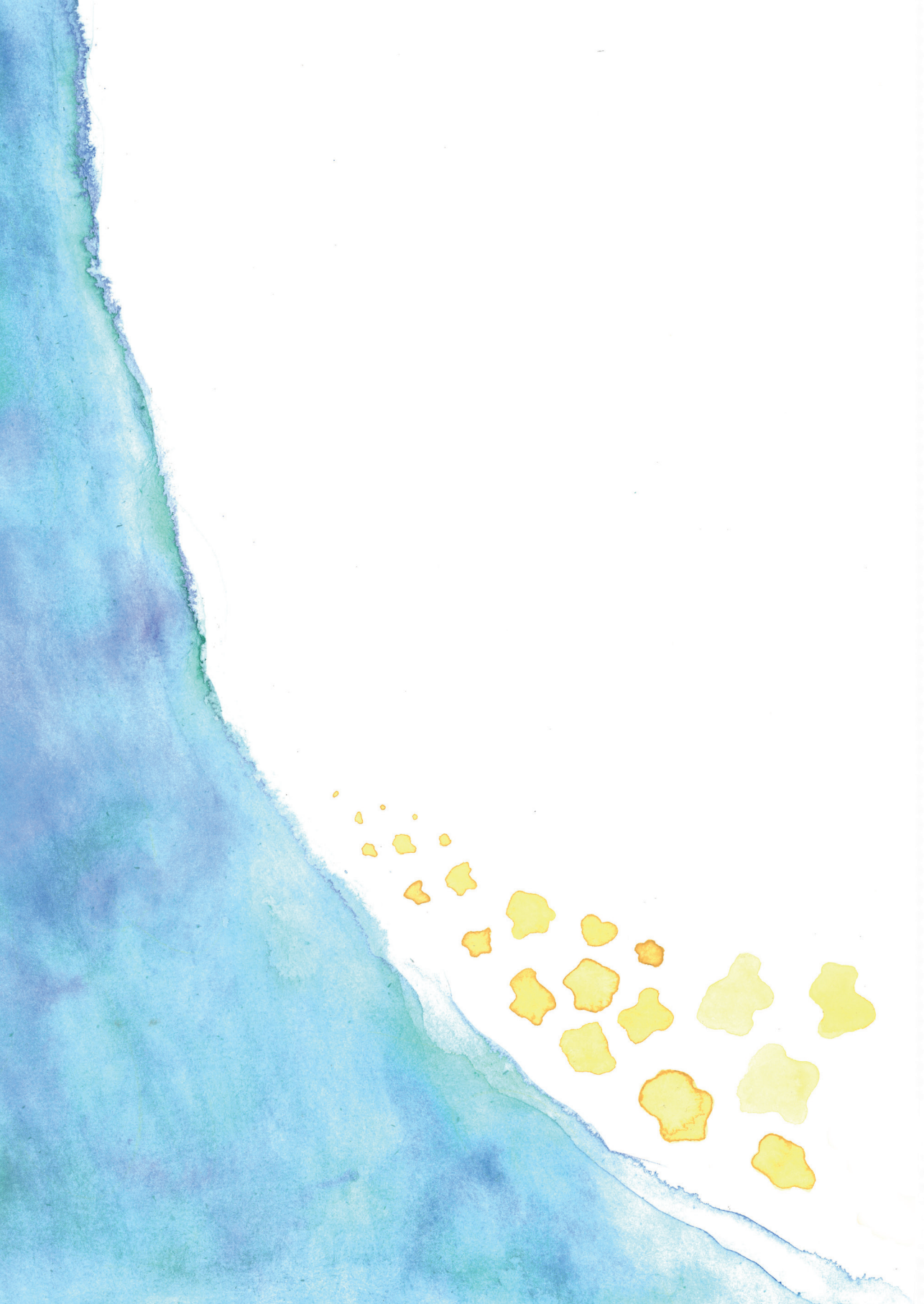
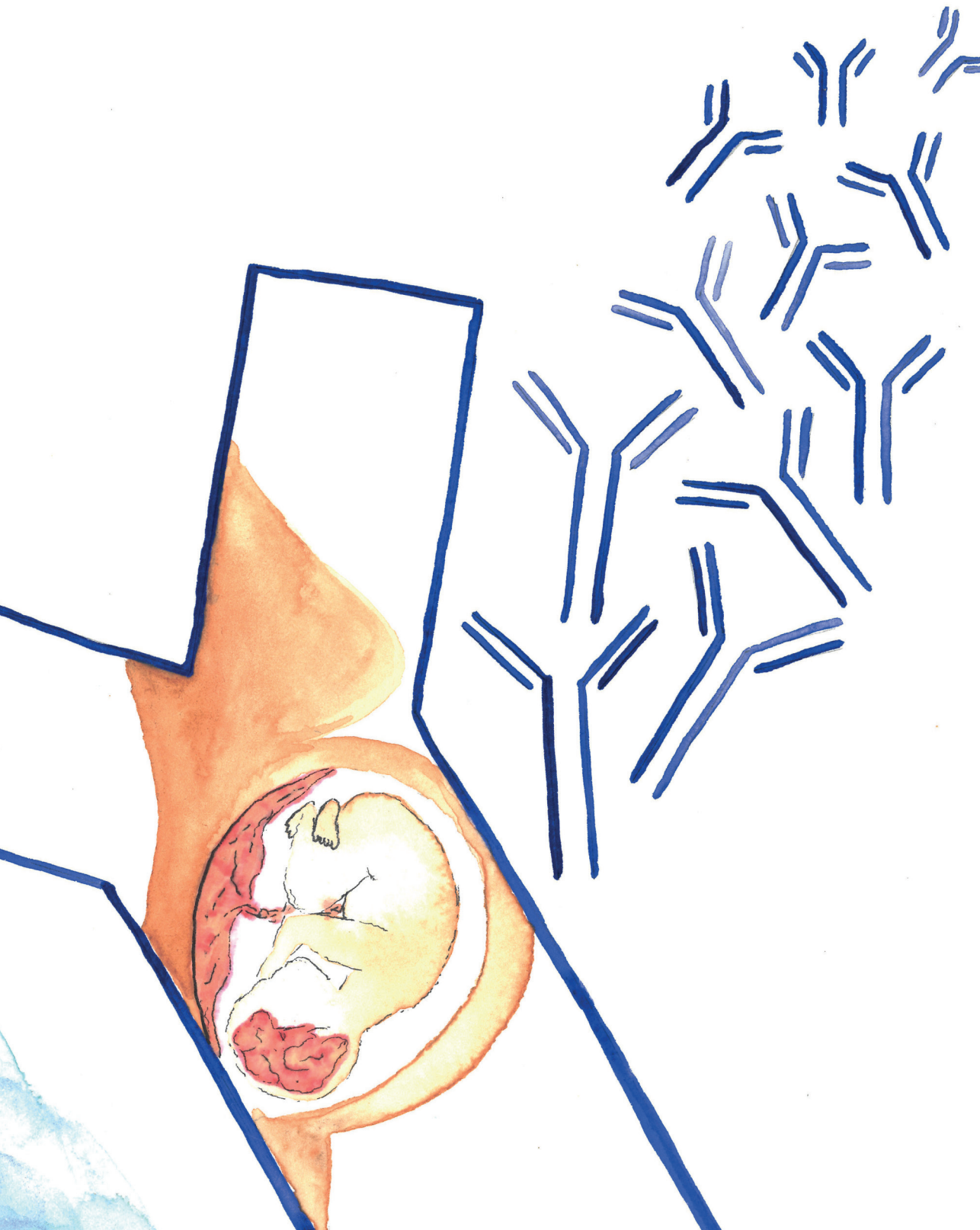
Fetal and neonatal alloimmune thrombocytopenia: the proof of the pudding is in the eating

Vos, T.W. de



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.

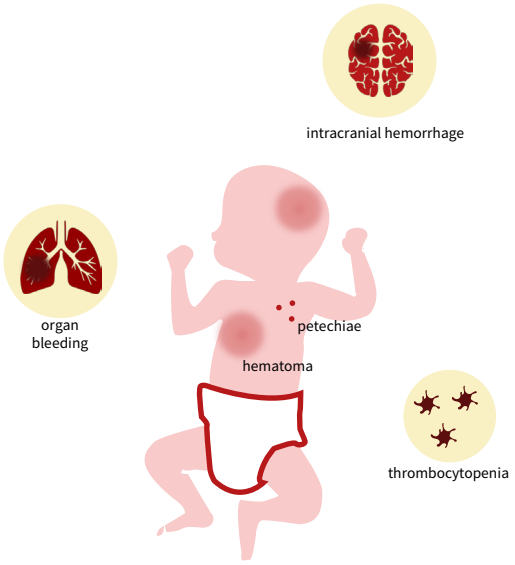


FIGURE 1. Clinical outcome in FNAIT

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.

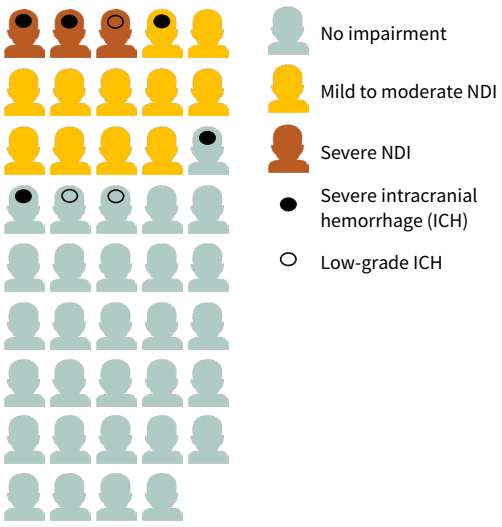


FIGURE 3. Neurodevelopmental impairment (NDI) in newly diagnosed FNAIT cases.

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

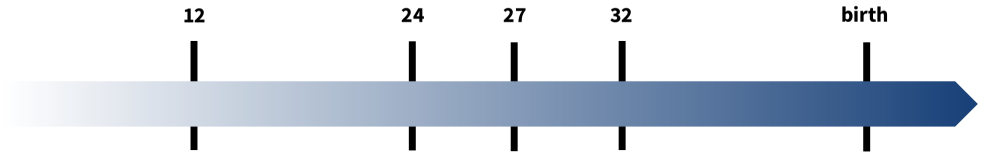
	Mother HPA-1ab	Mother HPA-1bb
Observed – n (%)	19 (86)	3 (14)
Expected – n (%)	20 (92)	2 (8)

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

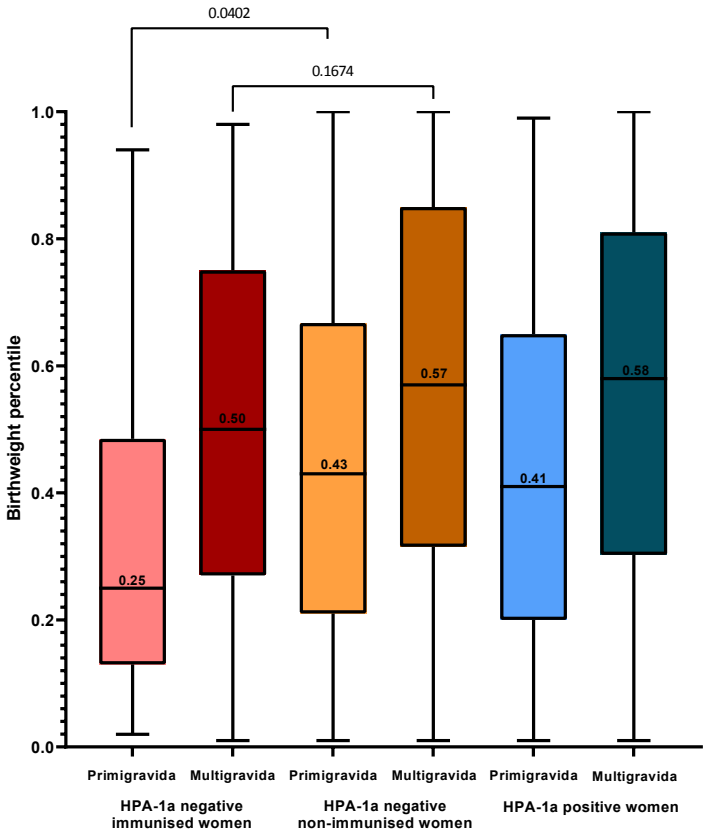


FIGURE 5. Birthweight percentile stratified for primigravida and primiparous women
This figure shows birthweight percentiles in the HIP study stratified by primigravida and multigravida pregnancies. Median percentiles were compared using the Kruskal-Wallis Test.
Abbreviation: HPA, human platelet antigen.

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.

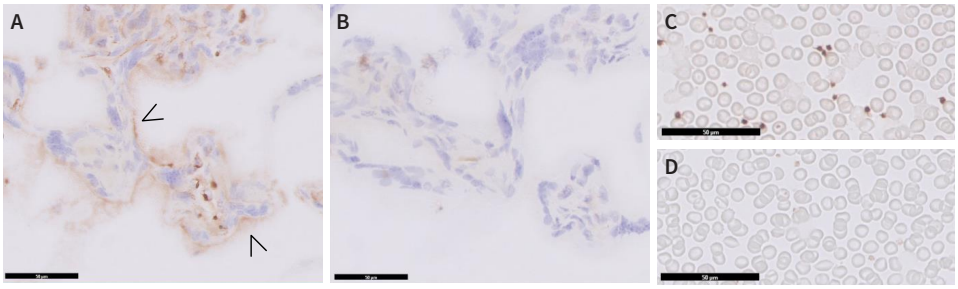


FIGURE 6. Binding of anti-HPA-1a to placenta and platelets

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]

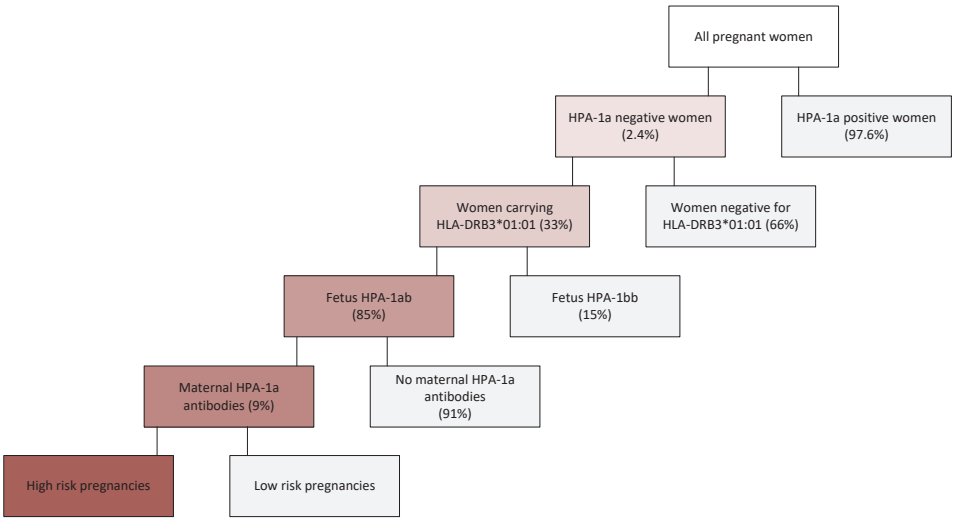


FIGURE 7. Flowchart selection of population at risk
Figure 7 shows a proposal in which women with a high-risk pregnancy are selected from the total pregnant population of pregnant women.
Abbreviations: HPA, human platelet antigen; HLA, human leukocyte antigen.

*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).

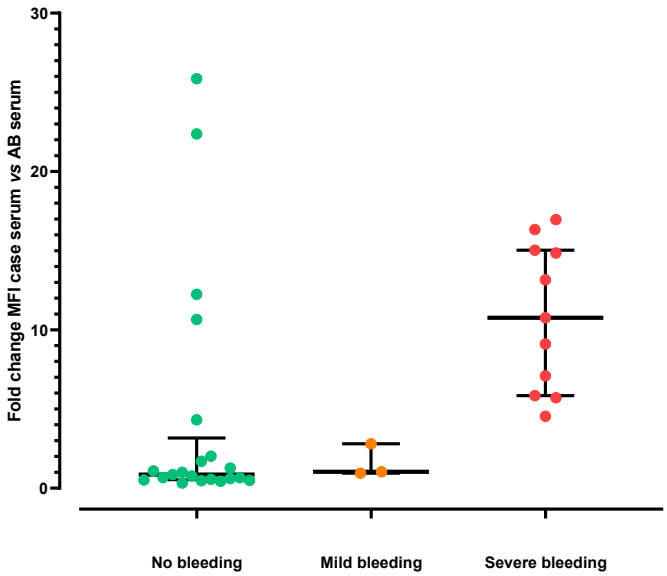


FIGURE 8. Endothelial reactivity and clinical outcome

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.

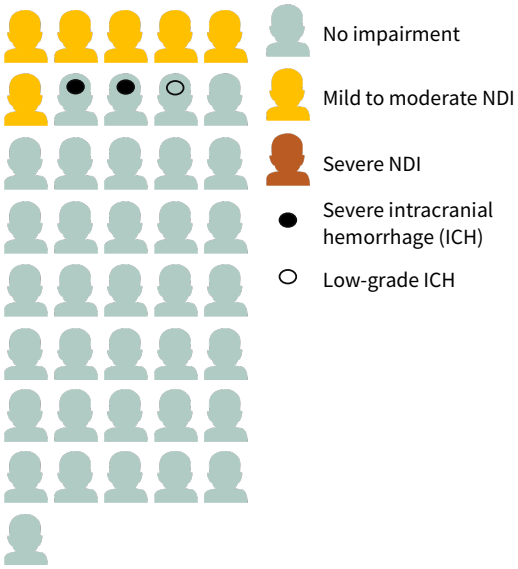


FIGURE 9. Neurodevelopmental impairment after antenatal IVIg treatment in FNAIT

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.

REFERENCES

1. Kamphuis MM, Paridaans NP, Porcelijn L, Lopriore E, Oepkes D. Incidence and consequences of neonatal alloimmune thrombocytopenia: a systematic review. Pediatrics. 2014;133(4):715-21.
2. Dreyfus M, Kaplan C, Verdy E, Schlegel N, Durand-Zaleski I, Tchernia G. Frequency of immune thrombocytopenia in newborns: a prospective study. Immune Thrombocytopenia Working Group. Blood. 1997;89(12):4402-6.
3. Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion. 2004;44(8):1220-5.
4. Van Loghem Jj J, Dorfmeijer H, Van Hart M, Schreuder F. Serological and genetical studies on a platelet antigen (Zw). Vox sanguinis. 1959;4(2):161-9.
5. Leeksma OC, Giltay JC, Zandbergen-Spaargaren J, Modderman PW, van Mourik JA, von dem Borne AE. The platelet alloantigen Zwa or PlA1 is expressed by cultured endothelial cells. British journal of haematology. 1987;66(3):369-73.
6. Felding-Habermann B, Cheresh DA. Vitronectin and its receptors. Current opinion in cell biology. 1993;5(5):864-8.
7. 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):2077-86.
8. Kapur R, Heitink-Pollé KM, Porcelijn L, Bentlage AE, Bruin MC, Visser R, et al. C-reactive protein enhances IgG-mediated phagocyte responses and thrombocytopenia. Blood. 2015;125(11):1793-802.
9. Yougbare I, Zdravic D, Ni H. Angiogenesis and bleeding disorders in FNAIT. Oncotarget. 2015;6(18):15724-5.
10. 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. Molecular immunology. 2009;46(3):406-15.
11. Santoso S, Wihadmadyatami H, Bakchoul T, Werth S, Al-Fakhri N, Bein G, et al. Antiendothelial αvβ3 Antibodies Are a Major Cause of Intracranial Bleeding in Fetal/Neonatal Alloimmune Thrombocytopenia. Arteriosclerosis, thrombosis, and vascular biology. 2016;36(8):1517-24.
12. Yougbaré I, Tai WS, Zdravic D, Oswald BE, Lang S, Zhu G, et al. Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. Nature communications. 2017;8(1):224.
13. Refsum E, Meinke S, Gryfelt G, Wikman A, Höglund P. Adding to the complexity of fetal and neonatal alloimmune thrombocytopenia: Reduced fibrinogen binding in the presence of anti-HPA-1a antibody and hypo-responsive neonatal platelets. Thrombosis research. 2018;162:69-76.
14. Liu ZJ, Bussel JB, Lakkaraja M, Ferrer-Marin F, Ghevaert C, Feldman HA, et al. Suppression of in vitro megakaryopoiesis by maternal sera containing anti-HPA-1a antibodies. Blood. 2015;126(10):1234-6.
15. Versiti. HPA Database 2020 [Available from: https://www.versiti.org/hpa.
16. Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S. The natural history of maternal immunization against foetal platelet alloantigens. Transfusion medicine (Oxford, England). 2004;14(6):399-408.
17. Curtis BR, Ali S, Glazier AM, Ebert DD, Aitman TJ, Aster RH. Isoimmunization against CD36 (glycoprotein IV): description of four cases of neonatal isoimmune thrombocytopenia and brief review of the literature. Transfusion. 2002;42(9):1173-9.
18. Ernstsen SL, Ahlen MT, Johansen T, Bertelsen EL, Kjeldsen-Kragh J, Tiller H. Antenatal intravenous immunoglobulins in pregnancies at risk of fetal and neonatal alloimmune thrombocytopenia: comparison of neonatal outcome in treated and nontreated pregnancies. American journal of obstetrics and gynecology. 2022.
19. 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 sanguinis. 2003;84(4):318-25.
20. 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. British journal of haematology. 2019;185(3):549-62.
21. Jocelyn LJ, Casiro OG. Neurodevelopmental outcome of term infants with intraventricular hemorrhage. American journal of diseases of children (1960). 1992;146(2):194-7.
22. 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).

23. Mao C, Guo J, Chituwo BM. Intraventricular haemorrhage and its prognosis, prevention and treatment in term infants. Journal of tropical pediatrics. 1999;45(4):237-40.
24. Winkelhorst D, Kamphuis MM, Steggerda SJ, Rijken M, Oepkes D, Lopriore E, et al. Perinatal Outcome and Long-Term Neurodevelopment after Intracranial Haemorrhage due to Fetal and Neonatal Alloimmune Thrombocytopenia. Fetal diagnosis and therapy. 2019;45(3):184-91.
25. Greenberg DA, Jin K. From angiogenesis to neuropathology. Nature. 2005;438(7070):954-9.
26. Hadders-Algra M. Two distinct forms of minor neurological dysfunction: perspectives emerging from a review of data of the Groningen Perinatal Project. Developmental medicine and child neurology. 2002;44(8):561-71.
27. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, et al. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? The Journal of clinical investigation. 1997;99(9):2139-51.
28. Bowman JM, Pollock JM, Penston LE. Fetomaternal transplacental hemorrhage during pregnancy and after delivery. Vox sanguinis. 1986;51(2):117-21.
29. 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 (PlA1, Zwa) as determined by antenatal screening. Blood. 1998;92(7):2280-7.
30. Murrieta-Coxca JM, Fuentes-Zacarias P, Ospina-Prieto S, Markert UR, Morales-Prieto DM. Synergies of Extracellular Vesicles and Microchimerism in Promoting Immunotolerance During Pregnancy. Frontiers in immunology. 2022;13:837281.
31. Valentin N, Visentin GP, Newman PJ. Involvement of the cysteine-rich domain of glycoprotein IIIa in the expression of the human platelet alloantigen, PlA1: evidence for heterogeneity in the humoral response. Blood. 1995;85(11):3028-33.
32. Levine P. The influence of the ABO system on Rh hemolytic disease. Human biology. 1958;30(1):14-28.
33. Zwiers C, Koelewijn JM, Vermij L, van Sambeeck J, Oepkes D, de Haas M, et al. ABO incompatibility and RhIG immunoprophylaxis protect against non-D alloimmunization by pregnancy. Transfusion. 2018;58(7):1611-7.
34. Szulman AE. The A, B and H blood-group antigens in human placenta. The New England journal of medicine. 1972;286(19):1028-31.
35. Ahlen MT, Husebekk A, Killie MK, Kjeldsen-Kragh J, Olsson ML, Skogen B. The development of severe neonatal alloimmune thrombocytopenia due to anti-HPA-1a antibodies is correlated to maternal ABO genotypes. Clinical & developmental immunology. 2012;2012:156867.
36. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(2):705-8.
37. Maloney S, Smith A, Furst DE, Myerson D, Rupert K, Evans PC, et al. Microchimerism of maternal origin persists into adult life. The Journal of clinical investigation. 1999;104(1):41-7.
38. Owen RD, Wood HR, Foord AG, Sturgeon P, Baldwin LG. EVIDENCE FOR ACTIVELY ACQUIRED TOLERANCE TO Rh ANTIGENS. Proceedings of the National Academy of Sciences of the United States of America. 1954;40(6):420-4.
39. Claas FH, Gijbels Y, van der Velden-de Munck J, van Rood JJ. Induction of B cell unresponsiveness to noninherited maternal HLA antigens during fetal life. Science (New York, NY). 1988;241(4874):1815-7.
40. Kjær M, Tiller H, Heide G, Kjeldsen-Kragh J, Skogen B, Husebekk A. Fetal exposure to maternal human platelet antigen-1a does not induce tolerance. An analytical observational study. PloS one. 2017;12(8):e0182957.
41. 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):833-9.
42. 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):870-7.
43. Dębska M, Uhrynowska M, Guz K, Kopeć I, Lachert E, Orzińska A, et al. Identification and follow-up of pregnant women with platelet-type human platelet antigen (HPA)-1bb alloimmunized with fetal HPA-1a. Archives of medical science : AMS. 2018;14(5):1041-7.
44. 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):1945-56.
45. Kjeldsen-Kragh J, Ni H, Skogen B. Towards a prophylactic treatment of HPA-related foetal and neonatal alloimmune

thrombocytopenia. Current opinion in hematology. 2012;19(6):469-74.
46. Kjaer M, Bertrand G, Bakchoul T, Massey E, Baker JM, Lieberman L, et al. Maternal HPA-1a antibody level and its role in predicting the severity of Fetal/Neonatal Alloimmune Thrombocytopenia: a systematic review. Vox sanguinis. 2019;114(1):79-94.
47. Jin JC, Lakkaraja MM, Ferd P, Manotas K, Gabor J, Wissert M, et al. Maternal sensitization occurs before delivery in severe cases of fetal alloimmune thrombocytopenia. American journal of hematology. 2019;94(8):E213-e5.
48. Mueller-Eckhardt C, Mueller-Eckhardt G, Willen-Ohff H, Horz A, Küenzlen 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):71-6.
49. Reznikoff-Etievant MF, Kaplan C, Muller JY, Daffos F, Forestier F. Allo-immune thrombocytopenias, definition of a group at risk; a prospective study. Current studies in hematology and blood transfusion. 1988(55):119-24.
50. Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Décary F. Alloimmunization to the PlA1 platelet antigen: results of a prospective study. British journal of haematology. 1990;74(2):209-15.
51. 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 : an international journal of obstetrics and gynaecology. 2003;110(5):492-6.
52. Doughty HA, Murphy MF, Metcalfe P, Waters AH. Antenatal screening for fetal alloimmune thrombocytopenia: the results of a pilot study. British journal of haematology. 1995;90(2):321-5.
53. 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. American journal of perinatology. 1996;13(7):423-31.
54. Maslanka K, Guz K, Zupanska B. Antenatal screening of unselected pregnant women for HPA-1a antigen, antibody and alloimmune thrombocytopenia. Vox sanguinis. 2003;85(4):326-7.
55. 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 obstetricia et gynecologica Scandinavica. 2012;91(1):79-86.
56. Kamphuis MM, Tiller H, van den Akker ES, Westgren M, Tiblad E, Oepkes D. Fetal and Neonatal Alloimmune Thrombocytopenia: Management and Outcome of a Large International Retrospective Cohort. Fetal diagnosis and therapy. 2017;41(4):251-7.
57. Fetal Growth Restriction: ACOG Practice Bulletin, Number 227. Obstetrics and gynecology. 2021;137(2):e16-e28.
58. Eksteen M, Heide G, Tiller H, Zhou Y, Nedberg NH, Martinez-Zubiaurre I, et al. Anti-human platelet antigen (HPA)-1a antibodies may affect trophoblast functions crucial for placental development: a laboratory study using an in vitro model. Reproductive biology and endocrinology : RB&E. 2017;15(1):28.
59. Cohen D, Colvin RB, Daha MR, Drachenberg CB, Haas M, Nickeleit V, et al. Pros and cons for C4d as a biomarker. Kidney international. 2012;81(7):628-39.
60. Rudzinski E, Gilroy M, Newbill C, Morgan T. Positive C4d immunostaining of placental villous syncytiotrophoblasts supports host-versus-graft rejection in villitis of unknown etiology. Pediatric and developmental pathology : the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society. 2013;16(1):7-13.
61. Nedberg NH, Turowski G, Guz K, Przytuła E, Uhrynowska M, Roald B, et al. Platelet alloimmunization is associated with low grade chronic histiocytic intervillositis - A new link to a rare placental lesion? Placenta. 2021;112:89-96.
62. Althaus J, Weir EG, Askin F, Kickler TS, Blakemore K. Chronic villitis in untreated neonatal alloimmune thrombocytopenia: an etiology for severe early intrauterine growth restriction and the effect of intravenous immunoglobulin therapy. American journal of obstetrics and gynecology. 2005;193(3 Pt 2):1100-4.
63. Dubruc E, Lebreton F, Giannoli C, Rabilloud M, Huissoud C, Devouassoux-Shisheboran M, et al. Placental histological lesions in fetal and neonatal alloimmune thrombocytopenia: A retrospective cohort study of 21 cases. Placenta. 2016;48:104-9.
64. Tiller H, Killie MK, Chen P, Eksteen M, Husebekk A, Skogen B, et al. Toward a prophylaxis against fetal and neonatal alloimmune thrombocytopenia: induction of antibody-mediated immune suppression and prevention of severe clinical complications in a murine model. Transfusion. 2012;52(7):1446-57.
65. Zhi H, Ahlen MT, Skogen B, Newman DK, Newman PJ. Preclinical evaluation of immunotherapeutic regimens for fetal/neonatal alloimmune thrombocytopenia. Blood advances. 2021;5(18):3552-62.
66. Winkelhorst D, Porcelijn L, Muizelaar E, Oldert G, Huiskes E, van der Schoot CE. Fast and low-cost direct ELISA for high-throughput serological HPA-1a typing. Transfusion. 2019;59(9):2989-96.

67. Avent ND, Martinez A, Flegel WA, Olsson ML, Scott ML, Nogués N, et al. The BloodGen project: toward mass-scale comprehensive genotyping of blood donors in the European Union and beyond. Transfusion. 2007;47(1 Suppl):40s-6s.
68. Ahlen MT, Husebekk A, Killie MK, Skogen B, Stuge TB. T-cell responses associated with neonatal alloimmune thrombocytopenia: isolation of HPA-1a-specific, HLA-DRB3*0101-restricted CD4+ T cells. Blood. 2009;113(16):3838-44.
69. 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):1344-52.
70. Kjeldsen-Kragh J, Fergusson DA, Kjaer M, Lieberman L, Greinacher A, Murphy MF, et al. Fetal/neonatal alloimmune thrombocytopenia: a systematic review of impact of HLA-DRB3*01:01 on fetal/neonatal outcome. Blood advances. 2020;4(14):3368-77.
71. Gleadall NS, Veldhuisen B, Gollub J, Butterworth AS, Ord J, Penkett CJ, et al. Development and validation of a universal blood donor genotyping platform: a multinational prospective study. Blood advances. 2020;4(15):3495-506.
72. Valentin N, Vergracht A, Bignon JD, Cheneau ML, Blanchard D, Kaplan C, et al. HLA-DRw52a is involved in alloimmunization against PL-A1 antigen. Human Immunology. 1990;27(2):73-9.
73. Braud V, Chevrier D, Cesbron A, Bignon J-D, Kaplan C, Valentin N, et al. Susceptibility to alloimmunization to platelet HPA-la antigen involves TAP1 polymorphism. Human Immunology. 1994;41(2):141-5.
74. Anani Sarab G, Moss M, Barker RN, Urbaniak SJ. Naturally processed peptides spanning the HPA-1a polymorphism are efficiently generated and displayed from platelet glycoprotein by HLA-DRB3*0101-positive antigen-presenting cells. Blood. 2009;114(9):1954-7.
75. Parry CS, Gorski J, Stern LJ. Crystallographic Structure of the Human Leukocyte Antigen DRA, DRB3*0101: Models of a Directional Alloimmune Response and Autoimmunity. Journal of Molecular Biology. 2007;371(2):435-46.
76. 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; quiz 58.
77. Kjeldsen-Kragh J, Ahlen MT. Foetal and neonatal alloimmune thrombocytopenia - The role of the HLA-DRB3*01:01 allele for HPA-1a-immunisation and foetal/neonatal outcome. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2020;59(1):102707.
78. Kjeldsen-Kragh J, Titze TL, Lie BA, Vaage JT, Kjær M. HLA-DRB3*01:01 exhibits a dose-dependent impact on HPA-1a antibody levels in HPA-1a-immunized women. Blood advances. 2019;3(7):945-51.
79. Nogués N. Recent advances in non-invasive fetal HPA-1a typing. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2020;59(1):102708.
80. Ouzegdouh Mammasse Y, Chenet C, Drubay D, Martageix C, Cartron JP, Vainchenker W, et al. A new efficient tool for non-invasive diagnosis of fetomaternal platelet antigen incompatibility. British journal of haematology. 2020;190(5):787-98.
81. Lewin A, Al Khan S, Beaudin L, Meilleur L, Clarke G, Richard L. Report on the 19th International Society of Blood Transfusion Platelet Immunology Workshop 2018. Vox sanguinis. 2020;115(8):767-82.
82. Porcelijn L, Huiskes E, de Haas M. Progress and development of platelet antibody detection. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2020;59(1):102705.
83. Winkelhorst D, Loeff RM, van den Akker-Van Marle ME, de Haas M, Oepkes D. Women's attitude towards routine human platelet antigen-screening in pregnancy. Acta obstetricia et gynecologica Scandinavica. 2017;96(8):991-7.
84. van der Ploeg CPB, Oomen P, van Lent M. Prenatale Screening Infectieziekten en Erytrocytenimmunisatie (PSIE) Procesmonitor 2019. In: Milieu RvVe, editor. 2021.
85. Kjaer M, Kjeldsen-Kragh J, Fiskum C, Leinan I, Skogen B, Husebekk A. Screening for fetal and neonatal alloimmune thrombocytopenia - lessons learned from a Norwegian screening program. Acta obstetricia et gynecologica Scandinavica. 2018;97(6):766-7.
86. Guo Y, Tian X, Wang X, Xiao Z. Adverse Effects of Immunoglobulin Therapy. Frontiers in immunology. 2018;9:1299.
87. Rossi KQ, Lehman KJ, O'Shaughnessy RW. Effects of antepartum therapy for fetal alloimmune thrombocytopenia on maternal lifestyle. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016;29(11):1783-8.
88. Kamphuis MM, Paridaans N, Porcelijn L, De Haas M, Van Der Schoot CE, Brand A, et al. Screening in pregnancy for

fetal or neonatal alloimmune thrombocytopenia: systematic review. BJOG : an international journal of obstetrics and gynaecology. 2010;117(11):1335-43.
89. Winkelhorst D, Oepkes D, Lopriore E. Fetal and neonatal alloimmune thrombocytopenia: evidence based antenatal and postnatal management strategies. Expert review of hematology. 2017;10(8):729-37.
90. Tiller H, Ahlen MT, Akkök Ç A, Husebekk A. Fetal and neonatal alloimmune thrombocytopenia - The Norwegian management model. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2020;59(1):102711.
91. Bertrand G, Petermann R, Kaplan C. Prediction of IVIG treatment efficiency in fetal/neonatal alloimmune thrombocytopenia. Blood. 2014;124(4):654-5.
92. 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. Journal of thrombosis and haemostasis : JTH. 2006;4(3):628-37.
93. 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):1296-305.
94. Sainio S, Javela K, Tuimala J, Koskinen S. Usefulness of maternal anti-HPA-1a antibody quantitation in predicting severity of foetomaternal alloimmune thrombocytopenia. Transfusion medicine (Oxford, England). 2013;23(2):114-20.
95. Bessos H, Killie MK, Seghatchian J, Skogen B, Urbaniak SJ. The relationship of anti-HPA-1a amount to severity of neonatal alloimmune thrombocytopenia - Where does it stand? Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2009;40(2):75-8.
96. Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, et al. Lack of Fucose on Human IgG1 N-Linked Oligosaccharide Improves Binding to Human FcγRIII and Antibody-dependent Cellular Toxicity*. Journal of Biological Chemistry. 2002;277(30):26733-40.
97. 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. British journal of haematology. 2016;174(2):310-20.
98. 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):471-80.
99. Thinn AMM, Wang Z, Zhou D, Zhao Y, Curtis BR, Zhu J. Autonomous conformational regulation of β(3) integrin and the conformation-dependent property of HPA-1a alloantibodies. Proceedings of the National Academy of Sciences of the United States of America. 2018;115(39):E9105-e14.
100. Mari G, Adrignolo A, Abuhamad AZ, Pirhonen J, Jones DC, Ludomirsky A, et al. Diagnosis of fetal anemia with Doppler ultrasound in the pregnancy complicated by maternal blood group immunization. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 1995;5(6):400-5.
101. Aertsen M, Diogo MC, Dymarkowski S, Deprest J, Prayer D. Fetal MRI for dummies: what the fetal medicine specialist should know about acquisitions and sequences. Prenatal diagnosis. 2020;40(1):6-17.
102. Alm J, Duong Y, Wienzek-Lischka S, Cooper N, Santoso S, Sachs UJ, et al. Anti-human platelet antigen-5b antibodies and fetal and neonatal alloimmune thrombocytopenia; incidental association or cause and effect? British journal of haematology. 2022.
103. Farley A, Lloyd S, Dayton M, Biben C, Stonehouse O, Taoudi S. Severe thrombocytopenia is sufficient for fetal and neonatal intracerebral hemorrhage to occur. Blood. 2021;138(10):885-97.
104. Coste T, Vincent-Delorme C, Stichelbout M, Devisme L, Gelot A, Deryabin I, et al. COL4A1/COL4A2 and inherited platelet disorder gene variants in fetuses showing intracranial hemorrhage. Prenatal diagnosis. 2022;42(5):601-10.
105. Bussel JB, Berkowitz RL, McFarland JG, Lynch L, Chitkara U. Antenatal treatment of neonatal alloimmune thrombocytopenia. The New England journal of medicine. 1988;319(21):1374-8.
106. Wabnitz H, Khan R, Lazarus AH. The use of IVIg in fetal and neonatal alloimmune thrombocytopenia- Principles and mechanisms. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2020;59(1):102710.
107. Urbaniak SJ, Duncan JI, Armstrong-Fisher SS, Abramovich DR, Page KR. Transfer of anti-D antibodies across the isolated perfused human placental lobule and inhibition by high-dose intravenous immunoglobulin: a possible mechanism of action. British journal of haematology. 1997;96(1):186-93.
108. Winkelhorst D, Murphy MF, Greinacher A, Shehata N, Bakchoul T, Massey E, et al. Antenatal management in fetal

and neonatal alloimmune thrombocytopenia: a systematic review. Blood. 2017;129(11):1538-47.
109. Herrmann A, Samelson-Jones BJ, Brake S, Samelson R. IVIG-Associated Maternal Pancytopenia during Treatment for Neonatal Alloimmune Thrombocytopenia. AJP reports. 2017;7(3):e197-e200.
110. Lakkaraja M, Jin JC, Manotas KC, Vinograd CA, Ferd P, Gabor J, et al. Blood group A mothers are more likely to develop anemia during antenatal intravenous immunoglobulin treatment of fetal and neonatal alloimmune thrombocytopenia. Transfusion. 2016;56(10):2449-54.
111. Rink BD, Gonik B, Chmait RH, O'Shaughnessy R. Maternal hemolysis after intravenous immunoglobulin treatment in fetal and neonatal alloimmune thrombocytopenia. Obstetrics and gynecology. 2013;121(2 Pt 2 Suppl 1):471-3.
112. Foundation JM. 10 Warning Signs of Primary Immunodeficiency 2022 [Available from: https://www.info4pi.org/library/educational-materials/10-warning-signs.
113. Driessen G. Richtlijn diagnostiek naar onderliggende aandoeningen bij kinderen met recidiverende luchtweginfecties. 2016 May 31st 2019.
114. Bussel JB, Vander Haar EL, Berkowitz RL. New developments in fetal and neonatal alloimmune thrombocytopenia. American journal of obstetrics and gynecology. 2021;225(2):120-7.
115. Robak T, Kaźmierczak M, Jarque I, Musteata V, Treliński J, Cooper N, et al. Phase 2 multiple-dose study of an FcRn inhibitor, rozanolixizumab, in patients with primary immune thrombocytopenia. Blood advances. 2020;4(17):4136-46.
116. 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. Clinical pharmacology and therapeutics. 2019;105(4):1031-9.
117. Newland AC, Sánchez-González B, Rejtő L, Egyed M, Romanyuk N, Godar M, et al. Phase 2 study of efgartigimod, a novel FcRn antagonist, in adult patients with primary immune thrombocytopenia. American journal of hematology. 2020;95(2):178-87.
118. Menon D, Barnett C, Bril V. Novel Treatments in Myasthenia Gravis. Frontiers in neurology. 2020;11:538.
119. El-Bohy M, Poowuttikul P, Secord E. Humoral Immune Deficiencies of Childhood. Pediatric clinics of North America. 2019;66(5):897-903.
120. Pyzik M, Rath T, Kuo TT, Win S, Baker K, Hubbard JJ, et al. Hepatic FcRn regulates albumin homeostasis and susceptibility to liver injury. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(14):E2862-e71.
121. Crowther CA, Middleton P, McBain RD. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. The Cochrane database of systematic reviews. 2013(2):Cd000020.
122. Kjær M, Geisen C, Akkök Ç A, Wikman A, Sachs U, Bussel JB, et al. Strategies to develop a prophylaxis for the prevention of HPA-1a immunization and fetal and neonatal alloimmune thrombocytopenia. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis. 2020;59(1):102712.
123. Baker JM, Shehata N, Bussel J, Murphy MF, Greinacher A, Bakchoul T, et al. Postnatal intervention for the treatment of FNAIT: a systematic review. Journal of perinatology : official journal of the California Perinatal Association. 2019;39(10):1329-39.
124. Farley AM, Dayton M, Biben C, Stonehouse O, Terreaux A, Taoudi S. Cerebral vasculature exhibits dose-dependent sensitivity to thrombocytopenia that is limited to fetal/neonatal life. Blood. 2022;139(15):2355-60.
125. Gafni A, Blanchette VS. Screening for neonatal alloimmune thrombocytopenia: an economic perspective. Current studies in hematology and blood transfusion. 1988(54):140-7.
126. Killie MK, Kjeldsen-Kragh J, Husebekk A, Skogen B, Olsen JA, Kristiansen IS. Cost-effectiveness of antenatal screening for neonatal alloimmune thrombocytopenia. BJOG : an international journal of obstetrics and gynaecology. 2007;114(5):588-95.