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. *Transfusion* 2005; **45**(12): 1945-1956.
21. Kjeldsen-Kragh J, Killie MK, Tomter G, Golebiowska E, Randen I, Hauge R, *et al.* A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. *Blood* 2007; **110**(3): 833-839.
22. Debska M, Uhrynowska M, Guz K, Kopec I, Lachert E, Orzinska A, *et al.* Identification and follow-up of pregnant women with platelet-type human platelet antigen (HPA)-1bb alloimmunized with fetal HPA-1a. *Arch Med Sci* 2018; **14**(5): 1041-1047.
23. Baber J, Kheyfets S, Sumfest J. A Rare Case of Neonatal Alloimmune Thrombocytopenia Causing Prolonged Postcircumcision Bleeding. *Urology* 2015; **85**(6): 1474-1476.
24. Jeronimo M, Azenha C, Mesquita J, Pereira DF. A rare manifestation of neonatal alloimmune thrombocytopenia. *BMJ Case Rep* 2014; **2014**.
25. Cook TJ, Qiu CC, Dickinson JE. A review of the contemporary management of fetal and neonatal alloimmune thrombocytopenia in an Australian tertiary obstetric hospital. *Aust N Z J Obstet Gynaecol* 2012; **52**(4): 321-326.
26. Borensztajn DM, Jansen S, Lopriore E, Boersma B. Thrombocytopenia in two newborn babies. Unexpected serious complications in full-term babies. *Ned Tijdschr Geneesk* 2010; **154**: A1922.
27. Nomura ML, Couto E, Martinelli BM, Barjas-Castro ML, Barini R, Passini Junior R, *et al.* Fetal genotyping for platelets antigens: a precise tool for alloimmune thrombocytopenia: case report and literature review. *Arch Gynecol Obstet* 2010; **282**(5): 573-575.
28. Ghevaert C, Campbell K, Walton J, Smith GA, Allen D, Williamson LM, *et al.* Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. *Transfusion* 2007; **47**(5): 901-910.
29. Paladini D, Maruotti GM, Sglavo G, Fratellanza G, Quarantelli M, Martinelli P. Massive fetal hemorrhage and fetomaternal alloimmune thrombocytopenia from human platelet antigen 5b incompatibility: an unusual association. *Ultrasound Obstet Gynecol* 2007; **29**(4): 471-474.
30. Rousseau J, Goldman M, David M. HPA-5b (Bra) neonatal alloimmune thrombocytopenia in Quebec: incidence and clinical outcome in 31 cases. *Transfusion* 2004; **44**(6): 844-848.
31. Abel M, Bona M, Zawodniak L, Sultan R, Masterson M. Cervical spinal cord hemorrhage secondary to neonatal alloimmune thrombocytopenia. *J Pediatr Hematol Oncol* 2003; **25**(4): 340-342.
32. Tomicic M, Dekovic M, Jaksic J, Stoini E, Drazic V, Grahovac B, *et al.* Neonatal alloimmune thrombocytopenic purpura caused by anti-HPA-1a alloantibodies. Case report. *Lijec Vjesn* 2001; **123**(3-4): 70-73.
33. Kankirawatana S, Kupatawintu P, Juji T, Veerakul G, Ngercham S, Chongkolwatana V, *et al.* Neonatal alloimmune thrombocytopenia due to anti-Nak(a). *Transfusion* 2001; **41**(3): 375-377.
34. Mokhtari M, Kaplan C, Gourrier E, Guyader AM, Lerailliez J. Neonatal alloimmune thrombopenia in anti-HPA-3a (Baka) immunization. *Arch Pediatr* 1997; **4**(4): 339-342.
35. Allen D. Neonatal alloimmune thrombocytopenia due to anit-HPA-5b(Br(a), Zav(a), Hca): the importance of third-generation platelet antibody detection techniques, a case report. *Transfus Med* 1992; **2**(4): 4.
36. Puig N, Muniz-Diaz E, Monteagudo E, Ribera A, Montoro JA. A second case of neonatal alloimmune thrombocytopenia by anti-HPA-4b (anti-Yuka) in a Caucasian family. *Transfus Med* 1993; **3**(2): 164-165.
37. Kaplan C, Morel-Kopp MC, Kroll H, Kiefel V, Schlegel N, Chesnel N, *et al.* HPA-5b (Br(a)) neonatal alloimmune thrombocytopenia: clinical and immunological analysis of 39 cases. *Br J Haematol* 1991; **78**(3): 425-429.
38. Kumpel BM, Sibley K, Jackson DJ, White G, Soothill PW. Ultrastructural localization of glycoprotein IIIa (GPIIIa, beta 3 integrin) on placental syncytiotrophoblast microvilli: implications for platelet alloimmunization during pregnancy. *Transfusion* 2008; **48**(10): 2077-2086.

39. Campbell S, Swann HR, Seif MW, Kimber SJ, Aplin JD. Cell adhesion molecules on the oocyte and preimplantation human embryo. *Hum Reprod* 1995; **10**(6): 1571-1578.
40. Gagnon R. Placental insufficiency and its consequences. *Eur J Obstet Gynecol Reprod Biol* 2003; **110 Suppl 1**: S99-107.
41. Jivraj S, Anstie B, Cheong YC, Fairlie FM, Laird SM, Li TC. Obstetric and neonatal outcome in women with a history of recurrent miscarriage: a cohort study. *Hum Reprod* 2001; **16**(1): 102-106.
42. D.W, editor. The Immunoassay Handbook. 4th edition ed. Waltham: Elsevier; 2013.
43. Yougbare I, Lang S, Yang H, Chen P, Zhao X, Tai WS, *et al.* Maternal anti-platelet beta3 integrins impair angiogenesis and cause intracranial hemorrhage. *J Clin Invest* 2015; **125**(4): 1545-1556.
44. Tiller H, Killie MK, Husebekk A, Skogen B, Ni H, Kjeldsen-Kragh J, *et al.* Platelet antibodies and fetal growth: maternal antibodies against fetal platelet antigen 1a are strongly associated with reduced birthweight in boys. *Acta Obstet Gynecol Scand* 2012; **91**(1): 79-86.
45. Christensen RD, Baer VL, Henry E, Snow GL, Butler A, Sola-Visner MC. Thrombocytopenia in Small-for-Gestational-Age Infants. *Pediatrics* 2015; **136**(2): e361-370.
46. Centraal Bureau voor de Statistiek. Geboorte; kerncijfers. Den Haag/Heerlen: CBS; 2017.
47. Committee prevention pregnancy immunisation. [Prevention of pregnancy immunisation]. *The Hague: Dutch Health Council* 1992; **50**: [Dutch].
48. Koelewijn JM, de Haas M, Vrijkotte TG, Bonsel GJ, van der Schoot CE. One single dose of 200 microg of antenatal RhIG halves the risk of anti-D immunization and hemolytic disease of the fetus and newborn in the next pregnancy. *Transfusion* 2008; **48**(8): 1721-1729.
49. Centraal Begeleidingsorgaan CBO. Richtlijn Bloedtransfusie. 2011. URL:<https://nvic.nl/sites/default/files/Richtlijnen%20aanmaken/CBO%20Richtlijn%20Bloedtransfusie.pdf> (accessed 22 may 2019).
50. Adams JMF, C.J. Guidelines for acute care of the neonate. Houston: Baylor College of Medicine; 2014.
51. Gibson BE, Todd A, Roberts I, Pamphilon D, Rodeck C, Bolton-Maggs P, *et al.* Transfusion guidelines for neonates and older children. *Br J Haematol* 2004; **124**(4): 433-453.
52. Baker JM, Shehata N, Bussel J, Murphy MF, Greinacher A, Bakchoul T, *et al.* Postnatal intervention for the treatment of FNAIT: a systematic review. *J Perinatol* 2019.
53. te Pas AB, Lopriore E, van den Akker ES, Oepkes D, Kanhai HH, Brand A, *et al.* Postnatal management of fetal and neonatal alloimmune thrombocytopenia: the role of matched platelet transfusion and IVIG. *Eur J Pediatr* 2007; **166**(10): 1057-1063.
54. Estcourt LJ. Platelet transfusion thresholds in premature neonates (PlaNeT-2 trial). *Transfus Med* 2019; **29**(1): 20-22.
55. Daffos F, Forestier F, Muller JY, Reznikoff-Etievant M, Habibi B, Capella-Pavlovsky M, *et al.* Prenatal treatment of alloimmune thrombocytopenia. *Lancet* 1984; **2**(8403): 632.
56. Regan F, Lees CC, Jones B, Nicolaides KH, Wimalasundera RC, Mijovic A. Prenatal Management of Pregnancies at Risk of Fetal Neonatal Alloimmune Thrombocytopenia (FNAIT): Scientific Impact Paper No. 61. *Bjog* 2019.
57. Ronzoni S, Keunen J, Shah PS, Kelly EN, Windrim R, Seaward PG, *et al.* Management and Neonatal Outcomes of Pregnancies with Fetal/Neonatal Alloimmune Thrombocytopenia: A Single-Center Retrospective Cohort Study. *Fetal Diagn Ther* 2019; **45**(2): 85-93.
58. Berkowitz RL, Kolb EA, McFarland JG, Wissert M, Primani A, Lesser M, *et al.* Parallel randomized trials of risk-based therapy for fetal alloimmune thrombocytopenia. *Obstet Gynecol* 2006; **107**(1): 91-96.
59. Rayment R, Brunskill SJ, Soothill PW, Roberts DJ, Bussel JB, Murphy MF. Antenatal interventions for fetomaternal alloimmune thrombocytopenia. *Cochrane Database Syst Rev* 2011; (5): Cd004226.
60. Van Der Lugt NM, Kamphuis MM, Paridaans NP, Figee A, Oepkes D, Walther FJ, *et al.* Neonatal outcome in alloimmune thrombocytopenia after maternal treatment with intravenous immunoglobulin. *Blood Transfus* 2015; **13**(1): 66-71.

61. Radder CM, Brand A, Kanhai HH. A less invasive treatment strategy to prevent intracranial hemorrhage in fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2001; **185**(3): 683-688.
62. Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust N Z J Obstet Gynaecol* 2001; **41**(1): 45-55.
63. Roy S, Nanovskaya T, Patrikeeva S, Cochran E, Parge V, Guess J, *et al.* M281, an anti-FcRn antibody, inhibits IgG transfer in a human ex vivo placental perfusion model. *Am J Obstet Gynecol* 2019; **220**(5): 498.e491-498.e499.
64. Ling LE, Hillson JL, Tiessen RG, Bosje T, van Iersel MP, Nix DJ, *et al.* M281, an Anti-FcRn Antibody: Pharmacodynamics, Pharmacokinetics, and Safety Across the Full Range of IgG Reduction in a First-in-Human Study. *Clin Pharmacol Ther* 2019; **105**(4): 1031-1039.
65. Killie MK, Kjeldsen-Kragh J, Husebekk A, Skogen B, Olsen JA, Kristiansen IS. Cost-effectiveness of antenatal screening for neonatal alloimmune thrombocytopenia. *BJOG* 2007; **114**(5): 588-595.
66. Lieberman L, Greinacher A, Murphy MF, Bussel J, Bakchoul T, Corke S, *et al.* Fetal and neonatal alloimmune thrombocytopenia: recommendations for evidence-based practice, an international approach. *Br J Haematol* 2019; **185**(3): 549-562.
67. Michie S, Dormandy E, Marteau TM. The multi-dimensional measure of informed choice: a validation study. *Patient Educ Couns* 2002; **48**(1): 87-91.
68. Marteau TM, Dormandy E, Michie S. A measure of informed choice. *Health Expect* 2001; **4**(2): 99-108.
69. Kjaer MB, G.; Bakchoul, T.; Massey, E.; Baker, J.M.; Lieberman, L.; Tanael, S.; Greinacher, A.; Murphy, M.F.; Arnold, D.M.; Baidya, S.; Bussel, J.; Hume, H.; Kaplan, C.; Oepkes D.; Ryan, G.; Savoia, H.; Shehata, N.; Kjeldsen-Kragh, J.; International Collaboration for Transfusion Medicine Guidelines. Maternal HPA-1a antibody level and its role in predicting the severity of Fetal/Neonatal Alloimmune Thrombocytopenia: a systematic review. *Vox Sang* 2019; **114**(4): 79-94.
70. Ghevaert C, Campbell K, Stafford P, Metcalfe P, Casbard A, Smith GA, *et al.* HPA-1a antibody potency and bioactivity do not predict severity of fetomaternal alloimmune thrombocytopenia. *Transfusion* 2007; **47**(7): 1296-1305.
71. Bertrand G, Martageix C, Jallu V, Vitry F, Kaplan C. Predictive value of sequential maternal anti-HPA-1a antibody concentrations for the severity of fetal alloimmune thrombocytopenia. *J Thromb Haemost* 2006; **4**(3): 628-637.
72. Sainio S, Javela K, Tuimala J, Koskinen S. Usefulness of maternal anti-HPA-1a antibody quantitation in predicting severity of foetomaternal alloimmune thrombocytopenia. *Transfus Med* 2013; **23**(2): 114-120.
73. Delbos F, Bertrand G, Croisille L, Ansart-Pirenne H, Bierling P, Kaplan C. Fetal and neonatal alloimmune thrombocytopenia: predictive factors of intracranial hemorrhage. *Transfusion* 2016; **56**(1): 59-66.
74. Loewenthal R, Rosenberg N, Kalt R, Dardik R, Landau M, Yahalom V, *et al.* Compound heterozygosity of HLA-DRB3*01:01 and HLA-DRB4*01:01 as a potential predictor of fetal neonatal alloimmune thrombocytopenia. *Transfusion* 2013; **53**(2): 344-352.
75. Wienzek-Lischka S, König IR, Papenkort EM, Hackstein H, Santoso S, Sachs UJ, *et al.* HLA-DRB3*01:01 is a predictor of immunization against human platelet antigen-1a but not of the severity of fetal and neonatal alloimmune thrombocytopenia. *Transfusion* 2017; **57**(3): 533-540.
76. Kjeldsen-Kragh J, Olsen KJ. Risk of HPA-1a-immunization in HPA-1a negative women after giving birth to an HPA-1a-positive child. *Transfusion* 2019; **59**(4): 1344-1352.
77. Kjeldsen-Kragh J, Titze TL, Lie BA, Vaage JT, Kjaer M. HLA-DRB3*01:01 exhibits a dose-dependent impact on HPA-1a antibody levels in HPA-1a-immunized women. *Blood Adv* 2019; **3**(7): 945-951.
78. Nimmerjahn F, Ravetch JV. Anti-inflammatory actions of intravenous immunoglobulin. *Annu Rev Immunol* 2008; **26**: 513-533.
79. Mizushima T, Yagi H, Takemoto E, Shibata-Koyama M, Isoda Y, Iida S, *et al.* Structural basis for improved efficacy of therapeutic antibodies on defucosylation of their Fc glycans. *Genes Cells* 2011; **16**(11): 1071-1080.

80. Kapur R, Kustiawan I, Vestrheim A, Koeleman CA, Visser R, Einarsdottir HK, *et al.* A prominent lack of IgG1-Fc fucosylation of platelet alloantibodies in pregnancy. *Blood* 2014; **123**(4): 471-480.
81. Sonneveld ME, Natunen S, Sainio S, Koeleman CA, Holst S, Dekkers G, *et al.* Glycosylation pattern of anti-platelet IgG is stable during pregnancy and predicts clinical outcome in alloimmune thrombocytopenia. *Br J Haematol* 2016.
82. van Gils JM, Stutterheim J, van Duijn TJ, Zwaginga JJ, Porcelijn L, de Haas M, *et al.* HPA-1a alloantibodies reduce endothelial cell spreading and monolayer integrity. *Mol Immunol* 2009; **46**(3): 406-415.
83. Santoso S, Wihadmadayati H, Bakchoul T, Werth S, Al-Fakhri N, Bein G, *et al.* Antiendothelial alphavbeta3 Antibodies Are a Major Cause of Intracranial Bleeding in Fetal/Neonatal Alloimmune Thrombocytopenia. *Arterioscler Thromb Vasc Biol* 2016; **36**(8): 1517-1524.
84. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell* 2011; **146**(6): 873-887.
85. Nowak-Sliwinska P, Alitalo K, Allen E, Anisimov A, Aplin AC, Auerbach R, *et al.* Consensus guidelines for the use and interpretation of angiogenesis assays. *Angiogenesis* 2018; **21**(3): 425-532.
86. Nakatsu MN, Hughes CC. An optimized three-dimensional in vitro model for the analysis of angiogenesis. *Methods Enzymol* 2008; **443**: 65-82.
87. Skogen B, Husebekk A, Killie MK, Kjeldsen-Kragh J. Neonatal alloimmune thrombocytopenia is not what it was: a lesson learned from a large prospective screening and intervention program. *Scand J Immunol* 2009; **70**(6): 531-534.
88. Killie MK, Husebekk A, Kjeldsen-Kragh J, Skogen B. A prospective study of maternal anti-HPA 1a antibody level as a potential predictor of alloimmune thrombocytopenia in the newborn. *Haematologica* 2008; **93**(6): 870-877.
89. Prophylis AS. Emergent BioSolutions to manufacture Prophylis AS developmental drug for fetal-neonatal alloimmune thrombocytopenia. 2016. <https://www.naitbabies.org/resources/profnait-project/> (accessed May 21 2019).

