Fetal and Neonatal Alloimmune Thrombocytopenia: evidence based screening

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Chapter 1

General introduction and scope of this thesis

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Fetal and Neonatal Alloimmune Thrombocytopenia

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most important and most frequent cause of fetal and early neonatal thrombocytopenia in term-born infants.[1] During normal fetal life, the platelet count progressively increases, and reaches a constant level by the end of the first trimester. As in adults, normal fetal and neonatal platelet counts range from 150 $\times 10^9$/L to 450 $\times 10^9$/L. Thrombocytopenia is defined as a platelet count below 150 $\times 10^9$/L.[2,3] The degree of the thrombocytopenia can be further classified into either mild (100 – 150 $\times 10^9$/L), moderate (50 – 100 $\times 10^9$/L), severe (< 50 $\times 10^9$/L) or very severe (< 20 – 30 $\times 10^9$/L). Of all term-born infants, 2% have platelet counts below 150 $\times 10^9$/L and 0.1 – 0.2% suffer from a severe thrombocytopenia.[4] Approximately half of the cases with severe neonatal thrombocytopenia are caused by FNAIT.[1,5] Other conditions that are associated with fetal/neonatal thrombocytopenia can be divided into causes that lead to an increased destruction or a decreased production of platelets (Table 1.1).

Table 1.1 – Causes of fetal and early neonatal thrombocytopenia

Increased destruction
Immune thrombocytopenia
Maternal autoimmune (ITP, SLE)
Fetal/Neonatal Alloimmune Thrombocytopenia (FNAIT)
Severe fetal hemolytic disease due to red cell alloimmunization
Alloimmune drug-induced (penicillin, anti-epileptica, quinidine, indomethacin)
Peripheral consumption
Hypersplenism
Kasabach-Meritt
Disseminated intravascular coagulation (DIC)
Thrombosis (e.g. aortic, renal vein)
Decreased production
Genetic disorders (TAR syndrome, trisomy 13,18,21, triploidy, Turner's syndrome, amegakaryocytosis, Wiskott-Aldrich, May-Hegglin, Bernard-Soulier, Alport syndrome)
Bacterial infection (GBS, E.Coli, Listeria, Syphilis)
Viral infection (CMV, parvo, rubella, HIV, HSV)
Parasite infection (toxoplasmosis)
Asphyxia
Placental insufficiency (pre-eclampsia, IUGR, diabetes, premature birth)

GBS, group-B Streptococcus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; HSV, Herpes Simplex Virus; ITP idiopathic thrombocytopenia; IUGR, intrauterine growth retardation; SLE, systemic lupus erythematosus; TAR, thrombocytopenia-absent radii syndrome.

Pathophysiology

FNAIT caused by alloantibodies against the foreign, paternally derived, fetal human platelet antigens (HPAs, figure 1.1). Consecutive conditions to be fulfilled are an incompatibility between mother and fetus, maternal alloantibody formation, active placental transport of antibodies into the fetal circulation and destruction of fetal cells. Exposure to the foreign, fetal HPA can occur physiologically as well as in pathological conditions. Fetal blood cells enter the maternal circulation, a phenomenon called fetomaternal hemorrhage (Figure 1.1B). This can occur spontaneously and often asymptomatically in healthy pregnancies, during or after delivery, as a result of invasive procedures or after abdominal trauma. In addition, the maternal circulation is exposed to the fetal placental tissue, in particular to the syncytiotrophoblast cells, which express the integrin β3 containing various HPA epitopes. Then, the exposure to the incompatible platelet antigens needs to be followed by a maternal immune response to result in the formation of platelet-specific alloantibodies (Figure 1.1C). Lastly, these alloantibodies, of the immunoglobulin G (IgG) subclass, can get in contact with the fetal HPA again, primarily by entering the fetal circulation through active transport across the placenta, by the neonatal Fc-receptor (FcRn, figure 1.1D). After entering the fetal circulation, these alloantibodies can cause destruction of fetal platelets and potentially damage other fetal cells containing the specific antigen involved.

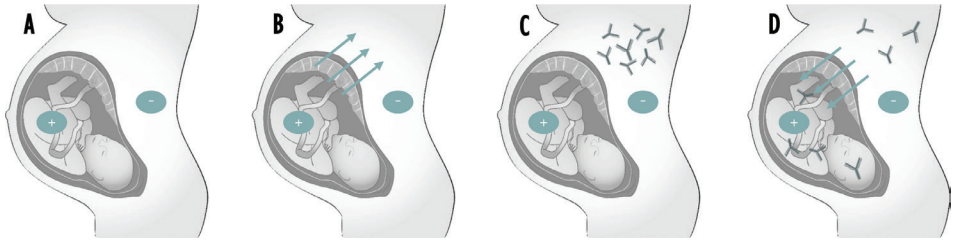

Figure 1.1 – Pathophysiology alloimmunization during pregnancy

A. Incompatibility between mother and fetus. **B.** Maternal exposure to fetal, paternally derived, antigens through fetomaternal transfusion. **C.** Maternal alloimmunization, formation of specific IgG alloantibodies. D. Transplacental transport of specific alloantibodies through FcRn into the fetal circulation.

Platelets

To date, 37 different HPAs have been identified and known to cause FNAIT. Various HPA epitopes are created by single nucleotide polymorphisms (SNPs) that result in small changes in the glycoprotein (GP) structure through an amino acid change.[6] The six different glycoprotein (GP) complexes (IIb/IIIa, Ib/IX, Ia/IIa and CD109), containing these 37 epitopes, are located on the platelet membrane. Twelve high-frequency HPAs are clustered into six biallelic groups; HPA-1, 2, 3, 4, 5 and 15 (Table 1.2).

The majority of HPAs are localized on GPIIb/GPIIIa (or integrin αIIbβ3), which is the most abundant membrane protein complex on the surface of platelets. In FNAIT in the Caucasian population, HPA-1a is the most targeted antigen, responsible for approximately 80% of the cases, followed by HPA-5b, which accounts for circa 10% of the cases (Table 1.3).[7,14-16] Genetic differences between ethnic populations lead to a variance in distributions of these incidences. For example, in the Asian population, anti-HPA-4b is the most frequently involved antibody, followed by anti-HPA-3a and anti-HPA-21b.[17-19] Furthermore, antibodies targeted against glycoprotein IV (also called CD36) are rarely seen in Caucasians (< 0.3% of cases), but are more frequently involved in FNAIT in African (7 – 8% of cases) and Asian (3 – 11% of cases) population.[13,20]

Endothelial cells

Glycoproteins containing the epitopes of HPAs are not solely present on platelets. Glycoprotein IIIa or integrin β3, containing the most HPAs, including HPA-1a, is expressed on the membranes of platelets in a heterodimer with GPIIb (integrin αIIb). In addition, integrin β3 can form a heterodimer with αV as well. This αVβ3 complex, still carrying the HPA-1a epitope, is scarcely expressed on platelets, but prominently present on the membrane of endothelial cells.[22-24] This raises an interesting dynamic: considering the fact that the pathogenic mechanism resulting in devastating intracranial hemorrhage (ICH) in FNAIT has never been adequately understood. Generally, alloantibodies are thought to enter the fetal circulation and cause bleeding complications and thrombocytopenia through destruction of fetal platelets. A theory that is not exactly airtight, given that severe bleedings have been described in only moderate thrombocytopenia and only a small proportion of severely thrombocytopenic new-borns actually suffer from bleeding complications. Additionally, research shows that mice that are completely lacking circulating platelets, survive in utero and do not bleed.[25] This combination of unexplained pathogenic bleeding mechanisms and the fact that the most involved antigen in FNAIT is present on endothelial cells has led to new insights. First, *in vitro* studies illuminated the direct interaction between anti-HPA-1a and human umbilical vein endothelial cells (HUVECs), demonstrated by a decreased HUVEC spreading as well as a decreased integrity of their monolayer in electric cell-substrate impedance sensing (ECIS) assays.[26] Second, a large *in vivo* study with both active and passive murine models of anti-HPA-1a mediated FNAIT showed that ICHs in these mice occurred regardless of platelet count. Also, HPA-1a antibodies inhibited angiogenic signaling, induced endothelial cell apoptosis and decreased vessel density in affected brains as well as retinas.[27] Lastly, a recent study with human sera of women with HPA-1a alloantibodies that caused FNAIT, suggested a correlation between the specific interaction and binding of the antibodies with αVβ3 only and whether or not an ICH had occurred in these pregnancies.[28]

Table 1.2 – Human platelet antigens and their prevalence

Antigen	Gene, chromosome, nucleotide change	Amino acid change	Allele or *phenotype*
HPA-1	*ITGB3*, 17, 196T>C rs5918	L33P	HPA-1a
			HPA-1b
			HPA-1b/b
HPA-2	*GP1BA*, 17, 482C>T rs6065	T145M	HPA-2a
			HPA-2b
			HPA2b/b
HPA-3	*ITGA2B*, 17, 2621T>G rs5911	I843S	HPA-3a
			HPA-3b
			HPA-3b/b
HPA-4	*ITGB3*, 17, 506G>A rs5917	R143Q	HPA-4a
			HPA-4b
			HPA-4b/b
HPA-5	*ITGA2,* 5, 16000G>A rs10471371	E505K	HPA-5a
			HPA-5b
			HPA-5a/a
HPA-15	*CD109*, 6, 2108C>A rs10455097	S682Y	HPA-15a
			HPA-15b
			HPA-15b/b
CD36, GPIV[¥]	CD36, 7, *variable*	*variable*	*GPIV negative*

GP, glycoproteint; HPA, human platelet antigen. Source: Immuno Polymorphism Database, https://www.ebi.ac.uk/ipd/hpa/freqs_2.html.

¬ Caucasian (French) population, n = 525-6135 7; ^North-African (Moroccan Berber) population, n = 104-112 8; °African (Egyptian) population, n = 367 9; ¤Sub-Sahara African (Congo), n = 125 10; ǂ African-American population (USA), n = 100 11; *Asian (Chinese Han) population. n = 1000 12; ¥ All phenotype numbers extracted from Curtis et al.13.

	Frequencies				
	Caucasian¬	North-African^	Sub-Sahara African¤	African-American‡	Asian*
	0.848	0.748	0.904	0.92	0.994
	0.152	0.252	0.096	0.08	0.006
	0.02	0.084	0.008	0	0
	0.92	0.818	0.776	0.82	0.952
	0.08	0.182	0.224	0.18	0.049
	0.006	0.028	0.040	0.03	0.001
	0.62	0.616	0.596	0.63	0.595
	0.38	0.384	0.434	0.37	0.406
	0.15	0.125	0.168	0.15	0.169
	1	1	1	1	0.996
	0	0	0	0	0.005
	<0.001	<0.001	0	0	0
	0.874	0.902	0.732	0.79	0.986
	0.126	0.098	0.268	0.21	0.014
	0.813	0.732	0.944	0.62	0.973
	0.455	0.861	0.701	-	0.532
	0.545	0.139	0.299	-	0.468
	0.23	0.221°	0.094	-	0.217
	-	<0.004	0.08	0.02	0.11

Table 1.3 – Alloantibodies involved in FNAIT

Authors	Number of patients	Alloantibody detected	Frequency	Alloantibody detected	Frequency
Mueller-Eckhart, 1989[15]	106	anti HPA-1a	90%	anti-HPA-3a	0.8%
		anti HPA-5b	8%	anti HPA-1a + 5b	0.8%
		anti HPA-1b	0.8%	anti-B	0.8%
Porcelijn, 2004	217	anti HPA-1a	73.7%	anti HPA-1b	1.4%
		anti HPA-5b	14.7%	anti HPA-15a	0.5%
		anti HPA-3a	4.6%	anti HPA-15b	0.5%
		anti Priv Ag	1.5%	anti A or anti B	2.8%
Davoren, 2004[16]	1162	anti HPA-1a	79%	anti GPIV (CD36)	0.4%
		anti HPA-5b	9%	anti HPA-4a	0.1%
		anti HPA-1b	4%	anti HPA-4b	0.1%
		anti HPA-3a	2%	anti HPA-6bw	0.1%
		anti HPA-5a	1%	Combinations	3.1%
		anti HPA-3b	0.8%		
Knight, 2011[21]	151	anti HPA-1a/b	81%	anti HPA-1a + 5b	5%
		anti HPA-5a/b	7%	Other	7%

FNAIT, fetal and neonatal alloimmune thrombocytopenia; HPA, human platelet antigen.

Placental function

In addition to platelets and endothelial cells, αVβ3 is also expressed on placental tissue by syncytiotrophoblast cells. Though there is no direct evidence, it has been suggested that anti-HPA-1a might induce placental insufficiency through interaction with these syncytiotrophoblast cells, possibly demonstrated by an association with intrauterine growth restriction (IUGR), as well as cases of intrauterine fetal demise (IUFD) in absence of bleeding problems.[29] Another correlation that has been suggested is one between FNAIT and miscarriages.[30] In addition, the expression of HPA-1a on placental tissue might lead to increased and early exposure to the fetal HPA-1a and might be a possible explanation for the high proportion of affected first pregnancies and first-born children in FNAIT.

Incidence

In the absence of population based screening, incidence and prevalence numbers have to be extracted from large prospective and preferably non-intervention studies. Such studies can be performed either postnatally, first screening for neonatal platelet count, followed by platelet-specific antibody testing, or antenatally, first screening for maternal HPA-type, then testing for antibody formation followed by assessment of neonatal outcome.

Postnatal estimation. When combining results from postnatal screening studies in all new-borns (n = 59,425), the incidence of severe FNAIT (platelet count < 50 × 10^9/L) was 0.04%, corresponding to 1 in 2500 new-borns, which led to an ICH in 25% of these cases.[31]

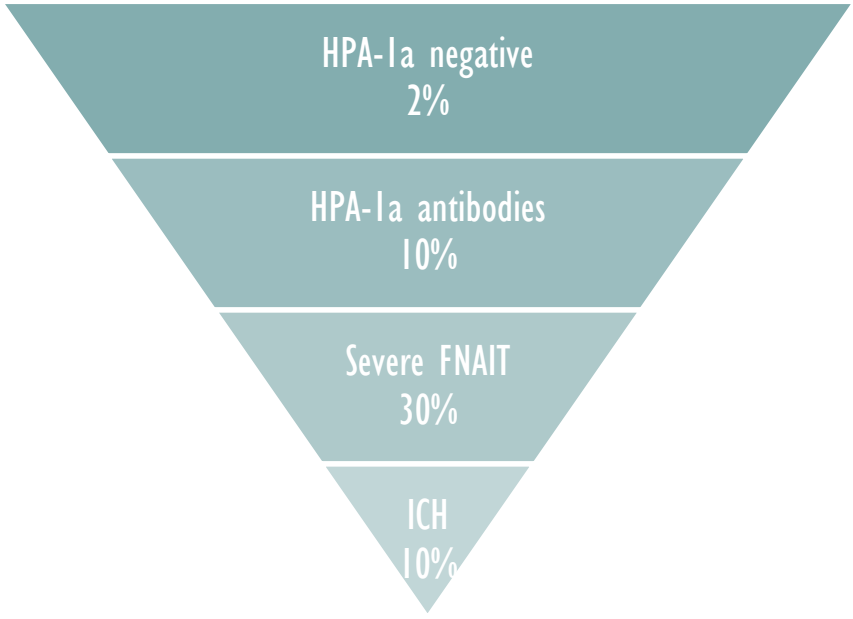

Figure 1.2 – Incidence of HPA-1a mediated FNAIT

FNAIT, fetal and neonatal alloimmune thrombocytopenia; HPA, human platelet antigen; ICH, intracranial hemorrhage.

Antenatal estimation. Antenatal cohort studies evaluating FNAIT incidence focus mainly on the predominant cause of FNAIT, HPA-1a alloimmunization. The largest prospective screening study, including 100,448 pregnant women in Norway, reported an incidence of HPA-1a negative women of 2.1%, leading to HPA-1a alloimmunization in 10.7%.[32] All alloimmunized women underwent an elective cesarean section at 2 – 4 weeks before term, so neonatal platelet counts and the incidence of bleeding problems are potentially an underestimation. They reported 58% of alloimmunizations to result in FNAIT, 33% in severe FNAIT and 2% of all alloimmunizations suffered from an ICH. A systematic review including ten prospective HPA-1a screening studies (n = 176,084 pregnant women), concluded that HPA-1a alloimmunization occurred in 9.7% of pregnancies at risk, leading to severe FNAIT in 31% of the cases and to perinatal ICH in 10% of the severe FNAIT cases (Figure 1.2).[33] While this review combined the largest screening studies performed thus far, only four of the ten studies did not perform an antenatal intervention. This leaves a total of 52,994 women observed, in which only a single ICH occurred. Therefore, no conclusions on natural history of the disease can be made and the above-mentioned estimates

are likely an underestimation of the true incidence of FNAIT caused by HPA-1a. The suggestion of underestimation was also put forth by an Irish study, that compared the diagnosed cases of FNAIT to the suggested number from the above mentioned screening studies.[34] Within eight years, 27 cases of FNAIT were diagnosed, all had bleeding manifestations and 20 were severely thrombocytopenic, which corresponds to 1 case of FNAIT in 16,500 live births. They compared this to estimated incidence in screening studies of 1 in 1,000 – 2,000 and therefore strongly suggested that FNAIT is currently underdiagnosed.

Clinical characteristics

FNAIT can have various clinical presentations. First, an asymptomatic thrombocytopenia might be detected as a chance finding without other signs of FNAIT. In these cases, FNAIT is usually only suspected after exclusion of other causes of fetal and early neonatal thrombocytopenia (Table 1.1). Second, mild bleeding symptoms might be present. These children might experience hematomas, petechiae, or small visceral bleeding. Also, transient hematuria or bloody stools might be seen.[15] Lastly, FNAIT can present with severe bleeding complications. Of these, an intracranial hemorrhage (ICH) is the most feared, due to its associated risk of lifelong disability and mortality.[35] An analysis of the short-term outcome of 43 cases of ICH showed that more than one-third (35%) resulted in perinatal death within four days of life and of the surviving children 82% suffered neurological disabilities.[35] Another cohort of consecutive cases of ICH at a single tertiary center showed an even higher mortality rate of 48%.[36] ICHs are estimated to originate before birth in over 80% of the cases, 67% of these bleedings started before 34 weeks' gestation and over half (54%) even before 28 weeks' gestation.[35,36] Also, the analysis by Tiller and colleagues showed that of the 43 cases of ICH, 23% occurred in first pregnancies, and 63% affected the first-born child.[35]

Diagnosis

In the absence of routine antenatal screening, suspicion of platelet alloimmunization leading to FNAIT usually arises in case of a clinically affected newborn (Figure 1.3A, 1.3B). Therefore, in the majority of the cases, diagnostic work-up is performed postnatally. However, antenatal suspicion and subsequent diagnostic work-up may be performed as well. This can be the case in antenatal ultrasound detection of fetal brain abnormalities, (Figure 1.3C, 1.3D) or because a sister of the pregnant women had a pregnancy complicated by FNAIT.

First, when HPA-alloimmunization FNAIT is suspected, diagnostic work-up should include HPA-typing of mother, father and child.[37] This way, possible HPA incompatibilities can be established. Second, an antibody screening should be performed to identify maternal platelet-specific alloantibodies, preferably using the MAIPA assay.[38] Additionally, tests for maternal autoantibodies, antibodies against private antigens expressed by the paternal platelets and Tpo levels can be part of the diagnostic work-up. Regulation of platelet production strongly

depends on the free plasma Tpo levels .[39-43] Therefore, plasma Tpo levels might be used to discriminate thrombocytopenia caused by megakaryocyte and platelet production failure (highly elevated Tpo levels) from thrombocytopenia caused by elevated platelet destruction as in ITP and FNAIT (normal or only slightly elevated Tpo levels).[44-48] FNAIT can be confirmed in case of a maternal-neonatal or maternal-paternal HPA incompatibility combined with the detection of alloantibodies for this specific HPA.

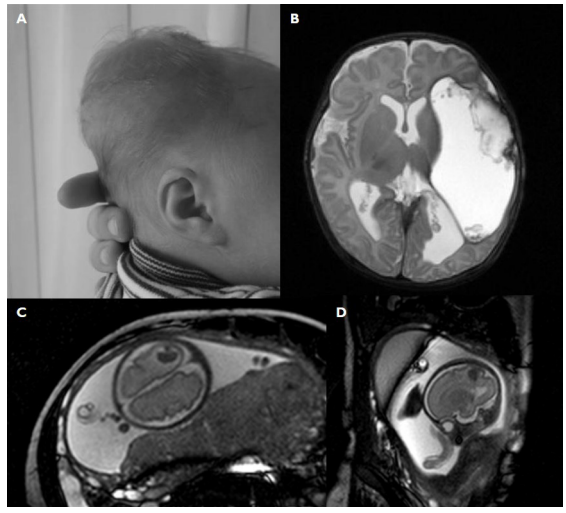

Figure 1.3 – Clinical aspects of FNAIT

A. Postnatal detection of FNAIT, large cephalic hematoma after vacuum assisted delivery, picture taken at 2 days of age. **B.** Postnatal detection of FNAIT, large intraparenchymal intracranial hemorrhage, Axial T2-weighted image of MRI obtained at 9 days of age showing porencephalic cysts both left parietal and right temporal. **C+D.** Antenatal detection of FNAIT, large intraparenchymal intracranial hemorrhage. T2-weighted image of MRI obtained at 28 weeks gestational age showing a hemorrhage left parietal.

Obstetric management

In current practice, preventive measures are virtually only available for subsequent pregnancies in women with known alloimmunization and diagnosed FNAIT due to a previously affected child. A rare exception concerns cases in which diagnostic work-up for FNAIT was performed following a sister with an affected child. Pregnancies at risk for FNAIT are best managed in a tertiary center with both obstetric and neonatal expertise in this disease. First, paternal genotype should be considered to assess the risk of an incompatible pregnancy. In case of paternal homozygosity, every next pregnancy for this couple will be incompatible by definition. In case of paternal heterozygosity, however, there is a 50% chance that the fetus is compatible with the mother and the pregnancy is not at risk to be complicated by FNAIT. In these cases, fetal genotype has to be determined to assess the need for monitoring and potential preventive treatment. For HPA-1a, the predominantly involved alloantibody, fetal status can be determined using non-invasive

testing of cell-free placental DNA in maternal plasma.[49] In recent years also non-invasive tests for other HPAs, based on massive parallel sequencing[50,51] or on digital droplet polymerase chain reaction (PCR, Hyland CA, personal communication) have been developed.

Risk assessment and monitoring

Once incompatibility between mother and fetus is confirmed, close ultrasound monitoring, specifically of the fetal brain, should be performed every 2-4 weeks. At this stage, clinicians should ideally be able to evaluate and monitor fetal disease severity as well as predict the occurrence of severe bleeding. Unfortunately, unlike in hemolytic disease of the fetus and newborn (HDFN), the red cell counterpart of FNAIT, there are no antenatal non-invasive diagnostic tests available to assess disease severity before severe bleeding complications occur. The only possibility is to assess fetal platelet count by fetal blood sampling (FBS), which means puncturing the umbilical cord. Besides the fact that this procedure is risky, in particular when platelets are low, platelet counts are not linearly correlated to disease severity. Because of this lack of reliable non-invasive diagnostic tools to guide obstetric management and treatment, several possible markers to select pregnancies at high risk have been suggested.

Antibody level. In some centers, antibody levels and titers are monitored by titration and quantification. While high titers do seem to be correlated to the severity of FNAIT, this is not a consistent relationship, and there are cases of severe hemorrhages with barely detectable antibody levels.[52-55] Therefore, monitoring antibody titers, if performed at all, is currently mostly in research setting and rarely influences obstetric treatment.

*HLA-DRB3*0101*. The HLA-DRB3*0101 genotype is positively correlated with the occurrence of alloimmunization in HPA-1a incompatible pregnancies.[56,57] However, besides this correlation to immunization, evidence on additional association with disease severity is inconsistent.[58-60]

Glycosylation. Another proposed predictive laboratory factor is the glycosylation pattern of the Fc-part of alloantibodies. Antibodies vary in glycosylation pattern, which influences the affinity and binding to Fc-receptors.[56,61] In FNAIT, a decreased fucosylation and increased galactosylation are reported to correlate to neonatal platelet counts and disease severity.[57]

Next to these laboratory parameters, clinical characteristics have been evaluated as well.[62] So far, the only clinical parameter directly correlated to disease severity is the occurrence of an ICH in a previous affected pregnancy. Estimated recurrence rate of an ICH, without the administration of preventive antenatal treatment, is as high as 79%.[63] Therefore, the only parameter that can guide the antenatal treatment regime is the occurrence of an ICH in a sibling.

Antenatal treatment

In current practice, without any tools to assess which alloimmunized pregnancies are at truly high risk for bleeding complications, preventive antenatal treatment is initiated in all incompatible pregnancies with known platelet-specific alloantibodies and an antigen-positive fetus. The preventive toolkit in these pregnancies consists of invasive and non-invasive treatment options.[64,65]

Fetal blood sampling – intrauterine platelet transfusion. The first prenatal strategy was adapted from the successful, and still routinely applied treatment of fetal anemia. In 1984, Daffos was the first to perform ultrasound-guided FBS followed by an intrauterine platelet transfusion (IUPT).[66] This strategy allowed both the assessment of fetal platelet count and the ability for direct treatment if necessary. Compared to serial intrauterine red blood cell transfusions as treatment for HDFN, there are two major differences to its application in FNAIT. First, the half-life of platelets is a few days, which is considerably shorter than that of red blood cells.[67] This results in the need for at least weekly fetal platelet transfusions. And even after a week, pre-transfusion platelet counts are often well-below 50×10^9/L, indicating that even weekly transfusions will not be enough to maintain safe platelet counts.[21,68,69] Second, cordocentesis in a thrombocytopenic fetus introduces a high risk of complications.[70,71] These complications include a high risk of bleeding, including exsanguination, due to this thrombocytopenic status. Also, fetal bradycardia is more often noted, which might possibly be attributed to the higher plasma volume transfused.[68]

Intravenous immunoglobulins. Endeavoring to replace this risky strategy with a safer non-invasive alternative, Bussel was the first, in 1988, to report the effect of maternal intravenous immunoglobulins (IVIg) in a pregnancy complicated by FNAIT.[67] The treatment, as well as the dose of 1.0 g per kg maternal body weight per week (g/kg/wk), was adapted from the treatment of idiopathic thrombocytopenic purpura (ITP), caused by platelet autoantibodies. Different strategies with regard to this dose (reduced to 0.5g/kg/wk or increased 2.0g/kg/wk) and timing of treatment have been investigated since.[72-74] Although there are several theories, the exact working mechanism of IVIg remains unsolved.[75] One theory states that the presence of IVIg might dilute and lower HPA-alloantibody levels in maternal serum, and would therefore result in a lower titer and level of antibodies. Another theory proposes that IVIg might compete with HPA-alloantibodies for FcRn on the placenta, leading to a lower number of antibodies transported into the fetal circulation. Further, this concept of competition might occur in the fetal circulation and spleen as well, leading to fewer antibodies binding to fetal platelets or fewer platelets destroyed in the spleen.[75-77] Despite IVIg being common practice in most specialized fetal therapy centers, officially, the use of IVIg in pregnancies at risk for FNAIT is still off-label. This might be because of uncertainty on the possible, long-term immunostimulatory or immunosuppressive effects of IVIg to the maturing fetal immune system. The only knowledge on neurodevelopmental outcome of children after intrauterine IVIg exposure during fetal life, reported no clinically apparent adverse effects in early childhood in 37 children.[76]

Corticosteroids. Another non-invasive treatment is the administration of corticosteroids. These can be administered either as single therapy or, more often, in addition to IVIg. When comparing IVIg to corticosteroids, both applied as singular treatment, corticosteroids are less efficient.[78,79] As an addition to IVIg, corticosteroids are thought to reduce possible headache complaints, an IVIg side effect, and support its efficiency. This strategy of adding steroids to IVIg treatment was first described by Bussel and colleagues.[80] They started with dexamethasone 1.5mg/kg – 5mg/kg, but because of limited beneficial effects and significant side effects such as oligohydramnios, dexamethasone was replaced by prednisone, which seemed to have less side effects at a dose of 0.5 mg/kg/day.[81] Evidence on the efficiency of adding corticosteroids to IVIg treatment seems inconsistent and no clear conclusions can be reached so far.

Mode and timing of delivery

The final part of antenatal management comprises the mode of delivery. A planned, near-term, cesarean section is often performed, in order to reduce the birth trauma with risk of bleeding problems. However, evidence for this rationale is lacking. Firstly, specific intrapartum risk of bleeding has never been proven and, in a small cohort analysis, vaginal delivery was not associated with the occurrence of ICH.[82] Second, in the analysis of 43 cases of ICH no intrapartum bleeding was detected and only 3/43 ICHs were thought to have occurred after delivery.[35] The majority of women are multiparous and a non-traumatic vaginal delivery is usually expected. So, in women with a previous vaginal delivery, without a sibling that suffered from ICH, a planned induction of labor at term can be considered. In contrast to women that previously delivered a child that suffered from ICH, a near-term planned delivery or CS can be offered. In case of vaginal delivery, it is recommended to avoid any potential traumatic events such as scalp electrode, scalp blood sampling or assisted vaginal delivery.

Neonatal management

Neonatal management is aimed at reducing bleeding tendency by increasing platelet counts.[83] Initial neonatal evaluation should always include clinical assessment, platelet count and cranial ultrasound. The combination of clinical and laboratory parameters determines the need for treatment. Various national guidelines differ in threshold for the start of treatment, 20×10^9/L, 30×10^9/L or 50×10^9/L.[2,84,85] A recent study on management of thrombocytopenia in preterm children demonstrated that a lower transfusion threshold was associated with better outcome.[86] Consensus does exist on the first choice of treatment when platelet count drops below threshold, is a platelet transfusion. Ideally, the transfused product contains platelets that lack the involved HPA (HPA-compatible or HPA-matched transfusion). An alternative can be a platelet transfusion with random-donor platelets. Kiefel and colleagues[87] showed in a small cohort that multiple random platelet transfusions can be sufficient in increasing platelet counts. IVIg can be administered as well, although its efficacy in treatment of neonatal thrombocytopenia is not clearly proven.[88,89]

Prevention

Primary prevention - prophylaxis

In HDFN, the red cell counterpart of FNAIT, the implementation of anti-D prophylaxis has led to a great decrease of mortality and morbidity caused by RhD immunization.[90] Historically, RhD, like HPA-1a in FNAIT, was the most frequently involved antigen of severe HDFN.[91,92] The possibility of immunoprophylaxis for HPA-1a immunization in FNAIT, as a prophylactic equivalent to anti-D, is debated for years and is an important focus for research. In vivo animal studies have reported that antibody mediated immune suppression can also occur in FNAIT mouse models.[93] In these murine studies, β3 integrin-deficient (β3-/-) mice are used to mimic HPA-1a negativity. After injection with HPA-1a/a or HPA-1a/b platelets to these β3-/- female mice, the administration of human anti-HPA-1a strongly reduced the β3 antibody response. Besides a drop in β3 antibody level of 90%, there were fewer miscarriages, fewer stillborn pups, fewer pups with ICH and the pups had significantly higher platelet counts.[94,95] Nevertheless, a few obstacles still have to be taken. First, without population-based screening, it is currently impossible to identify women that will benefit from a potential anti-HPA-1a prophylaxis. A collaboration between nine North-European institutions, PROFNAIT project, aims to develop such a prophylaxis. They have already collected plasma of HPA-1a immunized women and announced a collaboration for the manufacturing of NAITgam from these plasmas, to develop the first drug for the prevention of FNAIT. After successful development, a phase 1-2 study of NAITgam to demonstrate efficacy and safety of the drug will be planned.[96]

Secondary prevention – screening

Without the availability of a prophylaxis, disease burden caused by FNAIT, can only be prevented by timely detection of alloimmunization and treatment in pregnancies that are at high risk for developing bleeding complications. Because FNAIT can occur in first pregnancies, the only way to adequately identify all pregnancies is through prenatal population-based screening programs.

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

Figure 1.4 – Principles of early disease detection - Wilson & Jungner criteria[97]

Aim and outline of the thesis

Population-based screening in order to timely detect and prevent or treat FNAIT has been a debated topic over the past decades.[98-102] Similarities to its red cell counterpart, HDFN, together with the availability of an effective non-invasive preventive therapy, make it seem logical and desirable to instate such a screening program. However, differences to HDFN together with critical missing knowledge, hamper nationwide implementation. Despite efforts from various countries in performing large prospective studies, nationwide population-based screening for FNAIT has not been implemented thus far. The research presented in this thesis is aimed at gaining the evidence necessary to answer this decade-long question on the feasibility and efficacy of population-based screening to prevent morbidity and mortality caused by FNAIT. To guide this quest ten criteria or principles of screening or early disease detection, defined by James Wilson and Gunner Jungner (W&J), were used (Figure 1.4).[97] These ten criteria, that were published in a report in 1968 by the World Health Organization (WHO), became a 'public health classic' and still remain the gold standard for assessing the usefulness of population-based screening. Later, in 2008, driven by the development of new genetic screening possibilities, Andermann and colleagues[103] state 10 criteria as a synthesis of 50 lists of screening criteria that have been proposed (Figure 1.5). These, partly overlapping and mainly complementary, criteria were published in the bulletin of the WHO. Whereas they state themselves, that the value of the W&J criteria remains undisputed, we primarily focus on these. In this thesis, we will evaluate the existing evidence, formulate research questions based on missing knowledge and evidence, and contribute to the fulfilling of these ten screening criteria (Table 1.4).

Table 1.4 – Research questions and outline of this thesis

W&J	Research questions	Chapter
1	What is the incidence of the disease?	2, 9
	What is the long-term follow-up after ICH?	3
	Severe hemorrhage besides ICH?	4
2	What is the optimal antenatal management?	5
	What is the optimal postnatal management?	6
3	Are there facilities for diagnosis and treatment in place?	1
4	Is there a recognizable latent stage?	1
5	Is there a suitable test for HPA-typing?	7
6	Is screening acceptable for the population?	8
7	What is the natural history of the disease?	9
8	Is there effective treatment to halt pathologic changes?	5
	Is there a clear policy whom to treat or follow-up?	10
9	Is screening cost-effective?	1
10	Continuity of the screening process	1

HPA, human platelet antigen; ICH, intracranial hemorrhage; W&J, Wilson & Jungner.

1. *Important health problem*
   A health problem might be important from the community perspective (e.g. disease with a high prevalence) or from the individual perspective. The latter includes relatively rare diseases with very severe consequences. In *chapter 3* severe consequences that FNAIT can have besides the well-known and described ICH. Besides the high mortality and short-term morbidity caused by severe bleeding complications in FNAIT, these hemorrhages impact long-term outcome as well. In *chapter 4* we are the first to evaluate the short- and long-term outcomes with a neurodevelopmental follow-up study of cases of ICH.
2. *Accepted treatment for patients with recognized disease*
   In *chapter 5 and 6* we describe both the optimal postnatal and antenatal treatment in cases of FNAIT. *Chapter 5* is a systematic review of all available evidence on antenatal treatment strategies. In *chapter 6* we performed a nationwide cohort study to evaluate postnatal management and outcome of all newly detected cases.
3. *Facilities for diagnosis and treatment*
   In current practice, there is a lot of experience with diagnosing the disease, for which specialized laboratories exist. Additionally, in subsequent pregnancies with known immunizations facilities are instated to monitor the disease and administer preventive treatment.
4. *Recognizable latent or early symptomatic stage*
   An incompatible pregnancy with known alloimmunization, in which the fetus has no signs of bleeding, is a easily recognizable and latent stage of FNAIT.
5. *Suitable test or examination*
   In terms of screening there has to be a first-line test that identifies a population at risk for the disease that is screened for. In *chapter 7* we describe the development and validation of an HPA-1a ELISA, that can be used for high-throughput, low-cost, serological HPA-1a typing in a general pregnant population.
6. *Acceptable for the population*
   With increasing consumerism in current health care this sixth screening criterion is getting more and more important. In *chapter 8* we describe the results of a cross-sectional questionnaire study, using a validated model for assessing informed decision making to assess women's attitude towards a potential HPA-screening in pregnancy.
7. *Natural history should be understood*
   Despite a couple of prospective cohort studies, knowledge on the natural history of the disease is still lacking. Due to the intervention performed in most of the large prospective studies, no conclusions can be drawn from these results. Therefore, we designed and started the HIP-study (HPA-screening In Pregnancy): a large, nationwide, observational, prospective, cohort study. The study-protocol is described in *chapter 2*. Results of a 1-year interim analysis are described in *chapter 9*.

8. *Agreed policy whom to treat as patients*
   Despite a recognizable latent stage, that is alloimmunization, it is not feasible to treat all alloimmunized women as patients. This would probably lead to a major over-treatment. With the HIP-study we are able to collect data and plasma samples from immunized pregnancies with and without disease. The latter being a unique group, necessary for development of a risk assessment tool. In *chapter 10* we describe the effect of interaction between endothelial cells and disease severity.
9. *Costs should be economically balanced*
   Obviously, an adequate estimation on costs can only be performed when the design of the screening program in the Netherlands is known and an adequate estimation of the natural history of the disease can be made. Indeed, natural history represents the costs that can be prevented. Despite this missing information, several studies have estimated cost-effectiveness as accurately as possible and concluded population-based screening and intervention programs are likely to be cost-effective. It was stated that a potential national screening program as described by Kjeldsen-Kragh *et al*[32], which included performing a near-term cesarean section in all alloimmunized pregnancies, would save up to 210 – 230 quality-adjusted life years and could reduce health care costs by €1.7 million per 100,000 pregnant women.[104]
10. *Continuous process*
    In the era of the development of the W&J criteria were developed was that screening should be a continuous process and not a one-time exercise that would minimize the yield of the program. In current health care and practice, the doctrine of starting population-based screening is by definition a continuous process, until new insights prove otherwise.

1. **The screening program should respond to a recognized need**
2. **The objectives of screening should be defined at the outset**
3. **There should be a defined target population**
4. **There should be scientific evidence of screening program effectiveness**
5. **The program should integrate education, testing, clinical services and program management**
6. **There should be quality assurance, with mechanisms to minimize potential risks of screening**
7. **The program should ensure informed choice, confidentiality and respect for autonomy**
8. **The program should promote equity and access to screening for the entire target population.**
9. **Program evaluation should be planned from the outset**
10. **The overall benefits of screening should outweigh the harm**

Figure 1.5 – Synthesis of emerging screening criteria from over the past 40 years[103]

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