Fetal and neonatal alloimmune thrombocytopenia : towards implementation of screening in pregnancy

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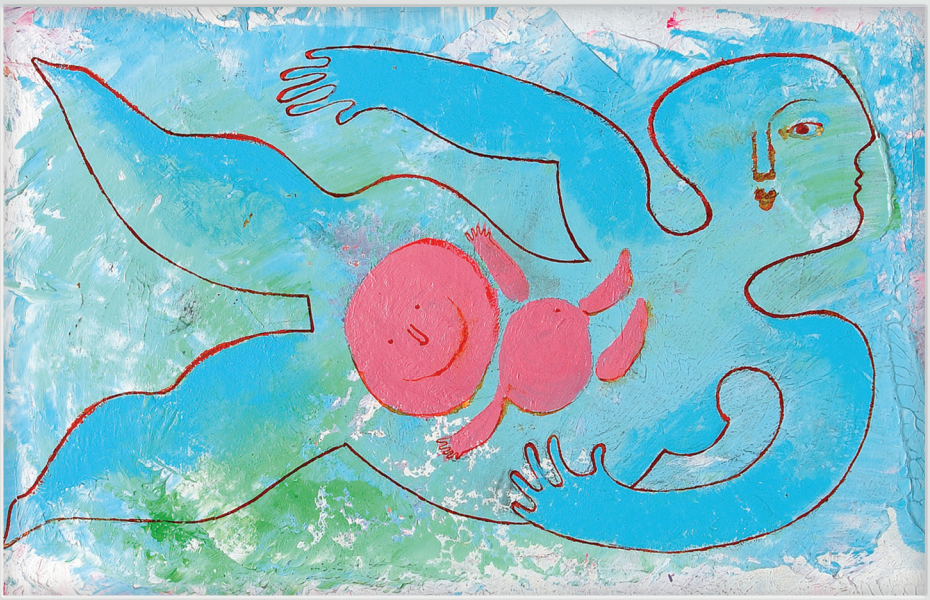

Fetal and Neonatal Alloimmune Thrombocytopenia

towards implementation of screening in pregnancy

Marije Kamphuis

“zolang het kind in mij lacht huil ik niet”

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COLOFON

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The studies described in this thesis were performed at the Department of Obstetrics of the Leiden University Medical Center, the Netherlands.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1

GENERAL INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by an immunological process in which the mother produces an antibody-mediated response against a platelet-specific antigen (human platelet antigen, HPA) that she herself lacks but that is present on the fetal platelets, inherited from the father. The fetal platelet antigens are expressed as early as 16–18 weeks of gestation.[1] The mother's antibodies [of the immunoglobulin G (IgG) type] can cross the placenta and bind to fetal platelets. The antibody-coated platelets are subsequently removed from the fetal circulation by the reticuloendothelial system, which results in fetal thrombocytopenia. These same antibodies also may inhibit platelet production.[2] The proportion of individuals belonging to a particular platelet antigen type varies according to race. The immunodominant antigen in Caucasian individuals is HPA-1a, which is responsible for 85% of the FNAIT cases, followed by HPA-5b.[3,4] Two percent of the Caucasians is HPA-1a negative. The reported incidence of FNAIT is estimated to be one in 1000-2000 births.[5-7]

FNAIT is a potentially devastating condition with intracranial haemorrhage (ICH) as most feared complication, which can lead to severe neurological sequelae including mental retardation, cerebral palsy, cortical blindness, seizures or even death. The clinical outcome seems to be more severe than for neonatal ICH from other causes.[8,9] However true specific data about the long-term neurodevelopmental, cognitive outcome and development of children suffering from ICH due to FNAIT is scarce.

Available data indicate that 50–80% of ICH cases happen in utero, and then mainly during the third trimester.[4] The highest risk for FNAIT-related complications in subsequent pregnancies seems to be among fetuses/neonates with siblings that experienced antenatal ICH, with a reported recurrence rate of 90%.[4,10] Among siblings with severe FNAIT and no ICH, data are still unclear, ranging from no risk to a 66% recurrence rate.[11,12] Furthermore, similar to red cell alloimmunisation, the severity of FNAIT is assumed to increase with each pregnancy, although strong evidence is lacking.

For several years, fetal blood sampling with intrauterine platelet transfusion was the standard treatment for FNAIT. However, in-utero platelet transfusion is an invasive procedure that carries a risk of fetal loss, especially for fetuses with a low platelet count.[13] Currently, administration of immunoglobulins (IVIG) to the pregnant mother, a varying degree of intrauterine monitoring and specific measures around birth is mainly offered to women affected by FNAIT.[14,15] There is no consensus about the dosage of IVIG, it varies from 0.5 (Netherlands), 1.0 (Sweden) to 2.0 g/kg per week in the USA. Some centres, particularly in the USA add steroids to the IVIG treatment. Other interventions, which may be used in conjunction to fetal therapy, are induction of labour, near term caesarean section, and delivery in a tertiary care centre with match platelets available for transfusion.

With the current lack of screening programs, the diagnosis of FNAIT is usually only established following the birth of a clinically affected child with signs of bleeding or coincidentally when thrombocytopenia is found with laboratory test for other reasons. As

a consequence, antenatal treatment modalities are nowadays only provided for women with a previously affected child.

If we truly want to prevent the burden of this disease, all at risk pregnancies should be identified in time to start effective preventive treatment and reduce severe adverse outcomes. This can only be realised when routine screening for HPA-type is offered. The question remains if the time is ripe to implement such a screening program or whether we need more detailed information about incidence, pathogenesis and natural course of this rare disease. The studies described in this thesis were designed to contribute to the implementation of a screening and intervention program for FNAIT.

OUTLINE OF THIS THESIS

The general aim of the studies described in this thesis is to contribute to the decision about implementation of screening for FNAIT in the healthcare program for pregnant women.

In chapter 2 a systematic review of the literature on antenatal screening studies for FNAIT is given. It provides a pooled estimate of the naive prevalence among pregnant women of HPA-1a negativity , the risk of HPA-antibody formation, thrombocytopenia and risk of adverse outcome.

In chapter 3 we systematically assessed the reported prevalence of severe thrombocytopenia in newborns secondary to NAIT with sub analysis of ICH due to NAIT.

In chapter 4 an overview is given on the current management of fetal and neonatal allo immune thrombocytopenia (FNAIT).

In chapter 5 we evaluated the rate and consequences of a late or missed diagnosis of FNAIT by assessing the clinical presentation of first affected children, the timing of diagnosis and the outcomes of subsequent children.

In chapter 6 we characterised pregnancies where the fetus or neonate suffered from ICH with special focus on clinical and laboratory characteristics and time of bleeding onset.

In chapter 7 the results of the NOICH trial are reported. This randomised trial comparing a lower dose of IVIG of 0.5 g/kg to the standard dose of 1 g/ kg showed no difference in frequency of neonatal ICH, platelet counts at birth, need for neonatal treatment and levels of cord blood levels of IgG. Unfortunately this trial had to be stopped prematurely, resulting in insufficient power to prove equivalence of the lower dose to the standard dose.

In chapter 8 we describe a cohort study that shows that treatment in pregnancies with FNAIT with a previous affected child without ICH, in a weekly dose of 0.5g/kg or 1.0 g/kg results in comparable neonatal platelet counts at birth and incidence of severe thrombocytopenia.

In chapter 9 we analysed the management and outcome of the largest international cohort of FNAIT-cases to date, with emphasis on different treatment modalities. Non-in-

vasive management using IVIG with or without additional steroids prevents bleeding in the fetus or neonate in virtually all cases.

In chapter 10 we aimed to determine the long-term outcome and cognitive development of a group of children with ICH due to FNAIT to clearly outline the burden of this disease.

In chapter 11, a general discussion of the overall results is presented. Furthermore, future perspectives and proposals for research are given.

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CHAPTER 2

SCREENING IN PREGNANCY FOR FETAL OR NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: SYSTEMATIC REVIEW

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ABSTRACT

Background

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a potentially devastating disease, which may lead to intracranial haemorrhage (ICH), with neurological damage as a consequence. In the absence of screening, FNAIT is only diagnosed after bleeding symptoms, with preventive options limited to a next pregnancy.

Objectives

To estimate the population incidence of FNAIT and its consequences to prepare for study design of a screening programme.

Search strategy

An electronic literature search using MEDLINE, EMBASE and Cochrane database, and references of retrieved articles. No language restrictions were applied.

Selection criteria

Prospective studies on screening for human platelet antigen 1a (HPA-1a) alloimmunisation in low risk pregnant women.

Data collection and analysis

Two reviewers independently assessed studies for inclusion and extracted data. Main outcome data were prevalence of HPA-1a negativity, HPA-1a immunisation, platelet count at birth and perinatal ICH. We aimed to compare outcome with and without intervention.

Main results

HPA-1a alloimmunisation occurred in 294/3028 (9.7%) pregnancies at risk. Severe FNAIT occurred in 71/227 (31%) of immunised pregnancies, with perinatal ICH in 7/71 (10%). True natural history data were not found, as interventions were performed in most screen-positive patients.

Author's conclusion

Screening for HPA-1a alloimmunisation detects about two cases in 1000 pregnancies. The calculated risk for perinatal ICH of 10% in pregnancies with severe FNAIT is an underestimation, because studies without interventions were lacking. Screening of all pregnancies together with effective antenatal treatment such as intravenous immunoglobulin may reduce mortality and morbidity associated with FNAIT.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a potentially devastating condition, which may lead to intracranial haemorrhage (ICH) in the fetus or neonate, often with death or major neurological damage as a consequence. In Caucasians, between 1.6% and 4.6% are negative for human platelet antigens (HPA) 1bb or 1a.[1] In this group, 85% of immunisations are caused by alloantibodies against HPA- 1a. Older literature suggests that most immunisations occur during pregnancy.[2-4] Recently, a large prospective study showed that most immunisations probably occurred during or shortly after birth.[5] The immunoglobulin G (IgG) antibodies against fetal HPA-1a can cross the placenta and cause destruction of fetal platelets. The chance of immunisation is correlated with maternal expression of human leucocyte antigen (HLA) DRB3*0101 type. Typing for this antigen may contribute to identifying the pregnancies at risk for FNAIT.[6]

Several studies aimed to identify a threshold of HPA-antibody level in the maternal serum below which severe FNAIT would not occur but with conflicting results.[7-9]

The reported incidence of FNAIT ranges from 1:350[10] to 1:1000[11] in the largest studies. The most severe complication is intracranial haemorrhage (ICH), leading to perinatal mortality in 1-7% and to surviving children with often severe neurological sequelae including mental retardation, cerebral palsy, cortical blindness and seizures in 14-26% of affected pregnancies. [4,12-14] Reviewing all published cases of ICH due to FNAIT, Spencer and Burrows found that 80.5% originated before birth.[4]

The recurrence rate of FNAIT in a subsequent pregnancy is estimated at 90%.[14] Radder et al. reviewed the scarce literature on ICH risk in subsequent, untreated pregnancies. In women with a previous child with ICH, the estimated recurrence risk was between 61-97%.[15] Furthermore, similar to red cell alloimmunisation, the severity of FNAIT is assumed to increase with each pregnancy.[14,16,17]

In the absence of screening, the disease is only diagnosed after the birth of symptomatic neonates, i.e. fetal or neonatal bleeding, or occasionally by chance in the case of neonatal blood tests for other reasons. As a consequence, antenatal treatment is currently provided only for women with a previously affected child. In such a pregnancy, preventive measures, such as fetal blood sampling with platelet transfusion or weekly intravenous infusions with immunoglobulins (IVIG), are taken. Until recently, insufficient specificity of the diagnostic tests and the risks of treatment precluded the introduction of population-based screening programmes. However, laboratory methods have improved, and IVIG treatment without any invasive testing was shown, at least in women with a previous affected child, to be both safe and effective.[18]

As a preparation to introduce a provisional national screening programme for FNAIT in a general population of pregnant women, we performed a systematic review of the literature.

METHODS

Research questions

We aimed to review all prospective screening studies in cohorts of low-risk pregnant women, where HPA-typing was performed to identify pregnancies at risk for FNAIT. The time range of publication was January 1980 to October 2008. Study data were pooled to the extent that study populations seemed comparable. The specific research questions were first: what is the incidence of HPA-1a negativity among pregnant women, what proportion of these women form HPA alloantibodies during pregnancy, what proportion of their offspring is affected by FNAIT, and how severely are they affected? Second, what are the effects of interventions in screen-positive pregnant women?

Definitions

HPA-1a status is defined straightforwardly, but the definition of FNAIT manifestations varies in the literature. Thrombocytopenia, in adults and children as well as in fetuses is defined as a platelet count < $150x10^9$/L. Severe thrombocytopenia, associated with an increased risk of bleeding is commonly defined as a platelet count < $50x10^9$/L, and therefore has been used in many studies on FNAIT as a single endpoint. However, only a minority of fetuses or neonates with such a low platelet count actually suffer from bleeding complications. The key complication of the disease to be prevented is ICH. However, even the use of ICH as an endpoint has become controversial through the application of ever-improving imaging modalities, which now may detect minute areas of haemorrhage that may be clinically irrelevant. The truly meaningful endpoint would be neurodevelopment, to be assessed later in life, e.g. Bayley score at 2 years of age or later. For our literature review, we aimed to identify studies that at least provided information of the incidence of HPA-1a negativity, HPA-alloantibodies, neonatal platelet counts and signs of bleeding in the newborns. As a definition of severe FNAIT we used a platelet count < $50x10^9$/L.

Search and study selection

Relevant literature was identified using the electronic bibliographic databases PubMed, Embase, and Cochrane. We used the following keywords: 'mass screening' [MESH] OR screen* AND 'Thrombocytopenia, neonatal alloimmune'[MESH] OR NAITP OR Alloimmune thrombocytopenia. We accepted original articles, short communications and letters to the editor. In addition a search was performed from the reference list of all identified articles. When needed, we contacted authors for additional, unpublished information. There were no language restrictions.

We subsequently excluded all non-prospective studies. Studies were excluded when screening was not done by HPA-typing, or when screening was applied in a specific or high-risk population.

Two of the authors (M.K. and N.P.) initially screened all the titles and abstracts of papers, identified by the review search strategy, for relevance. Only studies which were

obviously irrelevant were excluded at this stage. All other studies were assessed on the basis of their full text for inclusion versus exclusion by two reviewers independently (M.K. and N.P.) using the criteria indicated above. Discrepancies were to be resolved by discussion with a third reviewer but this proved to be unnecessary.

Methodological quality

The methodological quality of the articles included was assessed by evaluating the explicitness and clarity of the study question, level of detail of the selection criteria for pregnancies included and excluded, details of the laboratory tests used, details on the interventions offered and carried out both antenatally and postnatally, appropriateness of the methods used to detect fetal and neonatal bleeding complications and pregnancy outcome. An important aspect of screening studies is the assessment of the incidence of the disease in the screen-negative group. Therefore, we evaluated whether the studies reported platelet counts and bleeding complications in the HPA-1a- positive group, and in the HPA-1a- negative women without (detectable) HPA antibodies. Completeness of follow-up and explanation of reasons for loss to follow up were critically evaluated.

Outcome variables

We extracted the following primary outcome data: number of HPA-1a negative women detected by the screening, the incidence in that subgroup of HPA-alloantibody formation during pregnancy, number of fetuses and neonates with severe thrombocytopenia defined as a platelet count $<50x10^9$/L, incidence of intrauterine fetal death probably related to FNAIT, incidence of fetal or neonatal ICH, combined adverse outcome defined as perinatal mortality and morbidity associated with severe thrombocytopenia. In addition, we evaluated the use of HLA-typing, in particular assessment of the presence or absence of HLA-DRB3*0101, which is associated with the risk of antibody formation in HPA-1a-negative pregnant women.

From the selected articles, we only used the data on those pregnancies from which all relevant data on primary outcomes were available.

Some screening studies aimed to describe the natural history of the disease, while others included some type of intervention either antenatally, postnatally or by adapting the mode of delivery.

We planned to compare the total group of women screened without intervention with the group in which an intervention was carried out. We planned in addition to analyse the primary outcome parameters related to the types of interventions, divided in the following categories: antenatal transplacental medical treatment (IVIG and/or steroids), intrauterine platelet transfusions, altered time and mode of delivery (near-term caesarean section), availability of postnatal matched platelets for transfusion and/or IVIG.

Statistical analysis

Descriptive analysis of the outcome parameters was performed by dividing the total number of women with the outcome parameter by the total number of women screened. We compare the odds of having a fetus or neonate with ICH in women with HPA alloantibodies with and without any intervention, by calculating odds ratios and 95% confidence intervals.

RESULTS

The initial search of the databases revealed 660 studies. During the first screening 639 studies were excluded and 21 remaining studies were assessed on the basis of their full text for inclusion or exclusion using the criteria described above. After critical appraisal of the full text of the remaining 21 articles, ten studies were included in our review.[2,3,10-12,19-23] The main reason for exclusion was the use of case-finding through platelet counting in umbilical cord blood at birth or in neonates, with further analysis in those with low platelet counts instead of identifying pregnancies at risk by HPA-1a typing. The process of literature searching and study selection is illustrated in figure 2.1.

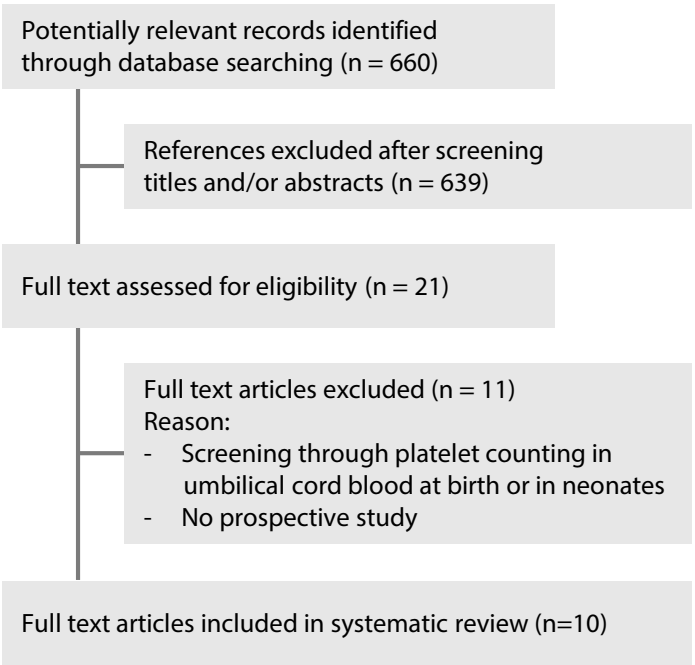

Figure 2.1 *Process from initial search to final inclusion*

No randomised controlled trials were found. Two of the ten studies were case-control studies, comparing outcome of the HPA-1a-negative women identified through the screening program with outcome of pregnancies of HPA-1a-positive women.[11,22] The other studies fulfilling our selection criteria were prospective cohort studies.

In the ten selected studies, a total number of 176 084 low-risk pregnancies comprised the screened populations with a range from 860 to 100 448. In table 2.1, the primary outcome data obtained from these ten studies are listed. In all studies, except one[20] both primigravida and multiparous women were included.

Table 2.1 *Outcome of screening studies for FNAIT included in the analysis*

Author, year	Women screened	HPA 1a negative (%)	Antenatal anti-HPA (%)	Intervention	Severe NAIT * (%)	ICH	IUFD
Mueller-Eckhardt 1985	1211	26 (2.1)	2/26 (7.7)	None	0	0	0
Reznikoff-Etievant 1988	860	27 (3.1)	0	None	0	0	0
Blanchette 1990	5000	81 (1.6)	3/50 (6.0)	NTCS, PP	1/3 (33)	1	0
Doughty 1995	3473	74 (2.1)	1/68 (1.5)	IUPT, IVIG, PP	2/1* * (100)	0	0
Durand-Zaleski 1996	2066	52 (2.5)	4/45 (8.9)	IVIG,ST	2/4 (50)	0	0
Williamson 1998	24417	598 (2.4)	36/385 (9.4)	PP	8/31 (26)	1	1***
Davoren 2003	4090	53 (1.3)	2/34 (5,9)	IUPT,PP	2/2 (100)	0	0
Maslanka 2003	8013	144 (1.8)	12/112 (10.7)	IUPT+IVIG	3/12 (25)	1	
Turner 2005	26506	327/19 127 (1.7)	25/318 (7.9)	PP	5/25 (20)	0	0
Kjeldsen-Kragh 2007	100448	2111 (2.1)	210/1990 (10.6)	NTCS, PP	48/147**** (33)	2	1*****
Total	**176 084**	**3493/168 705 (2.1)**	**294/3028 (9.7)**		**71/227 (31)**	**5**	**2**

ICH, intracranial haemorrhage; IUFD, intrauterine fetal death; PP, postpartum matched platelets available; IUPT, intrauterine platelet transfusion;
IVIG, antenatal intravenous immunogloblins; ST, antenatal corticosteroids.
The denominator in the columns differs as the result of drop-out or loss to follow up for these variables in the studies.
**Severe FNAIT was defined as a platelet count <50x10^9/L.*
***One pregnancy with two severely affected twin children.*
****IUFD related to fetal blood sampling in fetus with severe thrombocytopenia.*
*****Excludes second immunised pregnancies and those with HPA-1a-negative neonates.*
******One IUFD in a twin born at 31 weeks, with the other twin having severe thrombocytopenia.*

Methodological quality

A wide variation was found in the amount of detail provided on all major aspects. The articles ranged from a one page letter[21] to an 11-page report.[11] Only one paper[20] reported a sample size calculation, most others chose a fixed time period (e.g. 1 year), or lacked details on the basis for the size of the study population.

The primary investigators of all ten papers were haematologists or other specialists working in transfusion medicine, which might explain the generally detailed descriptions of the laboratory investigations. The various tests were either clearly described or references to the appropriate literature were provided in all papers.

Although all studies aimed to include only low-risk pregnant women, some series contained patients with previously affected pregnancies. Turner et al[11] found 3 women in their screened group with previously affected pregnancies, and excluded those from further analysis. Williamson et al[10] included two HPA-1a-negative women in their study group who were at first not typed as such but were known to have had affected children. In addition, one of the 33 women with positive HPA antibodies in their series had a diagnosis of NAIT in a previous child. Davoren et al[22] also included a woman who reported having had a previous child with petechiae at birth and a platelet count of 6 x 10^9/L. The index pregnancy in the study again ended in the birth of a child with petechiae, bruising and cord blood platelet count of 6 x 10^9/L. Kjeldsen-Kragh et al[23] included 16 women twice in their screening study, of which 14 gave birth in the second pregnancy again to an HPA-1a-positive child. These pregnancies are described separately in their article. Informed consent was commonly only obtained after identification of HPA-1a negativity, for follow-up and possible intervention.

The relative lack of obstetricians and paediatricians as co-authors could be the explanation for the absence or only rudimentary description of fetal examinations, obstetric management, clinical evaluations of the newborns and follow-up.

HPA-antibody detection

There was a large variation in maternal serum testing for antibody formation. In three studies the frequency of sampling was left unstated.[3,12,19] In the other studies the frequency ranged from twice during the pregnancy[20] to every 4 weeks.[23] Several women were found to have antibodies already at the first-trimester booking sample, all were multiparous. In the cohort reported by Williamson et al[10] eight women developed HPA-antibodies during their first pregnancy, two of whom already at 17 weeks of gestation. In the series by Turner et al[11], five of the 25 HPA-1a immunised women developed antibodies in their first pregnancy, two at 21 weeks, and the other three after 28 weeks of gestation.

Interventions

In the two smallest studies describing a total of 2071 pregnancies, no interventions in the screen-positive group were used. In the smallest study, none of the HPA-1a negative women developed HPA-1a antibodies.[19] In the study by Mueller-Eckhardt et al.[12] two women developed HPA-1a alloantibodies, both neonates had platelet counts

above 80 x 10^9/L. In the other eight studies, one or more types of interventions were offered to all HPA-1a alloimmunised pregnant women, a true nonintervention group was lacking.[2,3,10,11,19,20,22,23] Therefore, we had to omit the planned comparative analysis of the odds of having an affected child with or without intervention.

The eight studies in which interventions were performed in the screen-positive group used a wide variety of treatments, summarised in table 2.1. Only postnatal intervention, consisting of having matched platelets available for urgent transfusion, was used in two studies.[10,11] In six studies[3,11,20-23] one or more fetuses were treated with platelet transfusions, IVIG, corticosteroids or a combination of these. Two studies explicitly stated the offer of elective near-term caesarean section for women with HPA-antibodies.[2,23] In only two of the other eight studies, caesarean section rates in pregnancies with known HPA-antibodies were given. They were 36% in the study by Williamson et al.[10], and 33% in the study by Davoren et al.[22]

Perinatal mortality and neonatal morbidity

In the total study cohort where screening was applied 71 pregnancies were affected by severe FNAIT, with two perinatal deaths and five infants FNAIT-related ICH. Severe FNAIT occurred in the first pregnancy in 15/71 (21%), including three of the seven cases with adverse perinatal outcome (table 2.2). ICH was detected postnatally in four of five neonates, but all except one almost certainly occurred well before birth. Details on mode of delivery and platelet counts of this group are given in table 2.3.

Table 2.2 *Characteristics of all pregnancies complicated by severe FNAIT (platelet count < 50x10^9/L) in the ten screening studies*

Author, year	Primiparous	Multiparous
Mueller-Eckhardt 1985	0	0
Reznikoff-Etievant 1988	0	0
Blanchette 1990	0	1 (1 ICH)
Doughty 1995	0	2*
Durand-Zaleski 1996	2	_
Williamson 1998	4 (1 ICH)	3 (1 IUFD**)
Maslanka 2003	2 (1 ICH)	1
Davoren 2003	1	2
Turner 2005	0	5
Kjeldsen-Kragh 2007	6 (1 ICH)	42 (1 ICH, 1 IUFD)
Total	**15 (3 (ICH)**	**56 (2 ICH, 2 IUFD)**

ICH, intracranial haemorrhage; IUFD, intrauterine fetal death.
**One pregnancy with two severely affected twin children.*
***Death occurred after haemorrhage following fetal blood sampling at 29 weeks in an anaemic hydropic fetus with 6×10^9/L platelets.*
****Excludes pregnancies where mother has a previous affected child.*

Table 2.3 *Characteristics of the five HPA-1a alloimmunised pregnancies complicated by fetal or neonatal intracranial haemorrhage*

Case No. Author,yr	GA and mode of delivery	Detection of ICH	Occurrence of ICH	Platelet count	Long-term outcome*
1 Blanchette 1990	38 weeks, CS	Postnatal day 2	Antenatal < 36 wks	9 x 10^9/L	Mild cerebral palsy
2 Williamson 1998	37 weeks, CS	Postnatal day 1	Antenatal < 35 wks	4 x 10^9/L	Hydrocephaly, spasms, hypertonia, delayed development, mild optic atrophy
3 Maslanka 2003	38 weeks?	Postnatal	Antenatal likely < 36 wks**	34 x 10^9/L	Central neurological coordination dysfunction, recovered after rehabilitation
4 Kjeldsen-Kragh 2007	38 weeks, CS	Postnatal day 3	unknown	26 x 10^9/L	No clinical sequelae at 5 years
5 Kjeldsen-Kragh 2007	34 weeks, CS	Antenatal	Antenatal < 34 wks	13 x 10^9/L	Epilepsy with daily seizures at 7 months

GA gestational age; ICH intracranial haemorrhage; CS caesarean section
**Outcome description literally cited from publications.*
***Case 3 had fetal blood sampling at 36 weeks, platelet count 34×10^9/L followed by two intrauterine platelet transfusions, 119×10^9/L platelets at birth, gestational age and mode of birth unknown.*

HLA-typing

In four of the ten studies HLA-DRB3*0101 type was determined in the whole cohort.[3,10,11,21] These results are summarised in table 2.4. In the study Norwegian study of Kjeldsen-Kragh et al[23], only HPA-1a immunised women were HLA DRB3*0101 typed. They found that 12 of the 150 HPA-1a immunised women were HLA-DRB3*0101 negative.

Table 2.4 *Results of HLA DRB3*0101 typing in HPA-1a negative pregnant women*

Author, year	Prevalence of DRB3*0101 in HPA-1a negative women (%)	HPA-immunisation in DRB3*0101 positive women (pos. predictive value) (%)	No HPA-immunisation in DRB3*0101 negative women (neg. predictive value) (%)
Doughty 1995	22/71 (31)	2/22 (10)	49/49 (100)
Williamson 1998	123/385 ((32)	43/123 (35)	261/262 (99.6)
Maslanka 2003	41/122 (34)	12/41 (29)	81/81 (100)
Turner 2005	107/303 (35)	18/107 (17)	189/196 (96.4)
Total	**293/881 (33)**	**75/293 (26)**	**580/591 (98.1)**

HLA human leucocyte antigen; HPA human platelet antigen

DISCUSSION

Our analysis of studies describing screening pregnant women for FNAIT provides a pooled estimate of the naive prevalence among pregnant women of HPA-1a negativity (2.1%) and an estimate of the risk of negative women to show antenatal HPA-antibody formation (9.7%). The pooled data confirm that a significant proportion of severe disease occurs already in the first pregnancy. However, none of the studies reported on the true natural history of the disease. Understandably, the investigators offered interventions to women in whom they detected HPA antibodies, with the aim of reducing the incidence of the true clinical disease, which is fetal or neonatal bleeding. It seems safe to assume that the incidence of 31% severe fetal or neonatal thrombocytopenia in HPA-immunised women, with 10% severe adverse outcome, is an underestimation of the true risk in nonscreened populations. This was recently confirmed by a study from Norway, where the authors compared the detected infants with FNAIT in two groups, nonscreened versus a screened population of pregnant women. Their reported detection rate of FNAIT without screening was only 14% of the expected rate.[24]

Better estimations are unavailable because no randomised studies have been published. The largest and most recent study compared the outcome of a cohort with a historic control group for which they used outcome data from published screening studies.[9] About half of these studies were also included in our analysis, with the above described limitation of using interventions. The other half of their historic control group were studies screening 'low-risk' or 'randomly selected' neonates for thrombocytopenia, with subsequent maternal HPA-antibody testing in case of low platelet counts. Prenatal interventions were obviously not performed in this group, but postnatal treatment was generally available. The authors acknowledged the limitations of their control groups; however they considered a truly randomised design withholding intervention to one study-arm to be unethical. We conclude from our literature review that more reliable data on the natural history are both unavailable and not likely to be collected.

For many years, clinicians treating ICH in newborn children due to FNAIT considered antenatal screening as a measure to reduce the disease burden[2,25,26]. Our review adds essential information to this ongoing debate. Severe FNAIT occurs in about 40 per 100 000 pregnancies (of the estimated 210 HPA-1a immunised women) with, despite several interventions, severe ICH in three or four children per 100 000 pregnancies screened. The majority of these bleedings occurred *in utero*, before 36 weeks of gestation. Permanent neurological handicap in this group was common, associated with severe burden for affected individuals and their families, and with high costs for society. In previous literature reviews, estimated risks for ICH in HPA-immunised pregnancies ranged from 7 to 26%, which would mean three to ten per 100 000.[15] Again we must stress the fact that these figures are likely to be underestimations. This disease therefore seems to have enough burden of disability to consider a prevention program.

Although we consider the data of this review valuable for the design of a screening programme, several questions remain. The first aspect in a screening programme is to

how to identify women at risk for FNAIT. To timely select the 2.1% HPA-1a negative pregnant women, screening should start in the first trimester, the obvious choice being the use of the universally accepted 'booking sample'. Laboratory methods for large-scale, rapid, reliable and cheap assessment of HPA-1a antigens are needed. In most studies we reviewed, either an enzyme-linked immunosorbent assay or flow cytometry was used for phenotyping with, in more recent studies, a polymerase chain reaction-based method either as primary test or to confirm HPA-1a negativity. Large-scale primary genotyping could become cost-effective, however. Recent studies used a modified monoclonal antibody immobilisation of platelet antigens technique for antibody detection and quantification. Collaboration between reference laboratories in this field has let to highly reproducible results with this method.[27]

An option to optimise selection would be to assess the fetal HPA-type. About 30% of fathers are either HPA-1a negative (2%) or heterozygous HPA-1a1b. Recently, a reliable method became available for fetal HPA-typing using free fetal DNA in maternal plasma (M. de Haas, personal communication). This test would omit the use of paternal testing or amniocentesis for fetal testing. Its use, however logical, depends again on careful cost-effectiveness evaluation.

Whether or not to further narrow the screen-positive group by testing for HLA DRB3*0101 can only be determined by a detailed cost-effectiveness analysis, and also depends on logistic possibilities. As we showed in our review, a remarkably consistent prevalence of 33% was found. Although HPA-antibody formation is rare in DRB3*0101 negative women, the positive predictive value of only 26% questions its usefulness in a screening program. In the large Norwegian study, 10% of HPA-immunised women were found to be DRB3*0101 negative, again raising doubt on the value of testing for this allele.[23]

Furthermore as in any other screening programme it is significant to identify the window or presymptomatic stage of the disease. From the published data, we could not reliably determine the time between (detection of) antibody formation and occurrence of ICH. In many multiparous women, antibodies were already present early in pregnancy, whereas in first pregnancies several women had detectable antibodies already in the second trimester. In this large cohort of women without a previous affected child, the earliest ICH was detected at 34 weeks of gestation. It seems likely therefore, that a case finding strategy aiming to detect HPA-immunisation allows time for interventions preventing the clinical disease, which is fetal ICH.

A final option to further select the group requiring intervention would be to use a certain threshold of HPA-antibody level in the maternal serum. Only a few studies in our review discussed this subject, with conflicting conclusions.

Another as yet unsolved question in the debate on screening for FNAIT is what to offer the screen-positive group. From our review it seems clear that ICH may occur *in utero*, which makes antenatal intervention necessary. The large Norwegian study shows that near-term caesarean section with matched platelets available may reduce, but not eliminate, perinatal death and severe handicap due to ICH. Until recently, most ante-

natal interventions included the use of serial fetal blood sampling. The procedure-related fetal loss risks involved however, are 2-6%, which in a screening program would mean losing more fetuses to the intervention then would be saved from ICH-related adverse outcome.[28-30] Only recently, it was shown that at least in women with FNAIT and a previous affected child, non-invasive treatment using IVIG given to the pregnant women weekly in the third trimester was 100% effective and probably safe to mother and child.[8,18] If this intervention could be shown to be equally effective in first affected pregnancies, which theoretically seems likely, this would mean an important step towards an effective screening programme.

An interesting issue for debate is what to offer the HPA-1a negative women who do not become immunised during pregnancy. Killie et al.[31] recently reported that more than 75% of the immunisations occur during or after labour. Ideally, for this group, we would like to offer a prophylactic drug similar to anti-D in Rhesus immunisation. The development and testing of prophylactic drugs is likely to take many years before widespread clinical application is possible, so a management strategy for immunised women is urgently needed.

The choice for an elective caesarean section in pregnancies with FNAIT is another subject of debate. One study specifically addressed this subject, and concluded that there was insufficient evidence to support this choice.[32] Most studies in our review lacked sufficient information to reach any conclusion. None of the incidneces of ICH seemed to have occurred intrapartum, and such occurrences are extremely rare in the literature as well.[32] Two studies that reported mode of delivery had caesarean section rates around 35%, which is much higher than expected in a normal population. The Norwegian investigators defended their choice with three arguments. The first was the ability to deliver the child 2- 4 weeks prior to term. This is obviously also possible with the currently quite effective prostaglandin induction. Second, they refer to a radiologic study in which 26% of neonates born vaginally had signs of haemorrhage on magnetic resonance image, compared to 0% in the group delivered by caesarean section.[33] However, most of these bleeds were small subdural hematomas; none were symptomatic and most disappeared within 5 weeks. A more recent larger study by Rooks et al.[34] with a similar design showed the presence of such haematomas in neonates born after caesarean section. This type of bleeding is thought to originate from tearing of small veins by the (normal) movement of the skull bones during labour and delivery, and are quite different from intraventricular and intraparenchymal bleeding commonly seen in ICH due to FNAIT.[34] The third argument was that planned caesarean section would provide time for the blood bank to prepare matched platelets. This is, however, a matter of logistics and would be equally true for planned induction of labour. In conclusion, although not proven to be safer, we cannot exclude that elective caesarean section may prevent ICH. This intervention however is associated with maternal morbidity and increased risk of complications in subsequent pregnancies. A choice for elective caesarean section in a screening programme would, depending on country and culture, have consequences for acceptability for both pregnant women and clinicians.

Implementation of routine antenatal screening for FNAIT obviously depends on cost-effectiveness. Several studies provided calculations all reaching the conclusion that screening is likely to be cost-effective.[11,20,35,36] The major determinants of the costs are the initial HPA-typing, antibody detection in those at risk, and costs of interventions. Although these costs are considerable, even in the most expensive strategy (e.g. offering IVIG to all immunised women), they are easily outweighed by the savings assuming that most cases of perinatal ICH can be prevented. For a population of 200 000 pregnancies per year, such as in the Netherlands, the estimated costs of HPA-1a screening would be around 1 million euros/year. Testing the 4000 HPA-1a women for fetal HPA-type and presence of antibodies is probably feasible for 250 000 euro. If we elect the most costly intervention programme, around 350 women annually would receive IVIG for the last 10 weeks of pregnancy, which would cost around 5 million euros. Including costs of organisation a rough estimate of such a programme thus would be in the order of 7 million euros/200 000 pregnancies screened. The obvious benefits are prevention of cases of life-long severe neurological morbidity such as cerebral palsy, blindness, deafness, seizures and mental retardation.

Using calculations made for children with neurological handicap due to kernicterus, with an estimated annual additional cost of 50 000 euros, a conservative estimated life expectancy of 40 years and a conservative estimate of ten handicapped children born each year as a result of FNAIT, an 80% effective prevention programme could be cost-effective if total costs are below 16 million euros annually, or 2 million euros per case prevented. Given the estimated annual costs for a screening program in the Netherlands of 7 million euros, cost-effectiveness is reached when 4 cases of ICH are prevented each year. Assuming a high effectiveness of the proposed intervention programme, at least double that number are expected to be prevented, leading to net savings of at least 9 million euros per year.

In conclusion this review showed that severe FNAIT occurs in about 40 per 100 000 pregnancies. Despite several interventions severe ICH occurred in three or four children per 100 000 pregnancies screened**.** Large prospective studies without any intervention were not found, which means that the incidence of ICH in non-screened populations is likely to be higher. The majority of neonates with ICH had severe and life-long neurological sequelae. Intervention studies using antenatal IVIG suggest that ICH due to HPA-1a alloimmunisation is preventable without known risk for mother or child. These data indeed indicate that large-scale screening studies including comparison of several interventions are warranted.[37] Given the devastating outcome in severely affected pregnancies, such programs are likely to do more good than harm, and may be cost-effective.

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CHAPTER 3

INCIDENCE AND CONSEQUENCES OF NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: A SYSTEMATIC REVIEW

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ABSTRACT

Background

Neonatal alloimmune thrombocytopenia (NAIT) is a potentially devastating disease, which may lead to intracranial hemorrhage in the fetus or neonate, often with death or major neurological damage as consequences. There are no routine screening programs for NAIT. Preventive measures are only taken in a subsequent pregnancy. To estimate the population incidence of NAIT and its consequences we conducted a review of the literature. Our result may aid in the design of a screening program.

Methods

An electronic literature search using MEDLINE, EMBASE and Cochrane database, and references of retrieved articles. Eligible for inclusion were all prospective studies aimed at diagnosing NAIT in a general, nonselected newborn population, with sufficient information on platelet count at birth, bleeding complications and treatment. Titles and abstracts were reviewed followed by review of full text publications. Studies were independently assessed by 2 reviewers for methodological quality. Disagreements were resolved by consensus including a third reviewer.

Results

From the initial 768 studies identified, 21 remained for full text analysis, 6 of which met the inclusion criteria. In total, 59 425 newborns were screened, with severe thrombocytopenia in 89 cases (0.15%). NAIT was diagnosed in 24 of these 89 newborns (27%). In 6 (25%) of these cases an intracranial hemorrhage was found, all likely of antenatal origin.

Conclusions

NAIT is one of the most important causes of neonatal thrombocytopenia. Intracranial hemorrhage due to NAIT occurs in 10 per 100 000 neonates, commonly before birth. Screening for NAIT might be effective, but should be done antenatally.

INTRODUCTION

Low platelet count is a risk factor for bleeding complications. In newborns, the most feared bleeding is intracranial hemorrhage (ICH), which may lead to death or severe lifelong neurological handicap. Severe thrombocytopenia (platelet count < 50×10^9/L) is estimated to be present in 1:700 unselected newborns.[1,2] A major cause of isolated severe thrombocytopenia in term neonates is neonatal alloimmune thrombocytopenia (NAIT)[3], with an incidence of 1 in 1000 live births.[4] In NAIT, platelet destruction is caused by maternal immunoglobulin G allo-antibodies formed during pregnancy because of incompatibility of maternal and fetal (thus paternal) antigens on the platelet's surface. In Caucasians, 85% of immunizations are caused by alloantibodies against Human Platelet Antigens (HPA) 1a, commonly acquired during pregnancy.[5]

NAIT can be diagnosed either after clinical bleeding symptoms in the neonate, or after detection of a fetal intracranial bleeding on ultrasound examination. Thrombocytopenia may also be detected unexpectedly when blood is tested for other purposes. In the absence of skin bleeding, the diagnosis may be delayed or not be made at all, leaving the child at risk for ICH until spontaneous increase in platelet count occurs.

One option for early detection and therefore reduced time-to-treatment of NAIT would be to screen all neonates for this disease. Given the high disease burden for the affected child and the family, and high costs for health care and society in case of ICH with brain damage, prevention of even a few cases may make screening cost-effective. Alternatively, as has been advocated recently by several investigators, pregnant women could be screened for HPA-type and tested for allo-antibodies.[5,6,7]

The debate on screening for NAIT is complicated by the lack of a good population data on incidence of severe thrombocytopenia and bleeding complications.

Before considering a screening program, a reliable estimate of incidence and severity of NAIT in the general population is required. Several screening studies have been performed by using HPA-typing of pregnant women. We recently reviewed these studies, and found that in most, antenatal or intrapartum interventions were offered. Therefore, the true natural history, with unbiased outcomes in neonates, could not be derived from these studies.[5] We therefore performed a systematic review of studies on screening low risk, untreated newborns for thrombocytopenia. The aim of this study was to systematically assess the reported prevalence of severe thrombocytopenia in newborns secondary to NAIT with sub-analysis of ICH due to NAIT. The outcomes of this analysis can provide an essential element in preparation for a screening project, aimed at timely identification of fetuses or neonates at risk for NAIT to prevent the burden of this hazardous disease in the future.

METHODS

Data sources and study selection

We searched the following databases for studies on severe neonatal thrombocytopenia due to NAIT and intracranial hemorrhage: PubMed, Embase, and Cochrane. We used the following keywords: "Thrombocytopenia"[Majr:NoExp] OR "Thrombocytopenia, Neonatal Alloimmune"[Majr] OR "alloimmune thrombocytopenia"[ti] OR "NAIT"[ti] OR "NAITP"[ti] OR "FNAIT"[ti] OR "FNAITP"[ti] OR "neonatal thrombocytopenia"[ti] OR "HPA 1a"[ti] OR "HPA-1a"[ti]) AND ("Mass Screening"[Mesh] OR "screening"[all fields] OR "SCREEN"[all fields] OR "Incidence"[Mesh] OR "incidence"[all fields]. We accepted original articles, short communications and letters to the editor. In addition a search was performed from the reference list of all identified articles. When needed, we contacted authors for additional, unpublished information. There were no language restrictions.

The specific research questions were: what is the incidence of HPA-1a associated thrombocytopenia? and How severely are the neonates affected?

Studies were eligible for inclusion in this review if they fulfilled the following criteria: First, the study reported on low-risk newborns with severe thrombocytopenia, defined as a platelet count <50 x10^9/L, identified through screening. Second, the number of cases of severe thrombocytopenia caused by HPA-1a alloimmunization was clearly stated. Third the study reported on clinical signs of bleeding.

We subsequently excluded all nonprospective studies. Furthermore, studies were excluded when the method of screening was a method other than measuring platelet count in cord blood or when screening was not done in a low-risk unselected population. Because we wanted to identify the incidence of HPA-1a immunization in the Caucasian population, screenings studies in a non-Caucasian population, where HPA-1bb is rare, were excluded.

Data review and analysis

Two of the authors (MK and NP) initially screened all the titles and abstracts of articles, identified by the review search strategy, for relevance. Only studies clearly irrelevant were excluded at this stage. All other studies were assessed on the basis of their full text for inclusion versus exclusion by 2 reviewers independently using the criteria indicated earlier. Discrepancies were to be resolved by discussion with a third reviewer but this was proven unnecessary.

Statistical analysis

The main outcome of this systematic review was the pooled incidence of NAIT. We also wanted to assess the burden of this disease in a general population of newborns. We extracted the following primary outcome data from the selected studies: number of severe thrombocytopenic newborns (defined as a platelet count <50x10^9/L) detected by the screening, the incidence of NAIT in that group and the number of neonatal blee-

ding signs, ICH, and combined adverse outcome defined as perinatal mortality and morbidity associated with severe thrombocytopenia.

From the selected articles, we only used the information of pregnancies and newborns from which all relevant data on primary outcomes were available. Descriptive analysis of the outcome parameters was performed by dividing the total number of newborns with the outcome parameter by the total number of newborns screened. Numbers are given in rate per 100 000, with 95% confidence intervals (CIs).

Methodological Quality

An additional evaluation to decrease the risk of bias was performed by searching for components that could hamper accurate estimation of the true natural incidence of NAIT and the associated bleeding complications. The following study characteristics were evaluated: adequacy of inclusion and of outcome determination. For the evaluation of inclusion of patients, 1 point was given if (consecutive) nonselected patients were included; therefore details of the selection criteria for newborns included and excluded were studied. For outcome determination, 1 point was given if all patients included in the study were tested for thrombocytopenia, and 1 point was given if the laboratory tests to detect NAIT were clearly described. In addition, 1 point was given if no interventions, either antenatally and postnatally, were offered to prevent bleeding complications and 1 point was given if methods to detect bleeding complications were clearly shown.

Consequently each study could attain a maximum of 5 points. Studies that scored 0 or 1 point were considered to have a high risk of bias, studies with 2 or 3 points as intermediate risk, and studies with 4 to 5 points as studies with low risk of bias.

RESULTS

Systematic literature search

The initial search revealed 768 studies. During the first screening, 747 studies were excluded, and 21 studies were assessed on the basis of their full text for inclusion or exclusion using the criteria described. After critical appraisal of the full text of the remaining 21 articles independently by two authors (MK and NP) 6 studies were included in the review.[1,2,8-11] The main reason for exclusion was that screening was not performed in a low-risk population but in newborns selected by the history of siblings, who suffered from (F)NAIT during pregnancy or at birth. Another important reason for exclusion was the use of case finding through maternal HPA-1a typing in unselected pregnant women, with further analysis in those women who were typed negative for HPA-1a.

One prospective screening study was excluded because it was conducted in Brazil where, probably related to ethnicity, no cases of HPA-1a immunization were found.[12]

The process of literature searching and study selection is illustrated in figure 3.1.

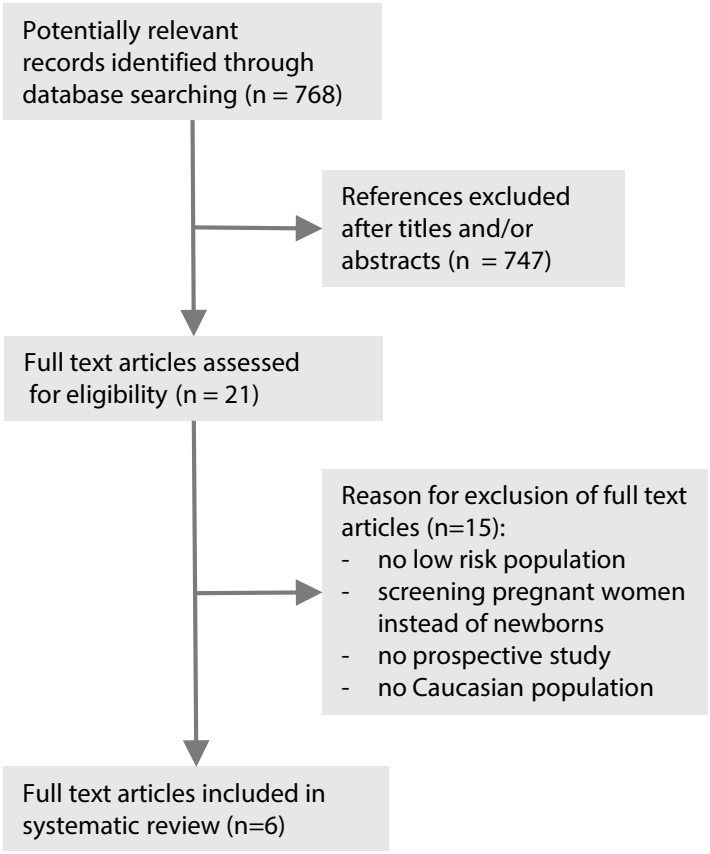

Figure 3.1
Process from initial search to final inclusion

Study characteristics

No randomized controlled trials were found. All included studies were prospective cohort studies, comparable in study design and information provided. The studies were published between 1993 and 2000. In the 6 selected studies, a total of 59 425 neonates were screened with a range from 933 to 24 101. Table 3.1 lists the primary outcome data obtained from these 6 studies.

Uhrynowska et al studied the incidence of thrombocytopenia in both unselected mothers and newborns. [11] De Moerlose et al and Burrows et al[8] measured platelet count in all mothers of the newborns screened. The other 3 studies focused on neonatal thrombocytopenia.[1,8,10]

One study analyzed 2 control groups to identify the incidence of alloimmunization in respectively thrombocytopenic mothers of non-thrombocytopenic newborns and non-thrombocytopenic mothers of non-thrombocytopenic newborn.[2] The difference

was statistically significant between the group of mothers with thrombocytopenic newborns and the control groups but not between the 2 control groups.

Only 1 paper reported a sample size calculation[1]; all the others chose a fixed time period, varying from 1 to 7 years.

Methodological quality and heterogeneity

Known HPA-alloimmunization

All study authors stated that they aimed to determine the true incidence of NAIT in an unselected population of newborns. In all series however, ≥ 1 babies were born to mothers already known to have antiplatelet alloantibodies, often with previously affected siblings. Dreyfus et al specifically mentioned that children born to women with previously affected children due to NAIT were not excluded.[1] Panzer et al included only those deliveries with an uncomplicated history of pregnancy and delivery, without further specification.[9] Only Burrows et al provided information about the history of the mothers with affected offspring.[8] In their study of 18 mothers found to have HPA-1a alloimmunization, 15 had a previous affected child, and 3 alloimmunized women were sisters of women with known NAIT.

Parity

Only in the study of Panzer et al[9] and Saino et al[10], was parity of the mothers mentioned.[9,10] In the study of Panzer et al, only the parity of the HPA-1a negative mothers (n = 11) was mentioned: 10 were primiparae, 2 had previous abortions and 1 had a third delivery with 4 previous abortions. None of these mothers were found to have HPA alloantibodies. In the study of Saino et al, (n = 4489) half of all included mothers were nulliparous, 33% had 1 previous child and 17% had ≥ 2 previous deliveries.

Gestational age

Saino et al included only full-term infants.[10] Uhrynowska et al included 6.8% preterm neonates.[11] In the study of de Moerloose et al, 19 neonates (0.23%) were premature.[2] Panzer et al stated that they only included deliveries with an uncomplicated history of pregnancy and delivery.[9] This suggests that premature deliveries were excluded. In the other 2 studies premature deliveries were not excluded, without specific numbers given.[1,8]

Laboratory testing details

The blood tests in all studies were clearly described, and references to the appropriate literature were provided. The number of total children born and the percentage of included newborns during the study period are mentioned in table 3.1. Cord blood samples were not taken from all included neonates. This percentage varied between 88 % and 99%. Main reasons for drop out were refusal of mothers, inability of caregivers

Table 3.1 *Outcome of postnatal screening studies for NAIT included in the analysis*

Author Year	Included/ Total newborns[a]	Newborns tested[b]	Severe thrombocytopenia (PC<50 x10^9/L)	Bias Assesment	Severe NAIT (PC<50 x10^9/L + HPA-antibodies)	ICH Antenatal origin	ICH Postnatal origin
Burrows 1993[8]	16068/16124	15932 (99%)	19 (0.12%)	4	10 (0.06%)	3 (0.02%)	0
Panzer 1995[9]	NA	933	4	2	0	0	0
Dreyfus 1997[1]	6081/8836	5632 (93%)	9 (0.16%)	2	4 (0.07%)	1 (0.02%)	0
De Moerloose 1998[2]	9485	8388 (88%)	10 (0.12%)	3	3 (0.04%)	1 (0.01%)	0
Sainio 2000[10]	4588/5285	4489 (98%)	11 (0.24%)	4	2[c] (0.04%	0	0
Uhrynowska 2000[11]	26275	24101 (90%)	36 (0.15%)	3	5[d] (0.02%)	1 (0.004%)	0
Total		**59475**	**89 (0.15%)**		**24 (0.04%)**	**6 (0.01%)**	**0**

PC, platelet count; NA, not available; ICH intracranial hemorrhage
[a] actual included cases versus total newborns in the study period
[b] Actual amount of newborns tested for thrombocytopenia
[c] 1 case PC 37 x10^9/L HPA-1a incompatibility, no anti HPA-1a antibodies detected
[d] 1 case PC 31 x10^9/lLHPA-1a incompatibility, no anti HPA-1a antibodies detected

to draw blood (workload), or technical problems (clotting of the blood sample). Only Dreyfus et al compared 100 of these nonsampled babies with 100 babies of the sampled cohort to examine a possible bias.[1] Comparison (screened versus non-screened) showed no significant difference in gender, ethnic origin, gestational age at delivery, parity, mode of delivery, Apgar score and resuscitation of the neonate. There was a significant difference found for birth weight (lower) and maternal age (higher) in the screened population. The difference in birth weight might bias the incidence of NAIT in the screened group. It has been reported that HPA-1a alloimmunization is associated with reduced birth weight,[12] and neonates with low birth weight are more prone to have thrombocytopenia and therefore are maybe more likely to be tested for thrombocytopenia. This may have biased to a higher incidence of NAIT.

In all studies, platelet count was measured in cord blood at birth, with confirmation from neonatal capillary or venous blood. The definition of neonatal thrombocytopenia varied among the studies ranging from 50×10^9/L[5] , to 100×10^9/L[8],to 150×10^9/L[1,2,8,9]. In our review we included only those cases with platelets < 50×10^9/L.

The diagnosis of NAIT was confirmed by the detection of anti HPA-1a antibodies in the mother of an HPA-1a incompatible infant. In the studies of Uhrynowska et al and Panzer et al, HPA-typing was done in all maternal plasma, followed by evaluation for HPA-1a antibodies in case of HPA-1a incompatibility.[9,11] In the other studies this was only done in case of confirmed neonatal thrombocytopenia.[1,2,8,9]

In the study of Burrows et al the percentage of alloimmunization (formation of anti HPA-1a antibodies) in case of severe thrombocytopenia was not described.[8] In 19 of the 15 932 pregnancies, parental mismatch for HPA-1a was noted after detecting a low platelet count in the newborn.

Antenatal and postnatal interventions

None of the studies excluded pregnancies already known to be complicated by NAIT. In these cases, various antenatal preventive interventions were described. Saino et al described 1 pregnancy with known HPA-1a alloimmunization treated antenatally with maternal intravenous immunoglobulins (IVIG) and a single intrauterine platelet transfusion.[10]

De Moerloose et al described 1 case in which fetal blood sampling was performed at 32 weeks' gestation for Rh-D alloimmunization.[2] Severe thrombocytopenia was incidentally discovered. Bleeding and persistent bradycardia occurred during the procedure, followed by emergency cesarean section. The neonate was found to have a severe intracranial hemorrhage and died after 3 days.

Burrows et al used antenatal IVIG therapy successfully in 16 pregnancies with known risk for NAIT.[8] The 3 neonates with severe ICH in their series were not born from mothers with a known history of NAIT.

Dreyfus et al described intrauterine fetal blood sampling in a pregnancy with known NAIT, which resulted in excessive bleeding and emergency cesarean delivery.[1] A severely thrombocytopenic child was born who suffered from ICH. The neonate was treated immediately with matched platelets and IVIG. A left subependymal hemorrhage resolved without sequellae.

Saino et al used IVIG and platelet transfusions in 2 neonates with unexpected thrombocytopenia.[10] Dreyfus et al treated 2 thrombocytopenic neonates from pregnancies without a history of NAIT with IVIG.[1]

The other 4 studies did not provide data on postnatal treatment.[2,8,9,11]

Evaluation of bleeding complications

Routine ultrasound on the screened neonates was not performed in any study to detect signs of intracranial bleeding. Only 2 articles noted that ultrasonography was done in all neonates with severe thrombocytopenia to exclude ICH.[8,9] Because ultrasound is not always routinely performed in the absence of clinical ICH symptoms, this may have led to underdiagnosing, and lower incidence, of ICH. Only Saino et al described all full-term intrauterine deaths and performed autopsies on all. None had signs of serious hemorrhage.[10]

Risk of bias assessment

Four studies (Dreyfus et al[1], de Moerloose et al[2], Panzer et al[9], and Uhrynowska et al[11]) were classified as intermediate risk (received 2 or 3 points) and 2 studies (Burrows et al[8], Sianio et al[10]) as low risk (received 4 points). No study received the maximum of 5 points.

Perinatal mortality and neonatal morbidity

In the cumulative cohort of the 6 included studies, with a total of almost 60 000 newborns tested, the pooled prevalence of severe thrombocytopenia (platelet count <50×10^9/L) in neonates was 150 per 100 000 (0.15%; 95% CI, 0.0012-0.0018). In 24 cases (27%) NAIT was the cause of thrombocytopenia. In 6 of 24 neonates, ICH was detected, with an incidence of 39 per 100 000 neonates, most likely all 6 of antenatal origin. All neonates with low platelets and ICH in these series were diagnosed with NAIT. Details of this group are listed in table 3.2. The study of Panzer et al was not included in this table because no cases of NAIT were found.

A sensitivity analysis was performed with 2 studies with the lowest estimates risk of bias. The pooled prevalence of severe thrombocytopenia was then calculated as 0.0015 (95% CI, 0.0013-0.0017), which is similar with the pooled data of all the studies.

Table 3.2 *Characteristics of neonates affected by HPA-1a alloimmunization*

Author Year	NAIT Cases	PC (×10^9/L)	Bleeding Signs	Neonatal illness	Intervention
Burrows 1993[8]	1-9	6-40	ICH (n=2)	--	Antenatal IVIG(n=7);2 ICH Cases, no antenatal intervention
	10	unknown	ICH	IUFD at 35 wks	-
Dreyfus 1997 [1]	1	46	-	IUGR	Postnatal IVIG
	2	50	-	-	-
	3	2	Petechiae	-	Postnatal IVIG
	4	20	bleeding after FBS	ICH/Porencephaly	Antenatal IUPT Postnatal IVIG
De Moerloose 1998[2]	1	48	Petechiae	-	unknown
	2	20	Petechiae	-	unknown
Sainio 2000[10]	1	18	Petechiae	-	Postnatal IVIG
	2	33	-	-	Antenatal IVIG+IUPT
	3	40	-	-	Postnatal IVIG
Uhrynowska, 2000[11]	1	47	-	-	Unknown
	2	41	ICH	Infection, prematurity	Unknown
	3	18	Petechiae	-	Unknown
	4	27	None	-	Unknown
	5	25	Petechiae	-	Unknown

FBS fetal blood sampling; IUFD intrauterine fetal death; IUGR intrauterine growth restriction; IUPT intrauterine platelet transfusion; IVIG intravenous immunoglobulin; — none.

DISCUSSION

Neonatal thrombocytopenia is a serious condition, requiring rapid diagnostic and therapeutic action to prevent bleeding complications. A major cause in otherwise healthy term newborns is NAIT.

Although this systematic review of screening studies in general populations produces the best possible estimate of the incidences of NAIT and NAIT-related ICH, underestimation remains likely. Older studies on risks for ICH in HPA-immunized pregnancies cited an incidence ranging from 7 to 26%, or 3 to 10 per 100 000 pregnancies.[13,14] However, true natural history information is lacking. Our previous review on screening studies performed in pregnant women at risk for FNAIT[5] showed that screen-positive cases and those already known to have alloantibodies against fetal platelets received, understandably, various types of interventions. The results of the current review showed a pooled incidence of severe NAIT of 63 per 100 000, with 6 cases of ICH per 100 000 (table 3.3). All cases of ICH reported in these 2 reviews were (likely) of antenatal origin. The difference in the incidence of ICH, 9.9% vs 25% (Fisher's Exact test $P = .087$) does not reach statistical significance because of limited numbers. However, the difference in our view is most likely explained by the use of interventions, which is only possible in the antenatally screened group.

Without routine screening in low-risk, general populations of pregnant women or newborns, the diagnosis of FNAIT is often missed. Tiller et al calculated that testing for FNAIT only in the presence of clinical symptoms would miss the diagnosis in 86% of cases.[15] Other study data showed that even in neonates born with severe thrombocytopenia, timely diagnostic testing for NAIT was not performed in 15% of the cases, with severe consequences for the subsequent pregnancies resulting in the occurrence of ICH.[16] Missing the diagnosis is obviously potentially hazardous for the neonate. In addition, the mother is unaware that in a subsequent pregnancy, the risks for fetal or neonatal bleeding complications are high. This is particularly important given the highly effective preventive measures that are currently available.[17]

Our review confirms previous reports suggesting that the majority of cases of ICH develop in utero.[5,18] A recent study of Tiller et al showed that most cases of ICH seem to occur before the 28th week of gestation.[19] Therefore, screening neonates with the

Table 3.3 *Comparision of Incidences of FNAIT and ICH in Antenatal versus Postnatal screening studies*

Studies	Number of subjects screened	Severe FNAIT[a]	ICH
Antenatal screening[b]	176 084	71 (0.04%)	7 (9.9%)
Postnatal screening[c]	59 425	24 (0.04%)	6 (25%)

[a] *Thrombocytopenia < 50×109/L*
[b] *Kamphuis et al*
[c] *Current study*

aim to timely detect and treat severe thrombocytopenia will have limited effectiveness. Neonatal screening may have some benefit particularly in the asymptomatic thrombocytopenic child, although the studies we analyzed were not designed to investigate this. An expected benefit would be to provide the mother with knowledge that FNAIT may occur in future pregnancies, and she should consult a fetal medicine specialist to help prevent complications.

Significantly more benefit can be expected from general screening of pregnant women for HPA type and anti-HPA alloantibodies. Intervention options for screen-positive pregnancies suggested by investigators are weekly IVIG infusions to the mother, timed near-term delivery by induction of labor or cesarean delivery, birth in a perinatal center with immediate availability of matched platelets and combinations of these measures. Several groups have published calculations of costs and potential benefits of screening and intervention, all coming to the same conclusion that such programs are likely cost-effective.[5-7,20-22] The main reason for cost-effectiveness, despite large-scale testing and, in the case of IVIG, expensive treatment, is the fact that the disease burden and costs for a child with lifelong severe neurological damage from ICH is excessive.

CONCLUSION

This review shows that NAIT is among the most important causes of severe thrombocytopenia in newborns, with ICH in at least 10 per 100 000 newborns. Given the antenatal origin of most intracranial bleedings, the best option to reduce the associated mortality and morbidity seems to be screening all pregnant women for HPA alloimmunization, followed by effective interventions.

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CHAPTER 4

FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: PRENATAL INTERVENTIONS

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ABSTRACT

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a potentially devastating condition, which may lead to intracranial haemorrhage (ICH) in the fetus or neonate, often with death or major neurological damage as consequence. In the absence of screening, preventive measures are only possible in the next pregnancy of women with an affected child.

Controversy exists on the best intervention to minimise the risk of ICH. Most centres have abandoned treatment with serial fetal blood sampling (FBS) and platelet transfusions, because of a high rate of complications and the availability of quite effective non-invasive alternatives. In pregnancies with FNAIT and a previous affected child without ICH, weekly intravenous administration of immunoglobulins to the mother appears close to 100% effective to prevent fetal or neonatal ICH. Some centres add prednisone; this combination leads to slightly higher platelet counts at birth. In pregnant women with a previous child with ICH, the recurrence risk seems particularly high, and more aggressive maternal medical treatment is recommended, starting earlier with immunoglobulins. Whether a higher intravenous immunoglobulin dose or the addition of prednisone is really necessary is unclear. What does seem to be clear is that the use of fetal blood sampling should be minimised, possibly even abandoned completely.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a potentially devastating condition, which may lead to intracranial hemorrhage (ICH) in the fetus or neonate, often with death or major neurological damage as consequence. The reported incidence of FNAIT ranges from 1:350 to 1:1000. [1,2] FNAIT is the most common cause of thrombocytopenia in term neonates. Thrombocytopenia is defined as a platelet count < 150x10^9/L. However, the risk of clinically significant bleeding problems is minimal unless platelets counts drop below 30 or even 20 x10^9/L. A generally accepted definition of severe FNAIT is a platelet count <50 x10^9/L. In this group ICH occurs in around 10% of fetuses and neonates.[3] Many cases, even of severe FNAIT, may therefore occur unnoticed.

The most severe complication is ICH, leading to perinatal mortality in 1-7% and surviving children with often severe neurological sequelae including mental retardation, cerebral palsy, cortical blindness and seizures in 7-26% of affected pregnancies.[3,4] Unlike in the pathophysiologically similar red cell alloimmunisation, fetuses in a first pregnancy can be severely affected. In the absence of any screening programmes, the disease is virtually always only detected after birth of an affected child. Preventive measures can than only be taken in the next pregnancy. There is still controversy on type and timing of various interventions in pregnancies with FNAIT. In this review article, we critically evaluate the literature on these preventive interventions for pregnancies known to be complicated with FNAIT.

PATHOPHYSIOLOGY

FNAIT is caused by an immunological process in which the mother produces an antibody-mediated response against a platelet-specific antigen that she herself lacks but that is present on the fetal platelets, inherited from the father. The fetal platelets are expressed as early as 16-18 weeks of gestation.[5]

The mother's antibodies [of the immunoglobulin G (IgG) type] can cross the placenta and bind to fetal platelets. The antibody-coated platelets are subsequently removed from the fetal circulation by the reticuloendothelial system, which results in fetal thrombocytopenia. These same antibodies also may inhibit platelet production.[6,7] The proportion of individuals belonging to a particular platelet antigen type varies according to race. Human Platelet Antigen (HPA)-1a is the immunodominant antigen in Caucasian individuals responsible for 85% of the FNAIT cases, followed by HPA-5b. [8,9]

IDENTIFYING PREGNANCIES AT RISK

Only around 10% of HPA-1a negative pregnant women actually produce HPA-antibodies despite carrying a HPA-1a-positive fetus.[3] There appears to be an association between maternal maternal Human Leucocyte Antigen (HLA) type and tendency to produce these antibodies. Most immunisations occur in women with HLA DRB3*0101, and in the absence of this phenotype, immunisation is rare. According to Bussel and Primiani the positive predictive value of this phenotype is only 35%, but negative predictive value would be 99.6%.[10] However in the largest prospective study thus far, by Kjeldsen-Kragh et al, 12 of 150 HPA-1a-negative women were HLA DRB3*0101 negative and 2/12 still gave birth to thrombocytopenic children.[11] At this point, the use of HLA typing to direct clinical management in our view is therefore questionable but more data are needed.

Several investigators aimed to detect a certain threshold of HPA-antibody level in the maternal serum below which severe FNAIT would not occur or which level would predict the degree of thrombocytopenia. The largest prospective study evaluating possible predictive value of HPA-antibody levels came from Norway.[12] Antibody quantitation was carried out in 160 pregnancies with FNAIT. In most multiparous women, the antibody levels decreased during pregnancy whereas in first immunisation a rise was commonly seen. Although there was a correlation between area under the curve of antibody levels measured in the second half of pregnancy and the neonatal PC, clinical application would be difficult. Using antibody levels taken at 22 and 34 weeks' gestation, and a cut-off level of 3.0 IU/mL, 8-18% of severe FNAIT cases would be missed.

In a recent large retrospective study by Kaplan's group, the area under the curve of maternal antibody concentration had some predictive value for a neonatal platelet count < $50x10^9$/L in pregnancies treated with (IVIG) and IVIG + steroids. With a not very impressive sensitivity of 64% and a specificity of 83%, and derivation of the cut-off level from the same population, it seems likely that in a prospective cohort the diagnostic accuracy of this test proves to be disappointing.[13]

Fetuses with FNAIT with PCs <$50x10^9$/L, have a potential risk of bleeding. The most feared complication, and the true disease that needs to be prevented, is ICH. The best estimate of the incidence of ICH comes from a systematic review of all prospective screening studies in unselected populations, encompassing a total of 176 084 pregnancies.[3] In 7/71 (10%) of the pregnancies with severe FNAIT (PCs <$50x10^9$/L), severe adverse outcome occurred with ICH, leading to death (*n*=2) or permanent neurological sequellae in most others. All except one of these bleedings occurred before birth. Even these prospectively derived data are an underestimation, as in all these studies, some form of preventive measure was taken in screen-positive pregnancies. Our best estimate is therefore that the incidence of clinically significant ICH due to FNAIT ranges between 3 and 10 per 100 000 pregnancies. Given the often life-long handicaps in survivors, a screening and intervention program would in most Western countries almost certainly be cost-effective.[3,14,15]

PRENATAL MANAGEMENT OF PREGNANCIES WITH FNAIT

With the current lack of screening programmes, we only need to focus on how best to manage pregnancies with known FNAIT, thus those pregnant women with a previously affected child. This indicates the value of recognising this diagnosis in relation to the prenatal management of subsequent pregnancies.

Commonly used prenatal treatments include serial fetal platelet transfusions or transplacental medical treatment using immunoglobulins and/or corticosteroids. Other options which may be used in conjunction to fetal therapy are near-term induction of labour, near-term caesarean section, and delivery in a tertiary care. In case of a vaginal birth, it is commonly advised to refrain from using forceps, ventouse, scalp electrodes and fetal scalp blood sampling. In pregnancies with timely diagnosed severe fetal ICH, termination of pregnancy (TOP) could be offered.

These modalities will be discussed in more detail. A suggested flowchart for the management of pregnancies with FNAIT is given in figure 4.1.

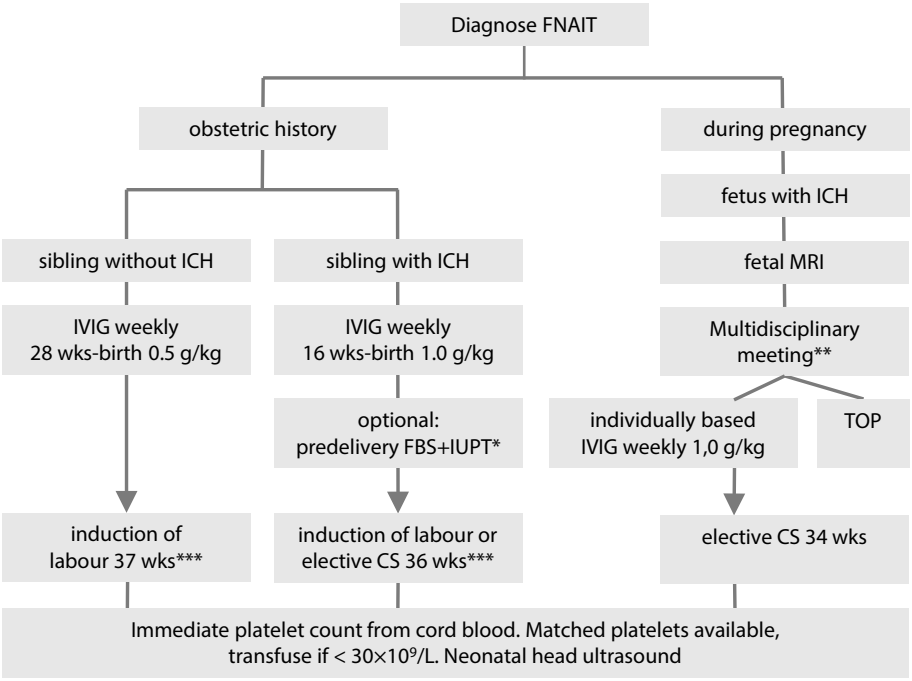

Figure 4.1 Flowchart for perinatal management of fetal and neonatal alloimmune thrombocytopenia
**with platelet transfusion if platelet count <50×10⁹/L*
*** consulting paediatric neurologist, neonatologist regarding outcome*
**** avoid any potential traumatic events, like scalp electrode, scalp bloodsampling or assisted vaginal delivery*
ICH intracranial haemorrhage; IVIG immunoglobulins; TOP termination of pregnancy, CS caesarean section; FBS fetal blood sampling; IUPT intrauterine platelet transfusion

INVASIVE FETAL THERAPY FOR FNAIT: FETAL BLOOD SAMPLING AND INTRAUTERINE PLATELET TRANSFUSION

Until 1984, the traditional management of subsequent pregnancies in women with a previous history of FNAIT consisted of an early elective caesarean section and transfusion of platelets after birth. Since the publication of Daffos et al[16], one of the pioneers of fetal blood sampling (FBS), several centres throughout the world started treating fetal thrombocytopenia in a similar fashion to anemia due to red cell alloimmunisation, by serial intrauterine platelet transfusions (IUPT). [17-19] In red cell alloimmunisation, this therapy has no alternative, and has been shown to be relatively safe in experienced hands, with procedure-related loss rates below 2% .[20] Two important differences with blood transfusion for fetal anemia must be stressed.

First, puncturing the umbilical cord in fetuses with a low PC increases the risk of complications, particularly exsanguination. In our experience and that of others, immediate platelet transfusion does not always prevent such a disaster.[21] All large centres involved in the treatment of FNAIT have reported fetal losses because of FBS and/or IUPT, except for a group from Germany who reported no fetal loss in 470 IUPTs for FNAIT, all done by a single operator.[22] Combining data from three relatively large series from experienced centres, the cumulative risk of fetal loss per pregnancy directly related to complications of FBS and IUPT was 6%.[21,23-25] Prolonged bradycardia is more often noted as compared to red cell transfusions, possibly related to a larger content of plasma.[26]

Second, Bussel et al, already pointed out that the half life of transfused platelets is very short, a few days only, which means that platelet transfusions would be required at least weekly if not more often.[27] This is clearly shown by the pretransfusion PCs in subsequent transfusions in patients treated with IUPTs. Commonly, these values are within a week again well below $50x10^9$/L, indicating that even weekly transfusions are likely inadequate to maintain safe PCs. [4,26,28]

With the mounting evidence that non-invasive treatment using immunoglobulins with or without steroids has high success rates, very few centres nowadays continue to use FBS and IUPT as the sole treatment of FNAIT. Some groups still use one or two diagnostic FBS to fine-tune and verify the medical therapy, or a predelivery fetal blood sample to enable vaginal birth. In the group of patients with an affected previous sibling without an ICH, which is the majority of cases referred to our national fetal therapy centre, we have refrained from any invasive procedure for the past 10 years.[29]

NON-INVASIVE MANAGEMENT OF FNAIT: IVIG AND STEROIDS

Concerns about the risks of invasive treatment have lead to the exploration of maternal treatment. The first report on clinical efficacy of IVIG in FNAIT dates from 1988, where Bussel *et al*. described the successful use of a weekly dose of 1 gr/kg maternal weight in seven pregnancies.[27] The treatment regimen was based on experience with idiopathic

Table 4.1 *Maternal IVIG to prevent ICH in Fetal and Neonatal Alloimmune Trombocytopenia: summary of the literature*

	Number of fetuses	Dose (g/kg/week)	ICH (n)	Plt count at birth ($x10^9$/L)	Neonatal Plt $<50 \times 10^9$/L
Lynch 1992	4	1.0	0	184 (57–322)	0
Birchall 2003	12	1.0	0	83	4 (33%)
Berkowitz 2007	37	2.0	1[a]	169 (14–312)	5 (14%)
Yinon 2006	24	1.0	0	118	<30: 2 (8%)
Van den Akker 2007	45	1.0	0	136	7 (16%)
Giers 2010	30	1.0	0	31[b] (6–117)	26 (87%)
Bertrand 2011	27	1.0	0	89 (95% CI 55–123)	12 (44%)
Knight 2011	17	n.s.	0	n.s.	n.s.

Studies included only if IVIG was given alone, without steroids or intrauterine platelet transfusions, in women with an affected previous child
without ICH.
GA gestational age; ICH intracranial haemorrhage; IVIG intravenous immunoglobulin; n.s. not stated; Plt platelet.
[a] Neonatal grade 1 subependymal bleeding, with platelet count at birth 133 $x10^9$/L, not considered treatment failure.
[b] Value obtained by fetal blood sampling just before birth, followed by single platelet transfusion.

thrombocytopenic purpura (ITP) patients. Several observational studies in women with a previous child affected by FNAIT suggested that this treatment is highly effective in this group (table 4.1).

How does IVIG work? This is in fact still unknown, although a combination of mechanisms seems likely. First, in the maternal circulation the IVIG will dilute the anti-HPA antibodies, resulting in a lower proportion of anti-HPA antibodies among the IgG transferred via the Fc-receptors through the placenta. Second, IVIG can block the placental receptor and decrease the placental transmission of maternal antibodies including anti-HPA-antibodies. Third, in the fetal circulation, IVIG may block the Fc-receptors on the macrophages and prohibit the destruction of antibody-covered cells.[30,31] However, other effects of IVIG, such as anti-idiotypic neutralisation of anti-HPA antibodies or suppression of antibody producing B cells, cannot be excluded.

IVIG is regarded as an expensive but safe and well-tolerated drug, although due to its production from plasma from multiple (>1000) blood donors, concern remains on risks for viral and other infections. Main dose-related side effects include headache and fever, whereas rarely more serious adverse events including chest pain, laryngeal edema, renal failure, aseptic meningitis and thrombotic complications have been reported.

The long-term side effects for mother and child are still unclear. As IVIG is known for its immunomodulating characteristics, there are some concerns. A study on short-term follow-up found a possible increase of IgE in children after maternal IVIG administration compared to the normal population. However, no clinically apparent adverse effects in early childhood could be demonstrated.[30]

Since Bussel's first report, the standard dose of IVIG in FNAIT has empirically been 1 g/kg given weekly, starting anywhere between 20 and 32 weeks of gestation. Only two studies on dose have been performed, with 0.5 g or 2 g instead of 1 g, without showing clear evidence for a particular dose.[32,33] There is theoretical and in vitro evidence that fetal IgG levels do not increase when the mother is given more than 0.5 g/kg/wk, suggesting a saturation of the placental IgG transfer.[30,34]

EFFICACY OF IVIG

Several hundreds of women with FNAIT have now been treated with IVIG, with a close to 100% success, with success defined as the absence of ICH (table 4.1). To our knowledge, only one often quoted well-documented case has been reported of fetal ICH despite 11 weeks of IVIG (1.0 g/kg) treatment.[35] Interestingly, this fetus was also managed by serial FBS and a total of 13 IUPTs, of which six were given prior to the detection of the ICH at 32 weeks. A second case, with less details but with remarkable similarities, was incorporated in a recent cohort study by Knight et al.[4] This pregnancy was managed with IVIG, steroids and IUPTs without improvement in PC, the infant had ICH. An outlier study is the one by Giers et al, describing a cohort of FNAIT pregnancies treated with IVIG, with a diagnostic FBS done before every weekly IVIG gift.[36] The 30 fetuses showed remarkable little response to the IVIG (table 4.1). Jim Bussel, in an accompanying editorial, suggested that the serial FBS might have increased sensitisation causing the low PCs in this cohort. [37] This may have played a role in the two above-mentioned cases of apparently failed IVIG treatment.

NON-RESPONDERS

However, in all studies reporting on IVIG in the treatment of FNAIT, around 20% (range 8 to 87, table 4.1) of fetuses do not seem to respond, with platelet counts remaining below $50x10^9$/L. There is currently no explanation for this phenomenon. The 'non-responders' may thus still be at risk for ICH, although surprisingly, this seems extremely rare in IVIG-treated fetuses. An intriguing hypothesis is that IVIG apart from often causing a rise in platelets, may aid in protection against bleeding.[29,38] A proposed mechanism supporting this hypothesis was described by Van Gils et al., studying the effects of HPA-1a antibodies on Fc-receptors on endothelial cells. This area certainly deserves further research.[39]

As many investigators consider the non-response to the commonly used IVIG dose of 1.0 g/kg maternal weight per week a failure of the treatment, other medication regimens have been studied. The two most common variants are increasing the dose of IVIG to 2 g/kg/week, and the addition of corticosteroids.

CORTICOSTEROIDS

In early studies by the group of Bussel, dexamethasone (3-5 mg/kg) was used in combination with IVIG 1.0 g/wk, but soon abandoned, due to both a limited effect and significant side effects for both mother and fetus (oligohydramnios).[40] A lower dose of dexamethasone (1.5 mg) with IVIG was then studied, and shown not to be superior to IVIG alone.[41] Since then, most investigators have used high dose prednisone to add to IVIG, or prednisone alone. The maximum tolerable dose of prednisone is 0.5 mg/kg per day.[10] Oligohydramnios is uncommon with prednisone treatment.

COMPARISON OF DIFFERENT APPROACHES

Although the end-point in many studies on the efficacy of various treatments often was, and should be, the occurrence of ICH, all studies published thus far were seriously underpowered to find any significant difference in this outcome.[42] Using the surrogate outcome of PC at birth, several randomised controlled trials by the group of Bussel, compared a variety of treatments in pregnancies with FNAIT, subdivided in groups perceived to have different risks of recurrence. In all pregnancies, one or more diagnostic FBS were done to monitor treatment. None of the treatment regimens proved to be significantly superior, although a trend towards higher PCs was observed for the use of 2.0 g/kg IVIG or the combined use of IVIG and prednisone in the cases with a previous prenatal ICH .[10]

When we compared our results of empiric or 'blind' treatment (without the use of diagnostic FBS) with 1.0 g/kg IVIG (Van den Akker et al, $n = 49$)[29] with two published comparable series by Birchall *et al* [23] ($n = 18$) and Berkowitz et al [25] ($n = 79$), both using IVIG or IVIG and steroids together with serial FBS for monitoring, we found that our non-invasive approach resulted in 100% survival without ICH, whereas in the other series ICH occurred twice, two cases of fetal loss due to FBS and 13-17% emergency caesarean sections during FBS, resulting in premature birth.[43]

A recent retrospective cohort study from the UK, describing 45 pregnancies with known FNAIT, two children were born prematurely by emergency caesarean section following complications of FBS.[4] One third of these cases was treated with IVIG alone, one third with combined IVIG, steroids and IUPT, 11% with IVIG and IUPT and 9% with IUPT alone, all in the years 2006-2008. This nicely illustrates the lack of consensus on the best treatment for FNAIT. The most important finding was that in pregnancies with known FNAIT and 1 year follow-up ($n = 28$), all children were alive and healthy, while in the group with unknown or unrecognised FNAIT ($n = 88$), disability or death occurred in 10%.

Similar results were reported recently from France and Switzerland.[44] Pregnancies with known FNAIT ($n = 92$) were most often treated with IVIG + steroids (59%), followed by IVIG alone (29%) and steroids alone (12%). Again, none of these pregnancies ended

in birth of a child with ICH. Neonatal PCs were highest in the group receiving IVIG + steroids (135 x10^9/L), followed by the group with IVIG alone (89x x10^9/L) and steroids alone (46x10^9/L). Severe thrombocytopenia defined as platelets < 50x10^9/L occurred in these groups in 27%, 44% and 73% respectively.

The intriguing question remains whether the favourable outcomes in the treated groups are due to actual treatment effects, or that the natural history is less similar to Rhesus disease than we commonly think, with possibly reduced risk in subsequent pregnancies. Antibody-level studies from Norway suggest that this might be true.[12] Only a randomised controlled trial including a placebo arm would provide appropriate evidence.

An elegant decision analysis model study by Thung and Grobman suggested that considering all options, empiric or 'blind' treatment with IVIG (thus without any FBS) would be the most cost-effective approach.[45]

MANAGEMENT OF PREGNANCIES PRESENTING WITH FETAL ICH

Finally we would like to discuss the management of pregnancies presenting with ICH secondary to FNAIT. It is a rare but problematic condition, therefore multidisciplinary approach including paediatric neurologist and neonatologist is advised. After detection of ICH on ultrasound, fetal magnetic resonance imaging (MRI) may further determine the extent of cerebral damage and the prognosis. In the few cases we have managed in our centre, we opted for treatment with IVIG until birth (around 34 weeks, by caesarean section) with the aim to protect the fetus from further bleeding while allowing for further (lung) maturation (figure 4.1). A separate article about these case series is in progress. For some couples termination of pregnancy (TOP) might be an option if cerebral damage is severe and seems incompatible with live. Finally, in cases with an expected poor prognosis, vaginal birth without fetal monitoring and refraining from neonatal interventions might be considered, meaning no intervention based on fetal condition. We do realise that some of these options may be restricted by law, varying by country.

PREVIOUS CHILD WITH ICH: VERY HIGH-RISK GROUP

Most reports support the assumption that pregnant women with a previous child with an intracranial bleeding comprise the highest risk group. Most clinicians caring for such pregnancies choose for a more aggressive approach as compared to the group in which the affected sib did not have an ICH. Over time, many centres have reduced their use of FBS and IUPT, as also or even more clearly, in this very high-risk group the invasive procedures do more harm than good. Kanhai et al was the first to describe completely non-invasive management using IVIG only in this group.[38] They treated 7 pregnancies with IVIG 1.0 g/kg/week, no ICH occurred, although PCs at birth were low (mean 27x10^9/L,

range 10-49). These finding again support a possible protective role against bleeding of IVIG in so-called 'non-responders'. Recently, Bussel et al, reported a relatively large series of FNAIT pregnancies with a previous child with ICH.[46] The 37 cases were subdivided in three risk-categories depending on the assumed timing of the ICH, and received IVIG 1.0 or 2.0 g/kg with or without prednisone in a complicated scheme. Only one pregnancy was treated with the use of IUPT. Three ICHs occurred, two in fetuses treated with IVIG 1.0 g/kg + prednisone 1mg/kg/day, and one in a fetus treated with 1.0 g/kg IVIG alone. No clear advantage from one type of treatment over another could be observed.

Following one of our studies[38] we continued our non-invasive protocol in this high-risk group. In order to allow a vaginal delivery we offer this small group of women pre-delivery FBS to determine the PC, in a set-up prepared for immediate caesarean section, if needed followed by platelet transfusion, in order to achieve a safe level for vaginal birth.

There is no consensus about the gestational age of starting prenatal treatment. Some groups start at 12 weeks, others at 16 weeks of gestation.[38,46] As fetal platelets antigens are fully expressed as early as 16-18w weeks we believe starting with IVIG at 16 weeks should both be safe and effective in preventing bleeding complications due to FNAIT (figure 4.1).[5]

DELIVERY, MODE AND TIMING

Caesarean section is often routinely employed for delivery in pregnancies with FNAIT. Evidence for a protective effect of caesarean section reducing the risk of ICH is lacking. Our recent systematic review showed that none of the cases of ICH seemed to have occurred intrapartum, and such cases are extremely rare in the literature as well.[3] The only study on this subject, by Van den Akker *et al* obviously underpowered with 32 FNAIT pregnancies with a sibling with thrombocytopenia but without an ICH, suggested that vaginal delivery was not associated with neonatal intracranial bleeding.[47] Most pregnant women with known FNAIT are multiparous, and a non-traumatic delivery is usually expected. We routinely advise near-term induction in multiparous women with known FNAIT and a sibling without ICH. In the small group of women with a previous child with an ICH, we offer elective caesarean section after steroids or predelivery FBS with matched platelets for transfusion at 36-37 weeks, although again, no evidence for such a policy exists.

The Norwegian investigators defended their choice of caesarean section in all women with FNAIT with three arguments. The first was the ability to deliver the child 2 - 4 weeks prior to term. This is obviously also possible with the currently quite effective prostaglandin or balloon induction. Second, they refer to a radiologic study in which 26% of neonates born vaginally had signs of haemorrhage on MRI, compared to 0% in the group delivered by caesarean section. [48] However, most of these bleedings were small subdural hematomas; none were symptomatic and most disappeared within 5

weeks. A more recent larger study by Rooks et al[49] with a similar design showed presence of such hematomas also in neonates born after caesarean section. This type of bleeding is thought to originate from tearing of small veins by the (normal) movement of the skull bones during labour and delivery, and are quite different from intraventricular and intraparenchymal bleeding commonly seen in ICH due to FNAIT. The third argument was that planned caesarean section would provide time for the blood bank to prepare matched platelets. This is however a matter of logistics and would be equally true for planned induction of labour. In conclusion, although not proven to be safer, we cannot entirely exclude that elective caesarean section protects against ICH. The associated maternal morbidity and increased risks in subsequent pregnancies must be weighed against perceived although possibly non-existent benefits.

POSTNATAL MANAGEMENT OF NEONATES WITH FNAIT

FNAIT in neonates has a variable course, in most cases thrombocytopenia resolves spontaneously in 1 to 16 weeks after birth. The most feared complication ICH mainly occurs in utero, so at birth the damage is already done.

The primary goal of the management of a neonate with FNAIT is to prevent or stop thrombocytopenic bleeding. Neonates without bleeding and a platelet count (PC) above 50×10^9/L may be closely observed without transfusions.[50]

Our protocol in Leiden advises not administer platelets when PC is above 30 and more recently even 20×10^9/L without any sign of bleeding.

There our two distinct treatment groups; those treated with IVIG prenatally and those diagnosed after birth in the presence of clinical symptoms. In the prenatally treated group a rapid rise in PC after 1 HPA-matched transfusion can be expected without the need of administering IVIG. In postnatally diagnosed cases random platelet transfusions can be used until matched platelets are available.[51] A study of Kiefel at al showed that serial random donor platelets could successfully be used in the majority of cases pending the availability of matched platelets.[52]

Transfusion of HPA 1a/5b negative platelets, if available from stock, will be effective in more than 95% of the cases seen in a Caucasian population.[50] Our bloodbank organisation can provide such units within a few hours anywhere in the country. IVIG could be added to increase and prolong the response to transfusions .[51] The use of corticosteroids is not supported by any evidence.[53]

CONCLUSION

The optimal prenatal therapy to prevent ICH in pregnancies complicated by FNAIT is still unclear. All published treatment modalities are associated with a significant percentage of failing to keep the PC in the safe range, which is above 30 or 50 $x10^9$/L.

However, the true goal of preventive measures is to prevent bleeding complications. In particular in the group of FNAIT pregnancies with a previous child without ICH, treatment with 1.0 g/kg IVIG appears highly successful. In this group, there is insufficient evidence for additional benefit of FBSs or steroids. Only small studies have been done using maternal medical treatment in FNAIT pregnancies with a previous child with ICH. IVIG with or without steroids was successful in this high risk group as well, although not 100%. Large prospective, preferably randomised studies are needed to provide evidence for optimal strategies.

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CHAPTER 5

DELAYED DIAGNOSIS OF FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: A CAUSE OF PERINATAL MORTALITY AND MORBIDITY

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ABSTRACT

Objective
To evaluate the rate and consequences of a late or missed diagnosis of fetal and neonatal alloimmune thrombocytopenia (FNAIT).

Design
Retrospective analysis of prospectively collected data of a national cohort.

Setting
National referral centre for fetal therapy in the Netherlands.

Population
Twenty-six women with pregnancies complicated by FNAIT and at least one previous pregnancy with a thrombocytopenic child.

Methods
Retrospective analysis of data from our electronic FNAIT database. In a consecutive cohort managed between July 2008 and July 2010, timing of first diagnosis of FNAIT was correlated to severity and outcome in the subsequent pregnancies.

Main outcome measures
Occurrence of delayed diagnosis of FNAIT, and possibly associated intracranial haemorrhage (ICH).

Results
In four of 26 cases, timely diagnostic testing for FNAIT was not performed despite fetal or neonatal thrombocytopenia or ICH. Down's syndrome, dysmaturity and birth trauma were perceived to be the cause of the thrombocytopenia or ICH. In two of these four subsequent, untreated pregnancies, severe fetal ICH occurred. The other 22 women were treated for FNAIT using intravenous immunoglobulin, all children are alive and well.

Conclusions
All neonates with thrombocytopenia at birth should be evaluated for FNAIT. Missing this diagnosis can have severe consequences for subsequent pregnancies.

INTRODUCTION

Thrombocytopenia is a common clinical problem in neonates: 1-5% of newborns present with this problem.[1] Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of thrombocytopenia in otherwise healthy term infants.[2] It is the result of maternal alloimmunisation to antigens on fetal platelets, inherited from the father. The maternal immunoglobulin G (IgG) antibodies cross the placenta and cause destruction of fetal/neonatal platelets, with resultant thrombocytopenia and associated risk of bleeding.

A potentially devastating condition, FNAIT may lead to intracranial haemorrhage (ICH) in the fetus or neonate, often with death or major neurological damage as consequence. The reported incidence of FNAIT ranges from 1: 500[3] to 1:1100.[4] With an annual birth rate of 185 000, the expected number of affected pregnancies in the Netherlands would range between 168 and 370. FNAIT is most often diagnosed after the birth of a clinically affected child with signs of bleeding. Coincidental detection of thrombocytopenia is not uncommon with laboratory tests for other reasons. Further testing then reveals the presence of alloantibodies in the maternal serum.

Currently, preventive measures are only possible in a subsequent pregnancy of women with a previously affected child and known FNAIT. Experience shows that FNAIT is not routinely taken into consideration by paediatricians as a possible cause of neonatal thrombocytopenia

This study was undertaken to evaluate the rate and consequences of a late or missed diagnosis of FNAIT by assessing the clinical presentation of first affected children, the timing of diagnosis and the outcomes of the subsequent children.

PATIENTS AND METHODS

Patients

The Leiden University Medical Centre is the national referral centre for pregnancies complicated by FNAIT in the Netherlands. We retrospectively evaluated prospectively collected data using our FNAIT database and neonatal records from all women and infants treated at our centre between July 2008 and July 2010.

Medical records were evaluated to obtain obstetric history, clinical presentation of the first affected child and timing of diagnosis of FNAIT.

Case definition

Early diagnosis was defined as a diagnosis of FNAIT made following clinical signs or suspicion after birth of a first affected child. Diagnosis was considered delayed if diagnostic testing for FNAIT was not performed after a first clinically affected child, with FNAIT being unknown at the outset of the subsequent pregnancy.

Study outcomes

Antenatal management and outcome in subsequent pregnancies of women with undiagnosed FNAIT in previous pregnancies were compared with those with early diagnosed FNAIT. Primary outcome was the occurrence of fetal or neonatal ICH. Secondary outcomes included other bleeding signs, the cord blood platelet count at birth and type of neonatal treatment.

Statistical analysis

Data are expressed as mean (SD) values for continuous variables or as median (range) for categorical variables. The Fisher's exact test and the Mann-Whitney test were used for comparison as appropriate. A P-value <0.05 was considered significant. Analyses were performed using SPSS 16.0 for Windows statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Between July 2008 and July 2010, 26 women were referred to the LUMC for management of pregnancies complicated by FNAIT. Table 5.1 outlines the characteristics of the study group.

Clinical presentation of older siblings

In the older siblings, 16 of the 26 children presented at birth with bleeding manifestations: skin bleeding only in 13, and ICH in three. Nine infants (35%) presented with

Table 5.1 *Characteristics of pregnancies with FNAIT following a previous affected child (n = 26)*

	Early diagnosis of FNAIT (n = 22	Delayed diagnosis of FNAIT* (n = 4)
Parity (before index pregnancy)		
Primiparity	18	3
Multiparity	4	1
Pregnancy loss		
One miscarriage	6	1
Recurrent miscarriage	2	0
Immature delivery	1	1
Type of HPA antibodies		
HPA-1a	17	2
HPA-5b	1	1
HPA-1a and HPA-5b	1	
HPA-1b	1	
HPA-5a		1
HPA-15a	2	

FNAIT fetal and neonatal alloimmune thrombocytopenia; HPA human platelet antigen
**Defined as diagnosis of FNAIT made only during or after the subsequent pregnancy*

asymptomatic thrombocytopenia, detected through blood drawing for other purposes. Seven had severe thrombocytopenia (platelet count <50 x10^9/L) and two infants had moderate thrombocytopenia (50 x10^9/L - 100x10^9/L).

Early diagnosis of FNAIT and primary outcome of index cases

In 22/26 cases, the diagnosis was made directly after birth of the first affected child. Four were multiparous women. Of these four women, two lost their child in the neonatal period due to unrelated congenital anomalies. Thrombocytopenia was first detected in their second child. The other two multiparous women first had asymptomatic children, with symptomatic thrombocytopenic children in the next pregnancy.

Intracranial haemorrhage occurred in one index case (1/22, 5%) of the early diagnosed group, The previous child of this mother had a low platelet count (5x10^9/L), without ICH. Ultrasound at 20 weeks of gestation showed no bleeding. Just before initiation of intravenous immunoglobulin (IVIG) therapy at 28 weeks of gestation, small intracranial bleedings were identified on ultrasound. The IVIG treatment (1.0 g/kg/week) was given as planned, and an asymptomatic child was born at 36 weeks of gestation. Magnetic resonance imaging confirmed several minor bleeding sites. The child is developing normally at 1year of age.

Late diagnosis of FNAIT and primary outcome of index cases

In four of 26 cases, there was a long delay in diagnosing FNAIT. Despite the birth of a child with a low platelet count or ICH, the possible diagnosis of FNAIT was not considered and no tests for FNAIT were performed. In the delayed diagnosis group, two of the four (50%) subsequent children suffered from ICH. One neonate died 2 hours postpartum due to a massive subarachnoidal haemorrhage. The other child had no clinical sequelae at 1 year of age. The four cases are summarised in table 5.2.

Table 5.2 *Characteristics of pregnancies with delayed diagnosis of FNAIT*

Case	A	B	C	D
Parity (at index pregnancy)	G3P2	G2P1	G2P1	G2P1
Clinical presentation of first affected child	ICH	Asymptomatic	Asymptomatic	Asymptomatic
Platelet count of first affected child	Unknown	30 × 10^9/L	67× 10^9/L	11× 10^9/L
Presumed cause of ICH/thrombocytopenia	Birth trauma	Down syndrome	IUGR	IUGR
Gestational age at diagnosis in index pregnancy	20-week scan (diagnosis of ICH)	After birth at 32 weeks (ICH)	35 weeks	13 weeks
Antenatal management of index pregnancy	IVIG 1.0 g/kg/wk for 15 weeks	_	IVIG 1.0 g/kg/wk for 3 weeks	IVIG 0.5 g/kg/wk for 4 weeks*
Clinical outcome of index pregnancy	CS, 38 weeks	CS for fetal distress at 32 weeks	Vaginal birth at 38 weeks. Healthy	CS for fetal distress at 32 weeks. Healthy
Platelet count at birth of index pregnancy	158 × 10^9/L	11 × 10^9/L	16 × 10^9/L	184 × 10^9/L

IUGR intrauterine growth restriction; ICH intracranial haemorrhage; CS caesarean section.
**IVIG planned for 10 weeks but preterm birth.*

Table 5.3 Data on neonatal outcome, antenatal and neonatal treatment and type of delivery in index pregnancies with FNAIT

Pregnancy outcome	Early diagnosis of FNAIT (n = 22)	Delayed diagnosis of FNAIT (n = 4)	P-value
ICH, n (%)	1 (5)	2 (50)	0.051
Perinatal death, n (%)	0	1 (25)	0154
Platelet count at birth, $\times 10^9$/L	66 (6-278)	57 (11-54)	0.561
Severe thrombocytopenia ($<50\times10^9$/L) n(%)	9 (41)	2 (50)	0.386
Haemorrhagic symptoms excluding ICH, n (%)	2(9)	1(25)	0.355
Antenatal treatment			
IVIG, n (%)*	21 (95)	2 complete (50), 1 incomplete, 3 weeks	0.271
Neonatal treatment			
Platelet transfusion only, n (%)	4 (18)	1(25)	0.445
Platelet transfusion and IVIG, n (%)	3 (14)	0	0.592
None, n (%)	15 (68)	3 (75)	0.437
Mode of delivery			
Vaginal, n (%)	13 (59)	1 (25)	0.206
Caesarean section, n (%)	9 (41)	3 (75)	

Values given in numbers (percentage) or median (range)
FNAIT fetal and neonatal alloimmune thrombocytopenia; ICH intracranial haemorrhage; IVIG intravenous immunoglobulin: 0.5 g/kg/wk from 28 weeks when affected sibling had no ICH, or 1.0 g/kg/week from 16 weeks if sibling had ICH.
**In one early diagnosis, a predelivery fetal blood sample was taken followed by one intrauterine platelet transfusion and a vaginal birth.*

Secondary outcomes of index cases

In table 5.3 , the platelet count at birth, bleeding signs other than ICH, antenatal and neonatal treatment are given.

DISCUSSION

In this cohort study of 26 women with FNAIT, delay of diagnosis was identified in four cases (15%). Two of these four fetuses suffered from severe ICH. Several factors were presumed to have caused the low platelets in the previous pregnancies—Down syndrome, intrauterine growth restriction and birth trauma—and kept the clinicians from requesting the appropriate investigations.

The most feared complication of fetal or neonatal thrombocytopenia is ICH. Untreated newborns with FNAIT are reported to be affected by ICH in 7-26% of pregnancies.[6-8] After the birth of a child with ICH, the recurrence rate of ICH is as high as 79% (CI 61-79%).[9-11] Surviving children suffer from severe neurological sequelae including mental retardation, cerebral palsy, cortical blindness and seizures in 14-26%.[5,8] As safe

and nearly 100% effective prenatal treatment is available, using IVIG,[12] it is of clinical importance to detect neonates with FNAIT.

In our study, one ICH occurred in the group with known FNAIT, just before to starting IVIG. This child is now developing normally at 1 year of age. The mother had received IVIG for 8 weeks, which could be the reason for the good long-term outcome. However, as this is the only failure in over 10 years and more than 120 women treated with noninvasive management (IVIG only), we have not changed our policy of starting IVIG at 28 weeks.

The limited number of pregnancies means that our analysis has limited power to detect statistically significant differences. However, these illustrative examples of delayed diagnosis show that missing the diagnosis of FNAIT can have devastating consequences for subsequent children, including perinatal death.

More than one-third of the neonates in our study with FNAIT presented with asymptomatic thrombocytopenia. A Norwegian study showed that in the absence of a routine screening program, only 14% of affected neonates would be identified. [13] Our data confirm their conclusion that for effective prevention of ICH due to FNAIT, routine screening of all pregnant women for human platelet antigen 1a (HPA-1a) antibodies needs to be implemented.

The same recommendation was given by Knight *et al.*[5] following a national study in the UK which showed significantly more children diagnosed with ICH when FNAIT was unknown or unrecognised at the onset of pregnancy compared with pregnancies with known diagnosis of FNAIT (at 1 year of age 10% versus 0% severe disability or death).

The debate on implementation of routine antenatal screening for FNAIT depends mostly on cost-effectiveness. Several studies provide calculations that all reach the conclusion that screening is likely to be cost-effective.[4,8,14,15,16] However, until then, given the serious consequences of missing the diagnosis FNAIT, we recommend platelet antibody detection for all cases with (suspected) antenatal fetal haemorrhage and neonatal thrombocytopenia. We propose a limited serological investigation for antenatally suspected cases, by maternal HPA-1a typing and screening for the clinical important HPA antibodies. This can be extended for newborns with proven thrombocytopenia with a cross-match between maternal serum and paternal platelets, maternal and neonatal (or paternal) HPA typing and platelet autoantibody investigation depending on the differential diagnosis.

CONCLUSION

Delay in diagnosing FNAIT, resulting in withholding appropriate preventive measures in the subsequent pregnancy, may lead to perinatal death or severe brain damage in newborns . We recommend that in all neonates with thrombocytopenia at birth, the diagnosis FNAIT should be considered, followed by appropriate testing. Screening all pregnant women for HPA-type would be even more effective.

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CHAPTER 6

FETAL INTRACRANIAL HAEMORRHAGES CAUSED BY FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: AN OBSERVATIONAL COHORT STUDY OF 43 CASES FROM AN INTERNATIONAL MULTICENTRE REGISTRY

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ABSTRACT

Objective

To characterise pregnancies where the fetus or neonate was diagnosed with fetal and neonatal alloimmune thrombocytopenia (FNAIT) and suffered from intracranial haemorrhage (ICH), with special focus on time of bleeding onset.

Design

Observational cohort study of all recorded cases of ICH caused by FNAIT from the international No IntraCranial Haemorrhage (NOICH) registry during the period 2001-2010.

Setting

13 tertiary referral centres from nine countries across the world.

Participants

37 mothers and 43 children of FNAIT pregnancies complicated by fetal or neonatal ICH identified from the NOICH registry was included if FNAIT diagnosis and ICH was confirmed.

Primary and secondary outcome measures

Gestational age at onset of ICH, type of ICH and clinical outcome of ICH were the primary outcome measures. General maternal and neonatal characteristics of pregnancies complicated by fetal/ neonatal ICH were secondary outcome measures.

Results

From a total of 592 FNAIT cases in the registry, 43 confirmed cases of ICH due to FNAIT were included in the study. The majority of bleedings (23/43, 54%) occurred before 28 gestational weeks and often affected the first born child (27/43, 63%). One third (35%) of the children died within 4 days after delivery. 23 (53%) children survived with severe neurological disabilities and only five (12%) were alive and well at time of discharge. Antenatal treatment was not given in most (91%) cases of fetal/ neonatal ICH.

Conclusions

ICH caused by FNAIT often occurs during second trimester and the clinical outcome is poor. In order to prevent ICH caused by FNAIT, at risk pregnancies must be identified and prevention and/or interventions should start early in the second trimester.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of intracranial haemorrhage (ICH) in relation to thrombocytopenia in term born infants.[1] FNAIT is caused by maternal alloantibodies directed against fetal platelets due to incompatibility in human platelet antigens (HPAs). ICH due to FNAIT is reported to occur in 1: 12 500 – 25 000 births.[2,3] The clinical outcome is often more severe than for neonatal ICH from other causes.[1,4] The ICH recurrence rate in subsequent pregnancies is reported to be 79%.[5] Therapies have been developed that can reduce the incidence of ICH, such as weekly high-dose intravenous immunoglobulin (IVIG).[6,7,8] It is therefore considered important to identify ICH caused by FNAIT in order to treat the mother in subsequent pregnancies. Gestational age at time of bleeding onset is a key factor when antenatal treatment options are discussed since treatment to prevent ICH needs to be started before ICH is likely to occur. No study has specifically addressed this. Identifying common denominators of human HPA-alloimmunised pregnancies complicated by ICH may serve as a future tool to help identify pregnancies at high risk.

The No IntraCranial Haemorrhage (NOICH) registry is a multinational registry including 592 pregnancies complicated by FNAIT from 13 tertiary referral centres across the world. This study presents the results of an in-depth evaluation of all recorded cases of ICH caused by FNAIT from this registry. The aim of the study was to characterize pregnancies where the fetus or neonate suffered from ICH with special focus on clinical and laboratory characteristics and time of bleeding onset.

MATERIALS AND METHODS

Study design and inclusion criteria

All centres that had registered FNAIT cases in the NOICH registry from 2001-2010 were invited to participate in this observational cohort study. Pregnancies recorded in the NOICH registry as complicated by fetal or neonatal ICH were identified, and included if both the diagnosis of FNAIT and ICH were confirmed. STROBE guidelines were followed as appropriate.

A case was defined as FNAIT if: 1) Incompatibility between maternal and paternal/ fetal HPA type was confirmed and maternal anti-HPA antibodies were detected, 2) HPA-incompatibility between the mother and father was confirmed and the fetus/ neonate suffered ICH and 3) anti-HPA antibodies were detected in the mother but data on fetal/ paternal HPA genotype was missing.

Neuroradiological images were recovered and reviewed as electronic copies in a picture archiving and communication system (PACS) using standard display programs. One Norwegian participant and two of the Dutch participants had MR-studies performed in utero. All available neuroradiological images were re-evaluated for this study by an experienced independent paediatric neuroradiologist (OF). The focus of the neu-

roradiological evaluation was primarily to confirm the ICH diagnosis, second to use imaging to assist in assessing time of bleeding onset and finally to classify the type of bleeding. When images were not available, written reports of the imaging evaluations for the patient files by others were used to evaluate if the ICH diagnosis was correct. Cases where an ICH could not be confirmed were not included in the study.

In cases where information from normal imaging preceding abnormal imaging results was available we were certain of a window in time during which the haemorrhage did occur. In most participants we did not have the benefit of initial normal imaging. Here we used instead well recognised imaging principles[9] in judging the age of haemorrhage from its appearance on CT or MRI and estimated time of onset of bleed from this assessment. According to the classification commonly used in the study of causes of cerebral palsy we classified the haemorrhages as either intraventricular (IVH) and/or periventricular (PVH) or as parenchymal, in brain parenchyma not associated with the central ventricular system.[10] The neuroradiological information of likely time of onset was matched to all other clinical information available.

In cases where the fetus or neonate died, autopsy reports were retrieved and studied to evaluate whether ICH diagnosis was certain or unlikely. All reports from post-mortem examinations were evaluated by an experienced perinatal pathologist (NP). Only cases evaluated to be certain ICH were included.

Laboratory data

All laboratory data except maternal anti-HPA-1a antibody levels were collected from the NOICH registry database (http://www.NOICH.org).

Maternal anti-HPA-1a antibody levels, except for the Finnish cases (four pregnancies), were measured at the National reference laboratory of clinical platelet immunology in Tromsø, Norway, by quantitative MAIPA.[11] Reproducibility between Norwegian and Finnish quantitation was secured by double analysis of some sera samples in both Norway and Finland. In cases where several anti-HPA-1a antibody level measurements were available, the highest maternal anti-HPA-1a antibody level measured during pregnancy or postpartum was included in the study.

The lowest platelet count recorded in the fetus or new-born before any platelet transfusions were given, was included.

Clinical data

Clinical data was mainly collected from the NOICH registry database (http://www.NOICH.org). Additional clinical information was retrieved from the original medical records by each country coordinator.

The pregnancy where ICH was detected for the first time was referred to as the index case. A subsequent ICH event was defined if ICH was recorded in any subsequent pregnancies after the index ICH case.

Statistics

All data were analysed using SPSS software (V.18.0 SPSS Inc, Chicago, Illinois, USA). The $P<0.05$ was considered significant. Means with 95% confidence intervals (CI) or median with range were calculated for all continuous variables. Independent sample t-test and Kruskal-Wallis test were used as appropriate. One-way of variance test was used to test clinical outcome in relation to time of bleeding onset.

RESULTS

From the NOICH registry recording 592 FNAIT cases, 66 pregnancies from 57 women were initially identified with suspected ICH. In 23 cases ICH or FNAIT diagnosis could not be confirmed. In total, 43 confirmed cases of ICH due to FNAIT were recorded from 37 mothers. The 43 cases studied were included from the Netherlands, Finland, Sweden, Norway and the UK (figure 6.1).

Laboratory characteristics of ICH cases

HPA-1a alloimmunisation was found to be the cause in 39 of 43 ICH cases (91%). Anti-HPA-5b antibodies were found in two further cases. In one woman with records of two ICH cases, incompatibility in the gpIa/IIa system was confirmed and in one of her ICH pregnancies anti-HPA-5a antibodies were detected.

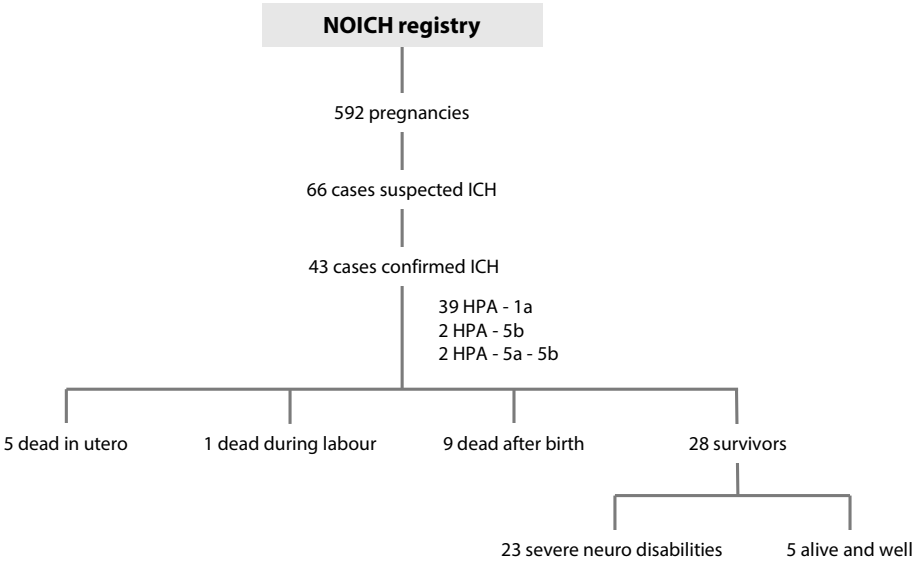


Figure 6.1
Flow diagram of the study population

Data on maternal anti-HPA-1a antibody levels during pregnancy or after delivery were available for 15 (35%) of the FNAIT pregnancies complicated by ICH. The median (range) highest anti-HPA-1a antibody level was 105 IU/mL (39 to 264 IU/mL). In two pregnancies we have serial anti-HPA-1a antibody level measurements starting in the first trimester, and in both these pregnancies the anti-HPA-1a antibody levels fell from the first to the third trimester. The ICH occurred between 28 and 34 weeks in these pregnancies. The median anti-HPA-1a antibody levels measured during the first, second, third trimesters and postpartum did not differ significantly from each other or from the overall median highest antibody level (data not shown).

The median (range) lowest fetal/ neonatal platelet count for ICH cases was $8x10^9$/L (1 to 27). In comparison, median (range) neonatal platelet count for previous or subsequent pregnancies where no ICH was detected, was $17x10^9$/L (1 to 199) and significantly higher when compared with ICH cases (Kruskal-Wallis test, P=0.004).

Clinical characteristics of ICH pregnancies

Maternal characteristics for all ICH cases are shown in table 6.1. In the group of the 37 index cases, 26 (70%) cases were first born children. However, most mothers had been pregnant before they had their first child: Eight women had one or more first trimester miscarriages and six women had one or more second trimester losses. For two women, we lack data on gravida status. The mothers were primigravidae in only 10/37 (27%) index cases. In total, ten mothers (23%) experienced one or more second trimester miscarriages (altogether 20) before or after the ICH case.

Table 6.1 *Maternal characteristics of ICH-affected pregnancies*

Maternal characteristic	
Maternal age in years, mean (95% CI)	29.3 (27.6-31.0)
Obstetrical history	
Primigravida, n (%)	10 (23)
First-born child, n (%)	27 (63)
2nd trimester miscarriage, n (%)	10 (23)
Pre-eclampsia in this pregnancy, n (%)	3 (7)
Vaginal delivery of ICH neonate, n (%)	22* (51)
Caesarean section of ICH neonate, n (%)	20* (47)
Fetal/ maternal treatment	
IVIG, n (%)	4 (9)
Steroids, n (%)	1 (2)
Intrauterine platelet transfusion, n (%)	3 (7)
No treatment	36 (84)

ICH intracranial haemorrhage; IVIG intravenous immunoglobulin.
**Mode of delivery was not known for one ICH pregnancy*

Antenatal treatment was given in 4/43 (9%) of the pregnancies complicated by fetal/ neonatal ICH. In three cases IVIG was given, two of these fetuses received additional intrauterine platelet transfusions. One mother received corticosteroids as single treatment from 6 weeks gestation onwards, due to two previous second trimester miscarriages (not identified as FNAIT related). For the 37 index cases, only one mother received antenatal treatment. In this case, IVIG treatment was started from week 7 due to autoimmune thrombocytopenia in the mother.

Twenty out of 43 mothers with pregnancies complicated by fetal/ neonatal ICH were delivered by caesarean section (CS); CS was performed in nine cases with abnormal fetal cerebral ultrasound (US) as main indication.

A high proportion of the pregnancies complicated by ICH were ended preterm: 29 out of 43 children (67%) were born before 37 weeks and 4 children were born before 28 weeks. Abnormal US scan with detection of ICH was the main reason for premature delivery (table 6.2).

Five (12%) of the infants died in utero and one (2%) died during labour. Neonatal characteristics of the live-born children are shown in table 6.3. Nine children died within the first four days after delivery. Most of the survivors developed neurological sequelae: 8 infants were diagnosed with cerebral palsy, 10 were reported to be moderate/ severely mentally retarded or severely disabled, 7 neonates were reported to have epilepsy and 4 were blind or with severely reduced vision. In addition, one case of autism and one case of impaired hearing were reported. More than one neurological complication was reported in several cases. Five (14%) of surviving neonates with ICH were reported to be alive and well at time of discharge after delivery, but data on long-term clinical outcome is missing (figure 6.1).

The fetuses/ neonates were male in the majority (65%) of ICH cases. There was no significant difference in maternal anti-HPA-1a antibody levels, birth weight, APGAR scores or platelet counts when comparing boys and girls (Independent sample t test, P>0.05).

Ten (23%) ICH neonates were below the 10th percentile for birth weight and defined as small for gestational age (SGA) according to standard growth curves. All SGA cases except one were boys.

Table 6.2 *Preterm birth*

Reason for preterm delivery	Number of pregnancies (% of total)
Abnormal US scan (ICH detected)	15* (52)
Intrauterine fetal demise	5* (17)
Intrauterine growth restriction/ pre-eclampsia	3 (10)
Spontaneous preterm birth	4 (14)
Not known	2 (7)
Total	**29** (100)**

**One case was a complication during cordocentesis*
***Including one twin pregnancy*
ICH intracranial haemorrhage

Table 6.3 *Clinical outcome characteristics of ICH cases*

Characteristic	Result
All ICH cases	
Gestational age at delivery, median (range) in weeks	35 (23-42)
Birth weight, mean (SD) in grams	2274 (832)
Sex, female/ male	14/28
Stillborn	6 (14)
Live-born children	37 (86)
APGAR score <7 after 5 minutes, n (%)	8 (23)
Lowest platelet count (range) x10^9/L*	8.9 (1-27)
Neonatal death, n (%)	9 (21)
Alive and well at discharge, n (%)	5 (12)
Alive with neurological sequelae, n (%)	23 (53)

**Data available for 32 pregnancies*
ICH intracranial haemorrhage

Bleeding characteristics

Neuroradiological studies were used to review and confirm 19 ICH cases. The remaining 24 ICH cases were confirmed using descriptions of radiographic material by local radiologists.

The estimated time of bleeding onset for each ICH case is shown in figure 6.2. The figure demonstrates that many bleedings started early, and that in most cases several weeks passed between bleeding onset and delivery. More than half (23/43, 54%) of the bleedings happened around gestational week 28 or earlier. In 16 (70%) of the 23 cases with bleeding onset before the third trimester we do not know the exact time of bleeding onset, only that the bleeding started no later than 28 weeks (figure 6.2). Twenty-nine of 43 bleedings (67%) started before 34 gestational weeks. No cases of intrapartum ICH bleedings were confirmed. The time of bleeding onset in the 10 primigravid cases were not different from the multigravid ICH cases.

There was no difference in clinical outcome in relation to time of bleeding onset (data not shown). However, it is noteworthy that perinatal death occurred in two girls (14%) where the bleedings were found to have happened before 30 weeks. In boys, perinatal death occurred in 13 (46%) cases with bleedings happening also at a later gestational age.

Intraparenchymal haemorrhages were found in 11 out of 13 cases where the bleeding occurred during the third trimester or after delivery. Figure 6.3A illustrates intraparenchymal haemorrhage.

In five cases, multiple bleeding episodes were found. All second bleedings occurred after 33 weeks (range 33 to 37 weeks). These five cases were all due to HPA-1a alloimmunisation.

Fifteen ICH cases were classified as intraparenchymal and 13 cases as IVH/ PVH (figure 6.3B). Five cases were classified as miscellaneous (figure 6.3C), whereas 10 cases could not be classified. Among cases where ICH was found to occur before 28 weeks, all but one case was found to be IVH/PVH.

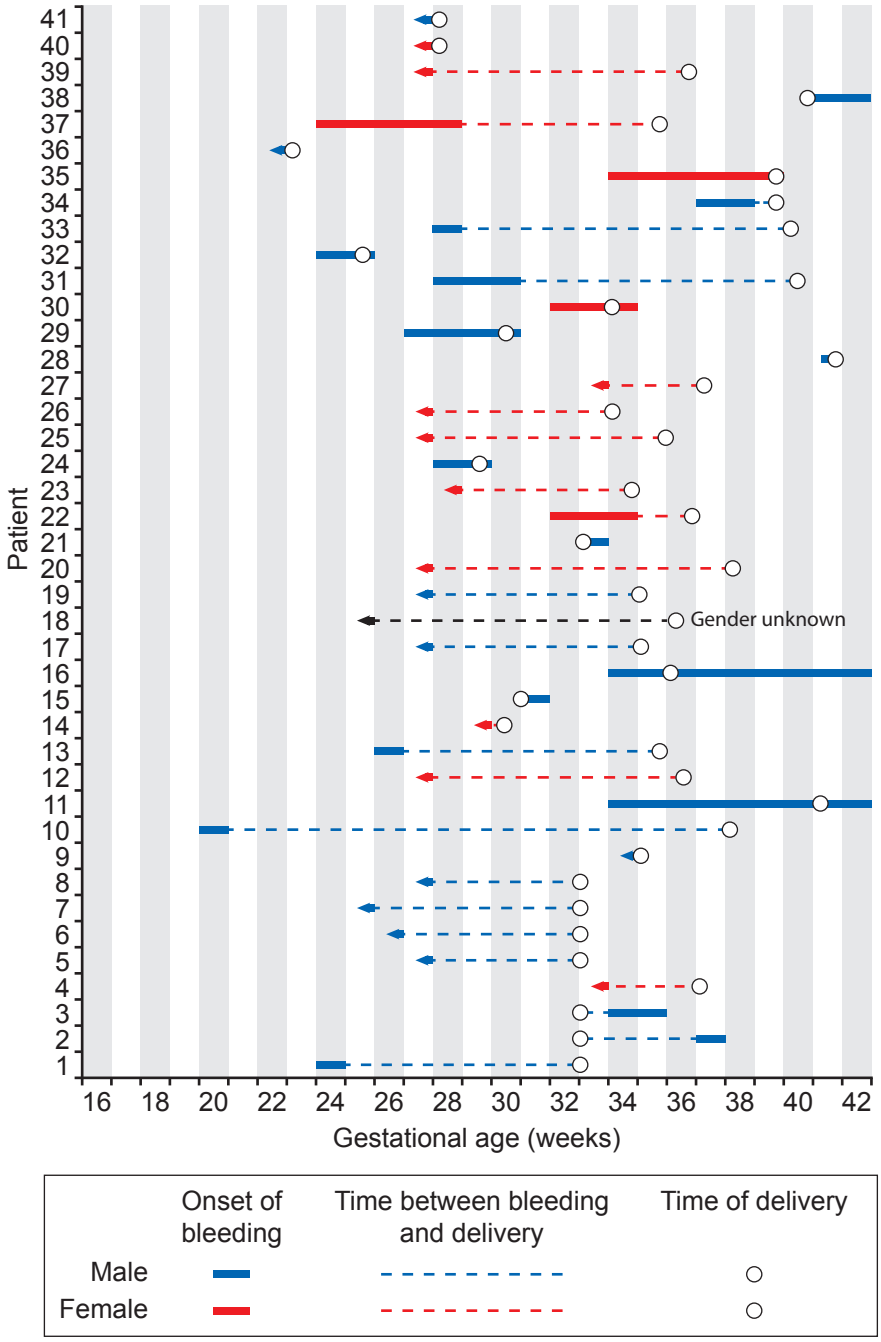
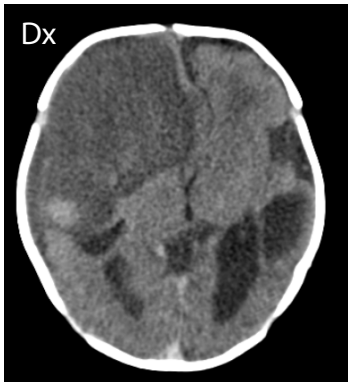
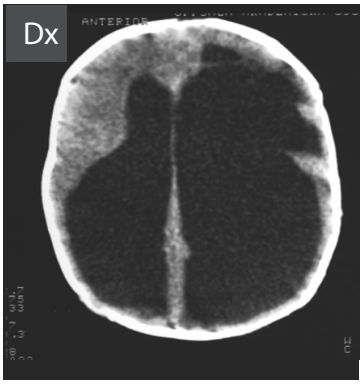


Figure 6.2
Estimated time period for onset of ICH is shown for 41 cases of ICH caused by FNAIT. An arrowhead to the left indicates that the earliest time of onset cannot be estimated, only that the bleeding occurred before the gestational age indicated on the x-axis.

a) Intraparenchymal hemorrhage



b) Intraventricular hemorrhage



c) Miscellaneous (Multiple intraparenchymal hemorrhages)

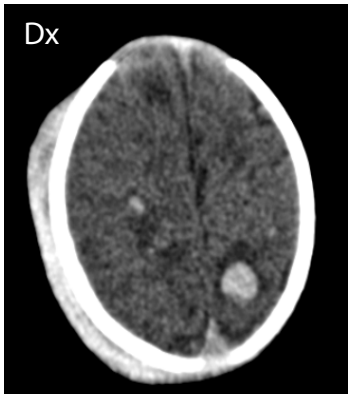


Figure 6.3

(A) CT scan shows a very large intraparenchymal haemorrhage with mass effect and occupying most of the right frontal lobe in a term baby. Note a second but smaller haemorrhage on the left side adjacent to the ventricle but separate from this. The attenuation of both haemorrhages indicates that the haemorrhage is several weeks old at the time of imaging. We estimated the right haemorrhage to be 4 - 6 weeks old and the left still a couple of weeks older. The pregnancy was considered normal, and no IVIG treatment was given. A live boy was born vaginally at 40 gestational weeks with petecchiae. The platelet count was 6x109/L.

(B)This CT shows marked ventriculomegaly, partly due to hydrocephalus, partly due to loss of brain tissue in the left hemisphere. Note the very large ventricle on the left side occupying most of the left hemicranium. The shape of the left lateral ventricle is irregular and that of residual from a previous intraventricular haemorrhage with a paraventricular atrophic defect caused by an old periventricular haemorrhagic infarction. This pattern is pathognomonic for this condition even though no actual blood can be detected. The interpretation of this image is that of an intraventricular haemorrhage associated with a periventricular hemorrhagic infarction timed at 28 – 30 gestational weeks or earlier. Hydrocephalus was diagnosed intrapartum because of breech presentation. A live boy was born at term with a platelet count of 4x109/L. The child has severe cerebral palsy and a hydrocephalus shunt.

(C)The CT scan shows multiple focal intraparenchymal haemorrhages throughout both cerebral hemispheres. Most haemorrhages are quite small. Note also the extensive extracranial subgaleal haemorrhage overlying the right hemicranium. All bleedings are of same age and maximum seven days old. It was noted that the fetus was small on US scan at 25 weeks, but otherwise the pregnancy was considered normal. At 42 weeks a live boy was born vaginally, with multiple petecchiaes and multiple retinal bleedings. The platelet count at delivery was 14x109/L, with nadir value 8x109/L. He received platelet transfusions. At time of discharge the boy was described as alive and well, but we do not have data on long-term clinical outcome.

Treatment and clinical outcome in subsequent pregnancies

ICH was detected in six (23%) subsequent pregnancies. One woman had records of ICH in two subsequent pregnancies after the index ICH case. In most subsequent pregnancies (20/26, 77%), no ICH was found.

In 19/ 26 (73%) of subsequent pregnancies, the mothers received antenatal treatment (IVIG). The intravenous immunoglobulin schedules varied greatly, with a median starting time at 18 weeks (range 16-35 weeks). In the six cases where ICH was detected in subsequent pregnancies, three received IVIG treatment. However, in one of these cases IVIG treatment was started *after* ICH was detected. Therefore, IVIG treatment failed to prevent ICH in two (11%) out of 19 cases. These two treatment failures were from the same woman. It should be commented that the obstetrical history of this woman is particularly severe with regards to FNAIT complications. The ICH recurrence rate was therefore 11% in IVIG treated pregnancies. Compared with historical data reporting 79% risk of ICH recurrence in FNAIT,[5] our data indicate that IVIG was effective in preventing ICH.

In seven cases, no IVIG treatment was given during a subsequent pregnancy after ICH was detected in a previous pregnancy. Five of these untreated pregnancies come from Norway, where IVIG is not routinely given as treatment during HPA alloimmunised pregnancies. ICH occurred in three of these seven cases.

DISCUSSION

The main findings of this study are that the majority of ICH bleedings occurred by the end of the second trimester and that clinical outcome was devastating for most cases. The high frequency of bleedings occurring before 28 weeks indicates that the fetus may be severely affected already in the second trimester.

This is the largest study to date on fetal/ neonatal ICH caused by FNAIT. For the first time, time of bleeding onset was assessed using clinical information together with radiographic imaging and autopsy reports. The in-depth study of both laboratory and clinical information was done in close collaboration between obstetricians, immunologists, perinatal pathologists and specialists in neuroradiology. Limitations of this study include that it is a retrospective cohort study, being subject to confounding and information bias. A bias toward inclusion of the more severe ICH phenotype may be considered. The ICH cases in this study were collected from five different countries and therefore reflect several institutions' clinical experiences. There is obvious heterogeneity in antenatal treatment for FNAIT between these countries. However, since most of the patients affected by fetal ICH did not receive antenatal treatment, we consider the study population uniform and representative of a larger population.

Some earlier studies have suggested the onset of bleedings to be in the third rather than the second trimester,[3,12] and are in variance with this study. The judgment of the onset of cerebral bleeding in this study was cautious, and the onset was probably in many cases even earlier than we report. However, previous studies included too few

cases to address this question in an adequate manner. Also, and more importantly, other studies report the gestational age when the ICH was diagnosed, and did not assess when the bleeding may have occurred. In a recent study by Bussel et al,[13] antenatal management to prevent recurrence of ICH caused by FNAIT was studied. Gestational age at the time of ICH is reported in this study, but without any data with regard to how the timing of ICH was assessed. These data are therefore difficult to assess, but in support of our data they report that as many as 8/37 (22%) of ICH cases in their study population occurred before 28 gestational weeks. Most of the ICH cases occurred in boys, and the bleedings were more often lethal when the fetus was male. The finding that 80% of the SGA neonates in our population were boys supports the recently published observation that birth weight in boys, but not in girls, was associated with maternal anti-HPA-1a antibodies.[14] Data on maternal anti-HPA-1a antibody levels in FNAIT pregnancies complicated by fetal/ neonatal ICH have not been published before.

Fetal ICH due to FNAIT often occurred in the first child. Most ICH cases will therefore not be recognised in time for treatment or prophylactic measures if we do not identify pregnancies at risk before the onset of the bleeding. The high number of first-born children in the study population may not necessarily mean that the risk of ICH caused by FNAIT is genuinely higher in the first-born child. The distribution could be skewed towards nulliparous women since these women may choose not to have more children due to high recurrence risk. Further, most of these women received antenatal treatment during the subsequent pregnancy thereby reducing the incidence of ICH in the younger siblings. Nevertheless, this finding challenges the current management strategy where antenatal treatment is given in subsequent pregnancies after FNAIT has been diagnosed in the first child. A majority of these children will suffer from bleeding already in the second trimester or in the early part of the third trimester. Possible interventions to reduce risk of ICH need to be introduced before the 20th week of gestation. In most children the ICH was either fatal or induced severe disabilities. Our findings therefore support the idea of identifying HPA-1bb mothers with anti-HPA-1a antibodies in all pregnancies[3,15] and working towards a prophylactic approach to prevent the immune response against HPA-1a.[16,17] The rate of prematurity and CS was high in our study. Many of these patients required delivery before term due to problems related to the fetal ICH such as fetal distress, lack of fetal movements, abnormal ultrasound scan etc. Thus, the present study does not indicate that FNAIT per se is associated with an increased risk for prematurity.

There were no confirmed cases of ICH occurring intrapartum in this study, and only two bleedings occurred after delivery. This could suggest that mode of delivery may not be so important in the prevention of ICH. However, the high CS rate in this study population may have contributed to the low number of intrapartum bleedings. Whether or not delivery by CS prevents ICH needs to be further addressed.[3,18] The present study suggests that IVIG treatment during pregnancy is protective in regard to ICH in most cases, which is in accordance with previous studies.[13] However, it is an open question whether IVIG would have protected the first born child from ICH, or whether there

is a genuine increased risk of ICH in the first born child. Further, it remains to be established if there is also a milder phenotype of ICH with discrete symptoms and better outcome. This question can only be addressed in prospective studies including general screening and repeated fetal US examinations. The maternal anti-HPA-1a antibody levels were extremely high among ICH cases in this study. Maternal anti-HPA-1a antibody levels may therefore be useful to identify pregnancies at risk for fetal ICH, but these findings need to be evaluated in larger prospective studies. Finally, why boys seem to be more susceptible to maternal anti-HPA-1a antibodies is currently not known and needs further investigation.

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CHAPTER 7

LOW-DOSE VERSUS STANDARD-DOSE INTRAVENOUS IMMUNOGLOBULIN TO PREVENT FETAL INTRACRANIAL HEMORRHAGE IN FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: A RANDOMIZED TRIAL

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ABSTRACT

Objective

Pregnancies at risk for fetal and neonatal alloimmune thrombocytopenia (FNAIT) are commonly treated using weekly intravenous immunoglobulins (IVIG), 1g/kg maternal weight. IVIG is an expensive multidonor human blood product with dose-related side effects. Our aim was to evaluate the effectiveness of IVIG at a lower dose, ie 0.5g/kg.

Methods

This was a randomized controlled multicenter trial conducted in Sweden, the Netherlands and Australia. Pregnant women with human platelet antigen alloantibodies and an affected previous child without intracranial hemorrhage (ICH) were enrolled. The participants were randomized to IVIG at 0.5 or 1 g/kg per week. The analyses were per intention to treat. The primary outcome was fetal or neonatal ICH. Secondary outcomes were platelet count at birth, maternal and neonatal IgG levels, neonatal treatment and bleeding other than ICH.

Results

A total of 23 women were randomized into two groups (low dose: n = 12; standard dose: n = 11). The trial was stopped early due to poor recruitment. No ICH occurred. The median newborn platelet count was 81×10^9/L (range 8–269) in the 0.5 g/kg group versus 110×10^9/L (range 11–279) in the 1 g/kg group (p = 0.644).

Conclusion

The risk of adverse outcomes in FNAIT pregnancies treated with IVIG at 0.5 g/kg is very low, similar to that using 1 g/kg, although our uncompleted trial lacked the power to conclusively prove the noninferiority of using the low dose.

INTRODUCTION

Fetal or neonatal alloimmune thrombocytopenia (FNAIT) is the result of platelet destruction in the fetus or neonate caused by maternal IgG allo-antibodies. In Caucasians, FNAIT is most commonly caused by the human platelet antigen (HPA)-1a.[1] Two percent of the Caucasian population is HPA-1a negative (HPA-1bb), thus, 1 in 50 Caucasian pregnant women is at risk to develop FNAIT. Actual sensitization occurs in 6-12% of HPA-1bb mothers, of whom 1 in 3 deliver a child with severe thrombocytopenia (<50x10^9/L).[2] The most devastating pathology associated with FNAIT is intracranial hemorrhage (ICH) in the fetus or newborn, often with death or severe, irreversible neurologic damage, which occurs in 10% of severely thrombocytopenic newborns.[2]

Until recently, repeated fetal blood sampling and intrauterine platelet transfusions was the only available treatment of fetuses with alloimmune thrombocytopenia. Bussel et al were the first, in 1988, to report beneficial effects of maternal administration of immunoglobulins (IVIG) in pregnancies with FNAIT. In all the 7 cases reported, the fetal platelet count increased substantially after treatment with IVIG 1 g/kg/wk.[3] This dosage was based on the dose used in the treatment of idiopathic thrombocytopenic purpura. No dose-finding studies for the treatment of FNAIT have ever been published.

In the past decade, several studies have been published supporting the safety and efficacy of noninvasive, IVIG-only treatment of FNAIT.[4-6] Currently, our standard treatment for pregnant women with FNAIT with a previous affected child without ICH is a weekly dose of 1 g/kg. Radder et al. reported that placental antibody transfer is not further increased with increasing IgG concentrations in the mother.[7] This suggests a limitation of the placental transfer via the Fc-receptor, likely due to saturation. We therefore hypothesized that a lower dose of IVIG might be equally effective in the treatment of FNAIT.

IVIG is an expensive multidonor human blood product, and although stringently tested, the risk of transmission of viral and other diseases remains. Dose-related maternal side effects have been described, including headache, fever, renal and cardiovascular dysfunction and aseptic meningitis. The aim of our study was to determine whether 0.5 g/kg per week of IVIG is as effective as 1 g/kg per week in the prevention of ICH in FNAIT.

SUBJECTS AND METHODS

Study design and participants

We performed an international, open-label randomized controlled trial in 4 tertiary care centers in 3 countries, to test the hypothesis that treating pregnant women with FNAIT, with an affected sib without ICH, with 0.5 gram IVIG/kg per week is just as effective as (non inferior to) the standard dose of 1 gram/kg per week.

Women with a singleton pregnancy with HPA allo-antibodies with a gestational age between 12 and 28 weeks, who had previously given birth to an affected sibling, with a platelet count < $150x10^9$ /L but without an ICH were included. HPA alloimmunization in the current pregnancy was confirmed by the presence of maternal anti-HPA antibodies, and either a homozygous father or detection of the offending HPA antigen in the fetus by amniocentesis in case of a heterozygous father.

Women with autoimmune thrombocytopenia, multiple pregnancies, fetuses and neonates with major congenital anomalies or chromosomal abnormalities, and women with a previous child with FNAIT and ICH were excluded. Patients with immunoglobulin-A deficiency were only excluded if they had a severe allergic constitution, and so were patients who ever had an allergic reaction to blood product due to anti-IgA antibodies. Each woman included in the study provided written informed consent accordance with institutional and national guidelines. The study was approved by the Leiden University Medical Center Medical Ethics Committee (MEC PP04.203), and by each centers' respective Institutional Review Board.

Randomization

Randomization was performed between 26 and 28 weeks' gestation, after stratification for center and for HPA-1a and non HPA-1a, by the Web-based randomization service integrated in the central trial database, provided by the Karolinksa Institute, Stockholm, Sweden (www.medscinet.com). The patients were randomized to either the low dose (0.5 g/g/kg maternal weight per week) or the standard dosage, 1 g/kg per week of IVIG.

Medication and management protocol

The brand of IVIG used was the IVIG with which the treating clinicians were accustomed to work in their centers. The medication was administered weekly, starting from 28 weeks' gestation until delivery over a period from 3 to 6 h according to the dose and tolerance.

The products utilized were Freeze-dried Immunoglobulin IV (CLB Sanquin, Amsterdam, The Netherlands) and Gammagard (Baxter International Inc., Deerfield, IL, USA). Side effects and complications were registered in the trial database.

Fetal ICH was ruled out by ultrasound before start of the treatment at 28 weeks. The ultrasound was repeated biweekly. The total IgG levels were measured before delivery in maternal serum and after delivery in neonatal cord blood. No fetal blood samplings were performed at any time. The choice of timing and mode of delivery was left to the discretion of the obstetrician. In case of a planned vaginal delivery, it was recommended not to use scalp electrodes, scalp blood sampling, or ventouse- or forceps-assisted delivery.

Directly after birth the platelet count in umbilical blood was tested automatically; in case of a count <$100x10^9$/L, a manual count was done. In all centers, HPA compatible platelets were available within 12 h after birth. A neonatologist examined the neonate directly after birth. Treatment was left to the discretion of the neonatologist. Within the

first days after birth, a cranial ultrasound of the neonate was performed, and all signs or suspicions of bleedings were recorded. The course of the neonatal platelet count was recorded, as well as any form of treatment of thrombocytopenia.

The primary outcome was fetal or neonatal ICH. Secondary outcomes were fetal platelet count at birth, the total IgG levels in maternal serum and cord blood , type of neonatal treatment needed and signs of bleeding other than ICH.

Statistical analysis

The study was set up as an equivalence trial. The null hypothesis was that the standard dose of 1 g/kg was superior. We wanted to test if the low dose of 0.5 g/kg was not inferior. We assumed that the probability of failure (prevalence of ICH) was 1%, if both groups were equal. Based on the assumption that the risk of failure in both groups was the same, we made a sample size calculation. We estimated a 5% specified maximal difference, meaning that the lower dose is inferior if the risk of failure is 5% higher than in the standard dose group. For a power of 80% and a one-sided 5% significance level, this means that 106 patients in each arm were needed to reject the null hypothesis. For the primary end point, the treatments are considered equivalent if the upper limit of the confidence interval of the difference was <5%.[8] The confidence interval for the difference in proportion was calculated using the quasi-exact method by Chen.[9]

Analysis was performed based on the intention-to-treat principle. For comparison of continuous data, the Kruskal-Wallis test was used; for categorical data we used the Fisher's exact test. Data are expressed as means (SD) or medians (ranges), and a p-value< 0.05 was considered significant. Calculations were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, Ill., USA)

RESULTS

The trial started in January 2005. At this point 15 centers in 8 countries agreed to participate. We estimated an inclusion period of 3 years. Despite regular contact between the Trial Steering Committee and the responsible investigators in these centers, only 4 centers, in The Netherlands, Sweden and Australia, managed to recruit patients.

Due to the low number of patients included until September 2007 (n=23), the Trial Steering Committee decided to end the study. Reasons for the slow recruitment were inability to obtain institutional review board approval, the low number of eligible patients (leading to loss of interest for the study) and being too busy with projects of higher priority.

The study group consisted of 23 pregnancies, from which all but 1 received and completed the allocated treatment. One patient requested to switch from 0.5 to 1.0 g/kg in week 34. Twelve patients were allocated to 0.5g/kg per week, and 11 patients to the standard dose of 1.0g/kg per week. All patientes were analyzed. Figure 7.1 shows the flow diagram of the trial.

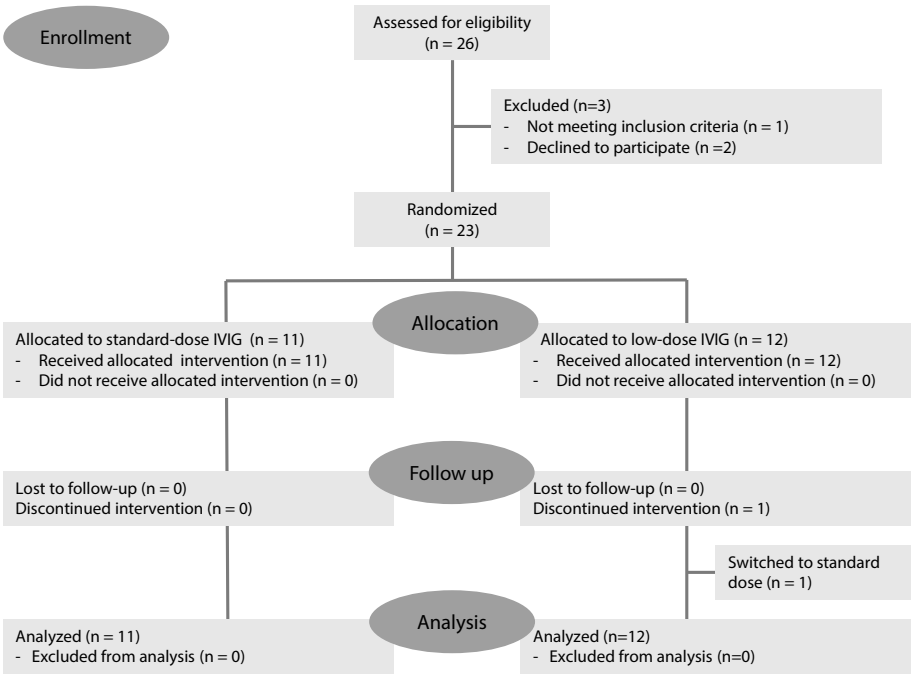

Figure 7.1
Consolidated Standards of Reporting Trials (CONSORT) flow diagram

Table 7.1 Characteristics of pregnant women with FNAIT randomized to 0.5g/kg or 1g/kg IVIG

	IVIG 0.5 g/kg/wk N=12	IVIG 1.0 g/kg/wk N=11	P-value
Maternal age	31 (29-39)	32 (24-43)	0.597
Parity	1 (1-2)	1 (1-3)	0.810
Nr. of doses IVIG	10 (7-11)	11 (7-12)	0.436
Caucasian	12	11	1.0
HPA-1a	11	11	0.338
PC sibling ($x10^9$/L)	17 (5-70)	11 (2-49)	0.532

Data are shown as numbers or medians (ranges).
FNAIT fetal or neonatal alloimmune thrombocytopenia; IVIG Intravenous Immunoglobulin; HPA-1a Human Platelet Antigen 1a.

The baseline characteristics of the included women are shown in table 7.1, showing both groups to be equal for all relevant parameters. Perinatal survival was 100%, and no ICH was observed. The difference in primary outcome is therefore 0%, with a 95% confidence interval of -25.2% to 23.6%. The platelet count at birth did not differ significantly (81 vs 110x10^9/L, p=0.644). The primary and secondary outcomes are given in table 7.2.

Table 7.2 *Outcome of pregnancies with FNAIT randomized to 0.5g/kg or 1g/kg IVIG*

	IVIG 0.5 g/kg/wk	IVIG 1.0 g/kg/wk	P-value
Fetal ICH	0	0	1.0
Neonatal ICH	0	0	1.0
PC at birth	81 (8-269)	110 (11-279)	0.644
Nadir of platelet count	71 (8-266)	110 (9-202)	0.943
Bleeding (non ICH)	0	0	1.0
Perinatal survival	12 (100%)	11 (100%)	1.0
IVIG in neonatal period	1 (8%)	0	0.338
Platelet transfusions in neonatal period	2 (17%)	3 (27%)	0.547
Maternal side effects	0	0	1.0
Fetal/neonatal adverse events	0	0	1.0
GA at birth	38^{+0} (34^{+3}-39^{+4})	38^{+0} (34^{+4}-38^{+5})	0.665
Vaginal birth	7 (58%)	10 (91%)	0.037
Planned CS	4 (33%)	1 (9%)	0.168
Emergency CS	1 (8%)	0	0.338
Birth weight (gram)	3087 (1940-3650)	3420 (2605-3750)	0.049
Platelet count:			
$<30x\ 10^9$/L	1 (8%)	2 (18%)	0.493
$<50x\ 10^9$/L	3 (25%)	4 (36%)	0.563
$<150x\ 10^9$/L	9 (75%)	7 (64%)	0.563

Data shown as number (%) or median (range).
ICH Intracranial Hemorrhage; PC platelet count; IVIG intravenous immunoglobulins; GA Gestational Age, CS caesarean section

Table 7.3 *IgG concentrations in maternal serum en neonatal cord blood samples of pregnancies complicated by FNAIT, treated with low dose or standard dose IVIG, compared to reference levels in normal pregnancies[10]*

	Type of treatment		
	0.5 g/kg/wk	1.0 g/kg/wk	Reference range
Cord blood IgG conc.	16.0 (14.4-21.1)	14.1 (12.9-18.4)	11.6 (7.5-15.9)
Maternal serum IgG conc.	19.4 (17.8-24.1)	26.2 (17.4-36.3)	8.0 (5.3-13.1)
Cordblood/serum ratio	0.82 (0.63-1.08)	0.61 (0.36-0.84)	1.4 (0.9-2.0)

Data shown in g/L, and median (range)

In table 7.3, the maternal serum and cord blood IgG levels are compared to the levels in the normal population, as expected[10] The maternal IgG serum levels were higher than in the normal population, as expected. The cord blood IgG levels were similar in the three groups. No serious side effects were reported in both treatment groups.

DISCUSSION

Both the low IVIG dose of 0.5 g/kg and the standard dose of 1.0 g/kg IVIG were associated with the absence of ICH or death in all the studied pregnancies with FNAIT in women having given birth to an affected sibling without ICH. The platelet counts at birth, the need for neonatal treatment and the cord blood levels of IgG were also similar. For statistically significant evidence of equivalence, the number of recruited pregnant women was too small.

Interpretation in light of other evidence

The results of our trial are consistent with the *in vitro* studies published by our group and others[7,11] showing a limitation, likely by saturation, of the Fc-receptor mediated transplacental transport of IgG. We confirmed in a clinical setting that a dose of 0.5 g/kg and a dose of 1 g/kg lead to similar IgG levels in the fetal circulation. In a recent review of the literature on the management of FNAIT, we found no studies using or evaluating a lower dose than 1 g/kg IVIG.[12] The dose of 1 g/kg used in most studies and protocols in the past decade is not based on dose-finding studies. Most users refer to Bussel et al[3], who first proposed treatment of FNAIT with IVIG and treated 7 women with a previously affected child (3 with ICH) with 1.0 g/kg per week after diagnostic fetal blood sampling showing a platelet count <100x10^9/L. All newborns appeared healthy, with platelet counts >30x10^9/L. Their choice for 1.0 g/kg was based on studies in patients with idiopathic thrombocytopenic purpura [13-15] and in neonates with alloimmune thrombocytopenia.[16,17] However, when analyzing these studies in detail, we found that the patients were actually treated with an IVIG dose of 0.4g/kg daily, for a period of several days. We assume that to permit administration once a week, this dose was recalculated to the 1 g/kg.

Three small randomized trials have been published comparing different regimens for the treatment of FNAIT in pregnant women with a previous child without ICH, all conducted by the same group from Cornell Medical College, New York, N.Y., USA. In the first study, IVIG at 1 g/kg only was compared with the same medication with added dexamethasone in 54 women, showing no benefit of dexamethasone. No case of ICH occurred.[18] In the second study, Berkowitz et al [19] studied 39 pregnant women, comparing 1 g/kg of IVIG with 0.5 mg/kg per day of prednisone. They used fetal blood sampling both before treatment and again during treatment to evaluate response, with addition of the medication given in the other arm in case of insufficient response. They found no difference in platelet counts at birth. Two fetal deaths of unknown cause (1 in each arm) occured 2 and 4 weeks after a fetal blood sampling, even though showing sufficient platelets. One fetal blood sampling was complicated by bradycardia and emergency caesarean section at 28 weeks' gestation. This neonate had a grade 3 ICH despite a platelet count of 68x10^9/L. One other neonate had a small grade 1 hemorrhage at term with a normal platelet count.

In the third study by this group[20], a total of 73 women were randomized to either IVIG 2.0g/kg per week (group A) or IVIG 1.0g/kg per week plus prednisone 0.5mg/kg per day (group B). At 32 weeks, fetal blood sampling was done, followed by either adding prednisone to group A or doubling the IVIG dose in group B. There was 1 small neonatal ICH in each group, apparently unrelated to FNAIT, since the platelet counts were normal. The platelet counts in both groups were similar. There were 4 complications of the fetal blood samplings: 2 emergency caesarean sections and 2 cases of preterm rupture of membranes within 24 h. Almost all women had moderate-to-severe side effects related to the treatments.

These small trials, just as underpowered as our own study, have limited meaning when trying to determine the optimal dose of IVIG. The most important conclusions from these studies in our view are that any IVIG treatment appears to effectively prevent thrombocytopenia-related ICH, and that fetal blood sampling in FNAIT is associated with more harm than good.[5]

The use of any medication in pregnancy, in particular substances that cross the placenta, should be carefully considered, balancing perceived benefit against potential harm to mother and fetus. Since most effects, both beneficial and harmful, are often dose-dependent, reducing the dose to the minimum effective level is an important principal. IVIG has been used successfully in many immune-related diseases since the 1950s. For a number of indications, double-blind, placebo-controlled trials have shown its efficacy.[21] In addition, IVIG is used off-label for a number of rare diseases, including FNAIT, only based on observational studies or small underpowered controlled trials. The drug is produced using pooled plasma donated by thousands of paid or unpaid volunteers, with some concern remaining about the transmission of viral or other infections despite meticulous testing protocols. Its highly successful use in FNAIT has now almost completely and justly replaced the hazardous invasive management protocol based on fetal blood samplings and intrauterine transfusions utilized in the past.[5]

Many aspects of IVIG treatment however, are still unclear. The presumed mechanisms of action, described as 'immunomodulatory and anti-inflammatory', are manifold, and in part still to be unraveled.[21] The increasing use of IVIG in many autoimmune and inflammatory diseases could lead to shortages of this already expensive human blood donor product. Future research may result in using only a specific fraction of the IVIG product for specific diseases, or it may lead to bioengineering of a protein with IgG-like properties. As IVIG is known for its immunomodulating characteristics, there are some, at least theoretical, concerns with its use in pregnancy. One study showed a possible increase in IgE in children after maternal IVIG administration. However, no clinically apparent adverse effects in early childhood could be demonstrated.[22] Severe maternal side effects of IVIG, including aseptic meningitis as well as renal and cardiovascular dysfunction, are uncommon, while mild discomfort such as headache is often reported dose-dependently. All these issues underline the importance of using IVIG in pregnancies with FNAIT in the lowest possible effective dose.

An important limitation to our trial is the low number of recruited women. The lack of power to prove equivalence means that the data must be interpreted with care. We do agree, however, with Kahn and Hills[23], commenting on trials stopped early, that the results have to be taken as they stand and need to be shared with the medical community. In the absence of other comparative studies, and the lack of justification for the 1 g/kg dose, we suggest that our data at least do not show any benefit of a dose >0.5 g/kg dose in the treatment of FNAIT.

Our study concerned only those women with FNAIT whose previously affected child did not suffer from ICH. For the much smaller group of pregnant women who had a previous child with ICH, the optimal dose of IVIG still needs to be determined. An adequately powered trial for this group, however, will be very difficult to perform, as we discussed in a previous article.[24]

CONCLUSION

We conclude that although our trial lacked sufficient power to prove equivalence to the standard dose of 1 g/ kg, administering an IVIG dose of 0.5 g/kg weekly appears safe and effective for pregnancies complicated by FNAIT in women with an affected sibling without ICH, reducing costs as well as long-term adverse events or side effects. We recommend using IVIG at a dose of 0.5 g/kg per week for the treatment of women with FNAIT without an affected sibling with ICH. A larger prospective study or randomized trial is needed to have sufficient power to prove the equivalence of a lower dose of IVIG compared to the standard dose of 1 g/kg. Adequately powered studies are also needed to find the optimal treatment protocol for pregnancies complicated by FNAIT for women with a previous child suffering from ICH.

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CHAPTER 8

LOWER-DOSE INTRAVENOUS IMMUNOGLOBULINS FOR THE TREATMENT OF FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA, A COHORT STUDY

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ABSTRACT

Background:

Intravenous immunoglobulins (IVIG) are the cornerstone in the treatment of pregnancies at risk for fetal and neonatal alloimmune thrombocytopenia (FNAIT). The most commonly used dose is 1.0 g/kg per week, not based on any dose finding study. IVIG is an expensive multidonor human blood product with dose-related side effects. Our aim was to describe the amount of severe thrombocytopenia according to two different doses of IVIG.

Study design and Methods

We performed a cohort study, where two dosage regimes of IVIG were evaluated in the treatment of pregnant women suffering from FNAIT with a previous affected child without intracranial hemorrhage (ICH). Cases, treated with 0.5 or 1.0 g/kg per week, were selected from the international multicenter No IntraCranial Hemorrhage (NOICH) registry. Outcome was neonatal platelet (PLT) count at birth and amount of severe thrombocytopenia. Furthermore the appearance of ICH was analyzed.

Results

A total of 109 women were included in the study, 46 in the 0.5 IVIG and 63 in the 1.0 IVIG group. There was no difference in PLT count at birth (mean, 112 vs. 119, crude difference 7; confidence interval [CI], -37.4-23.7) and incidence of severe thrombocytopenia (<30x109/L; n = 7/46 versus n = 7/63; odds ratio, 1.43 [CI 0.46-4.42]). No ICH occurred.

Conclusion

In pregnancies with FNAIT with a previous affected child without ICH, treatment with IVIG in a weekly dose of 0.5g/kg or 1.0 g/kg results in comparable neonatal PLT count at birth and degree of thrombocytopenia.

INTRODUCTION

Fetal or neonatal alloimmune thrombocytopenia (FNAIT) is a rare but devastating disease caused by platelet (PLT) destruction in the fetus or neonate through maternal (Ig)G allo-antibodies. In Caucasians, FNAIT is most commonly caused by the human platelet antigen (HPA)-1a.[1] Two percent of the Caucasian population is (HPA)-1a negative (HPA-1bb), thus, one in 50 Caucasian pregnant women is at risk to develop FNAIT. Actual sensitization occurs in 6 to 12% of HPA-1bb mothers, of whom one in three deliver a child with severe thrombocytopenia ($<50x10^9$/L).[2] The most feared complication is ICH (intracranial hemorrhage), often leading to death or severe, irreversible neurologic damage, which occurs in 10% of newborns with severe thrmobocytopenia.[2,3] Unlike in the pathophysiologically similar red blood cell (RBC) alloimmunization, fetuses in a first pregnancy can be severely affected. In the absence of screening programs, the disease is virtually always only detected after birth of an affected child. Preventive measures can then be taken in the next pregnancy.

Until recently, repeated fetal blood sampling and intrauterine PLT transfusions were the choice of treatment for fetuses with alloimmune thrombocytopenia. Bussel and colleagues were the first, in 1988, to report beneficial effects of maternal administration of immunoglobulins (IVIG) in pregnancies with FNAIT.[4] The conventional dose of 1.0 g IVIG/kg per week was not based on any dose-finding studies. The dose originated from studies in patients with idiopathic thrombocytopenic purpura and in neonates with alloimmune thrombocytopenia.[5-8]

In the past decade, several studies have been published supporting the safety and efficacy of non-invasive, IVIG-only treatment in FNAIT.[9-11] Currently, our standard treatment of pregnant women at risk for a child with FNAIT with a previous affected child without ICH is a weekly dose of 1 g/kg per week. Radder and coworkers reported that placental antibody transfer is not further increased with increasing IgG concentrations in the mother.[12] This suggests a limitation of the placental transfer via the Fc-receptor, likely due to saturation.

To test the hypothesis that a lower dose of 0.5 g/kg per week IVIG might be equally effective in the treatment of FNAIT, an international, open-label randomized controlled trial (RCT) in women with FNAIT, with an affected sibling without ICH was performed.[13] Unfortunately this trial was prematurely ended due to lack of inclusion and we therefore had to conclude that we had insufficient data to show equivalence between the two treatment regimens. The aim of this study was to describe the neonatal PLT count and clinical outcome of two different dosages of IVIG in a large cohort of pregnancies affected by FNAIT.

MATERIAL AND METHODS

Study design and participants

We performed a cohort study of pregnant women affected by FNAIT treated with two different dosages of IVIG (0.5 g/kg per week vs. 1.0 g/kg per week).

Because of the limited evidence for any particular dose we decided, after prematurely ending the RCT in 2007, comparing 0.5 g versus 1.0 g of IVIG for FNAIT, to continue offering 0.5 g of IVIG /kg per week to FNAIT women, with a previous child without ICH. These cases were collected in our international Web-based No IntraCranial Hemorrage (NOICH) database (http://www.NOICH.org), initially started to gather data for the RCT but kept open for caregivers to collect and share data of FNAIT cases from 2000 to 2010. From that same database, women were treated with 1.0 g/kg per week were selected. Inclusion period ranged from 2007 to 2015. The timing of starting IVIG treatment in pregnancy differed per center according their local protocol.

All cases were women with singleton pregnancies, who previously gave birth to an affected sib, with a PLT count of fewer than $150x10^9$ /L but without an ICH. HPA alloimmunization was confirmed by the presence of maternal anti-HPA antibodies, and either a homozygous father or detection of the offending HPA antigen in the fetus by amniocentesis or cfDNA testing in maternal plasma in case of a heterozygous father.

Women with autoimmune thrombocytopenia, multiple pregnancies, fetuses and neonates with major congenital anomalies or chromosomal abnormalities, and women with a previous child with FNAIT and ICH were excluded. Patients with immunoglobulin-A deficiency were only excluded if they had a severe allergic constitution, and so were patients who ever had an allergic reaction to blood product due to anti-IgA anti. Finally cases that participated in the NOICH trial were left out.[13]

Medication and management protocol

Eligible women were offered the lower dose of 0.5 g/kg IVIG, to be started at 28 weeks of gestation. The brand of IVIG used in Leiden was Freeze-dried Immunoglobulin (CLB Sanquin) and Gamma Gard (Baxter International, Inc) .The medication was administered weekly until delivery over a period from 3 to 6 hours, according to the amount required and tolerance. Ultrasound was used to rule out fetal ICH just before start of the treatment and repeated monthly. No fetal blood samplings were performed at any time. The choice for type of delivery, elective cesarean section or intended vaginal delivery, was left to the obstetrician with consent from the patient. Standard recommendations at vaginal delivery were not to use fetal scalp electrodes or fetal scalp blood samplings and to refrain from ventouse or forceps application. As in other alloimmunized pregnancies, we aim for a delivery around 37 weeks of gestation.

Baseline demographics, medical and obstetric history were recorded in the MedSciNet NOICH database (http://www.NOICH.org). Directly after birth the PLT count in umbilical blood was tested, first automatically and in case of a count $<100x10^9$/L, a manual count was done. HPA compatible platelets were available within 12 hours after

birth. A neonatologist examined the child directly after birth. Treatment was left to the discretion of the neonatologist. Within the first days after birth a cranial ultrasound of the neonate was performed, and all signs or suspicions of bleedings were recorded.

Statistical analysis

Patient characteristics are presented as medians with interquartile range (IQR) or numerical values in numbers with percentages or categories. Data analysis was generated using SPSS software (version 20; SPSS Inc., Chicago, IL, USA). A p value of 0.05 was considered significant. As data were not normally distributed data were analyzed using a Mann Whitney U test. Categorical data were analyzed using a chi-squared test. Correlation was analyzed using Spearman's correlation.

Outcome variables were reported as medians (PLT count at birth) with crude difference or numbers (severe thrombocytopenia) with percentages and odds ratio (OR). Confounding was prevented using regression analysis.

RESULTS

After ending of the trial in 2007, 46 cases were collected who received the lower-dose IVIG of 0.5 g/kg per week. Sixty-three cases could be selected from the NOICH database that were treated with 1.0 g/kg per week.

Baseline characteristics of the included cases and controls are shown in table 8.1, showing both groups to be equal for most relevant parameters, besides gestational age at start of IVIG treatment and total amount of IVIG per patient

Perinatal survival was 100%; no ICH was observed. Neonatal PLT count at birth did not differ significantly (112×10^9/L vs. 119×10^9/L; crude difference 7, confidence interval [CI], -37.4 to 23.7). Furthermore the number of cases of severe thrombocytopenia ($<30x10^9$/L) was not significantly different between the 0.5 IVIG group and the 1.0 IVIG group (n= 7 [15%] vs. n=7 [11%]; OR 1.43; CI 0.46-4.42). Neonatal PLT count at birth or amount of severe thrombocytopenia and gestational age at start IVIG were not correlated (p= 0.175 in Spearman's correlation).

Likewise regression analysis showed no significant difference in outcome. The outcome is given in table 8.2.

Table 8.1 *Demographic characteristics of patients with FNAIT treated with low-dose or higher dose-IVIG*

	0.5 g/kg IVIG (n= 46)	1.0 g/kg IVIG (n=63)	p value
Gravidity	3	3	
Parity	2	2	
Anti HPA-1a	36 (78)	52 (82)	0.41
5b	3	2	
1a/5b	1	2	
Other: 3a,15a (N)	6	7	
PLT count of sib	17 (8-30)	18 (13-35)	0.84
GA at start IVIG treatment	28 (28)	32 (27-34)	0.001#
Total number of IVIG treatments	10 (9.5-11)	7 (5-10)	0.53
Total amount of IVIG, g (dose × no. IVIG)	5 (4,75-5,5)	7 (5-10)	0.002
Mode of delivery			
Vaginal	25 (56)	30 (48)	0.39
Cesarean	20 (44)	31 (49)	
GA age at birth	38 (37-38)	38 (37-38)	

GA gestational age, IVIG immunoglobulins
continuous variables are reported as median (IQR), and categoric variables as numbers (%)
 # Spearman showed no correlation

Table 8.2 *Primary and secondary outcome*

	0.5 g/kg IVIG (n= 46)	1 g/kg IVIG (N=63)	Crude difference	Adjusted* difference
ICH	0	0		
PLT at birth (x10^9/L), mean (CI)	112 (87-138)	119 (102-137)	7 (-37,4-23,7) OR	0,349 (-31,2-31,9) Adjusted OR*
Severe thrombocytopenia (<30 x10^9/L)	7 (15%)	7 (11%)	1,43 (0,46-4,42)	1,15 (0,76-4,52)
Severe thrombocytopenia (<50 x10^9/L)	14 (30%)	12 (19%)	1,86 (0,76-4,52)	1,61 (0,65-4,0)
Other bleeding complications	0	0		

IVIG immunoglobulins
Continuous variables are reported as mean (CI), and categorical variables as number (%).
**adjusted for gestational age at start of IVIG*

DISCUSSION

Our findings show no difference in neonatal PLT count at birth or degree of thrombocytopenia between the two different dosages of IVIG treatment in pregnancies complicated by FNAIT with an affected sibling with no ICH. These results confirm earlier findings from our uncompleted RCT comparing the lower 0.5 dose with the standard 1.0 dose of IVIG.[13]

Until 1984, the traditional management of subsequent pregnancies in women with a previous history of FNAIT consisted of an early elective cesarean section and transfusion of PLTs after birth. Since the publication of Daffos and colleagues (1984), one of the pioneers of fetal blood sampling (FBS), several centers throughout the world started treating fetal thrombocytopenia in a similar fashion to anemia due to RBC alloimmunization, by serial intrauterine PLT transfusions.[14-17] In 1988, Bussel and coworkers reported on seven cases of FNAIT successfully treated by maternal administration of IVIG, using 1 g/kg per week.[4] This dosage was based on their experience with idiopathic thrombocytopenic purpura. A few subsequent studies confirmed effectiveness of this treatment.[10,11,18] Until recently however, the need to give such a high dose was never challenged.

Previous in vitro studies have shown a limitation, likely by saturation, of Fc-receptor-mediated transplacental transport of IgG, which leads to the suggestion that increasing the dose of IVIG does not result in a higher concentration of IgG in the fetal circulation.[12,19] This was supported clinically by the findings of our RCT.[13]

Until now there is no consensus about the gestational age of starting IVIG treatment and recommendations differ about using a particular dose, (varying from 0.5 to 2.0 g/kg per week). The treatment of FNAIT is usually only stratified according to the presence or absence of ICH in the previous child and the timing of its occurrence.[20] Generally a history with an affected child with ICH leads to a higher dose of IVIG and an earlier onset of IVIG treatment.

Besides our RCT, three small randomized trials have been published comparing different regimens for treatment of FNAIT in pregnant women with a previous child without ICH without any difference in bleeding complications or PLT count.[21-23] In one of these studies randomization was done between different dosages IVIG (2.0g/kg per week vs. 1.0g/kg per week plus 0.5mg/kg per day prednisone), in the other two the comparison was between IVIG and either prednisone or dexamethasone. All studies confirm effectiveness of noninvasive IVIG treatment in preventing thrombocytopenia-related ICH, and although views on the optimal dose may differ, it appears clear that there is no place left for invasive treatment using PLT transfusions.[10]

The number of women included in our study is still limited. However with a cohort of 109 women treated with IVIG for FNAIT, it is still one of the largest studies evaluating treatment modalities in such a rare disease as FNAIT. Unless trials are done using screening of populations as a means of subject selection, it seems unlikely that prospective studies will ever be significantly larger.

Analysis of our patient characteristics showed a significant difference in gestational age at start of IVIG treatment between the two treatment groups. It is conceivable that starting IVIG in an earlier stage of pregnancy leads to better neonatal outcome. However this association was not found with regression analysis and no significant difference in outcome was seen. Explanation for the difference in gestational age at start IVIG treatment is a change in treatment protocol for FNAIT over the years, starting at 28 instead of 32 weeks, implemented to have a uniform policy in several European centers.

The cases in this study were collected from different countries and therefore may reflect several institutions policies. Since most countries only stratify antenatal treatment according to the presence or absence of ICH in a previous child we consider the two study groups comparable. Therefore it is not likely that this had led to any selection bias.

The use of any medication in pregnancy, especially substances that cross the placenta, should be carefully considered, balancing perceived benefit against potential harm for mother and fetus. Since most effects, both beneficial and harmful, are often dose-dependent, reducing the dose to the minimum effective level is an important principal.

As IVIG is known for its immunomodulating characteristics, care should be taken using it in pregnancy. However, apart from one study suggesting an increase of IgE, no clinically apparent adverse effects in early childhood could be demonstrated.[24,25] Serious maternal side effects such as aseptic meningitis, renal and cardiovascular dysfunction, are uncommon. Mild discomfort such as headache is often reported, and appears dose-dependent. All these issues underline the importance of using IVIG in pregnancies with FNAIT in the lowest possible effective dose.

IVIG is produced from blood of thousands of human blood donors, and it is expensive. The working mechanism is not clear. Most likely, it acts on various levels, in maternal serum, at the level of placental transfer if IgG and in the fetal blood, blocking Fc-receptors on macrophages.[26]

Recent research by Yougbare and colleagues supports the hypothesis that IVIG may aid in protection against bleeding, instead of merely causing a rise in PLTs.[27] They showed that impairment of angiogenesis rather than thrombocytopenia is the critical cause of the ICH in FNAIT. In their murine-model study, ICH only occurred in fetuses and neonates with anti–β3 integrin–mediated, but not anti-GPIbα–mediated FNAIT, despite similar thrombocytopenia in both groups. Only anti–β3integrin–mediated FNAIT reduced brain and retina vessel density, impaired angiogenic signaling, and increased endothelial cell apoptosis.

This might be an explanation for the phenomenon of 'nonresponders', that is, fetuses not responding to IVIG with PLT counts remaining less than 50×10^9/L, reported to be approximately 20%.[10,28] Also in our study neonates were born with severe thrombocytopenia (< 30×10^9/L) despite IVIG treatment (respectively 15% in the 0.5 g of IVIG group and 11% in the 1 g of IVIG group). For PLT counts < 50 x 10^9/L the figures were 30% and 19% respectively. None of the differences between both groups were significant. The persistent occurrence of (severe) thrombocytopenia under antenatal treatment seems worrying but the most important finding is that neither in our study nor in several other studies did ICHs occur, suggesting other protective effects of IVIG.[10]

Without routine screening for FNAIT in all pregnancies, most women nowadays can only be treated in the pregnancy after the birth of an affected child. We would support implementation of routine HPA type and antibody screening in the near future.[2] Reducing overall costs of a screening and intervention program, by lowering the dose of IVIG for immunized women, may help to accelerate its introduction.

In conclusion, our study shows no difference in neonatal PLT count or degree of thrombocytopenia when two different dosages of IVG are compared in the treatment of pregnancies complicated by FNAIT with an affected sibling with no ICH.

This suggests that a lower dose of IVIG might be as effective as the more standard higher dose of IVIG in preventing severe thrombocytopenia. Further prospective studies are needed to confirm this.

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CHAPTER 9

FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA, MANAGEMENT AND OUTCOME OF A LARGE INTERNATIONAL RETROSPECTIVE COHORT

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ABSTRACT

Objective

To evaluate the management and outcome of a large international cohort of cases of pregnancies complicated by fetal and neonatal alloimmune thrombocytopenia (FNAIT).

Methods

This was an observational prospective and retrospective cohort study of all cases of FNAIT entered into the international multicenter No IntraCranial Haemorrhage (NOICH) registry during the period 2001–2010. We evaluated human platelet antigen (HPA specificity, antenatal and postnatal interventions performed and clinical outcome.

Results

A total of 615 pregnancies complicated by FNAIT from 10 countries were included. Anti-HPA-1a was the most commonly implicated antibody. Antenatal treatment was administered in 273 pregnancies (44%), varying from intrauterine platelet transfusion to maternal administration of immunoglobulins, steroids or a combination of those. Intracranial haemorrhage was diagnosed in 23 fetuses or neonates (3.7%). Overall perinatal mortality was 1.14% (n=7).

Conclusion

This study presents the largest cohort of cases of FNAIT published. Our data show that antenatal treatment for FNAIT results in favourable perinatal outcome. Over time, in most centres, treatment for FNAIT changed from an invasive to a complete non-invasive procedure.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by an immunological process in which the mother produces an antibody-mediated response against a platelet-specific antigen [human platelet antigen (HPA)] that she herself lacks but that is present on the fetal platelets, inherited from the father.[1] The mother's antibodies, of the IgG (immunoglobulin) type, can cross the placenta and bind to fetal platelets. The antibody-coated platelets are subsequently removed from the fetal circulation by the reticuloendothelial system, which results in fetal thrombocytopenia. These same antibodies also may inhibit platelet production.[2] The proportion of individuals belonging to a particular platelet antigen type varies according to ethnicity. The immunodominant antigen in Caucasians is HPA-1a, which is responsible for 85% of the FNAIT cases, followed by HPA-5b in 10%.[3] Two percent of the Caucasians is HPA-1a negative. The reported incidence of FNAIT ranges from 1:350 to 1:1000.[4] The most severe complication is intracranial haemorrhage (ICH), leading to lifelong handicap or even death. The clinical outcome is often more severe than that of neonatal ICH from other causes.[3-6] The majority of ICH bleedings seem to occur by the end of the 2nd trimester.[7]

Without routinely screening for HPA antibodies, the disease is nowadays diagnosed after birth of the first affected child. Subsequently, antenatal treatment can only be offered in following pregnancies to avoid recurrence of severe FNAIT.

The aim of this study was to analyse management and outcome of the largest international cohort of FNAIT-cases to date, with emphasis on the different treatment modalities.

MATERIAL AND METHODS

Data collection

The No IntraCranial Haemorrhage (NOICH) registry database (http://www.NOICH.org) was initially set up in 2001 for the NOICH study, an international multicentre randomized controlled trial comparing 0.5 with 1.0 g intravenous immunoglobulins (IVIG) for the prevention of bleeding in the fetus and neonate at risk for FNAIT. This study was prematurely ended in 2008 due to lack of inclusion.[8] The registry was kept open for fetal treatment centres worldwide to enter data on pregnancies complicated by FNAIT. Data were entered both retrospectively and prospectively by 13 tertiary referral centres from 10 different countries around the world (fig. 9.1.) An observational cohort study of these data was performed.

Cases

A case was defined as affected by FNAIT if incompatibility between maternal and paternal/fetal HPA type was confirmed and maternal anti-HPA antibodies were detected.

Initially, cases were entered into the database for inclusion of the NOICH randomized trial. All non-randomized and non-eligible cases from the participating centers were included as well. After having prematurely ended the trial the contributing centres kept collecting cases. These were mainly referred patients known to be at risk of FNAIT because of a previous affected child. Some cases originated from a previous Norwegian screening study of FNAIT.[9]

Fetuses and neonates with major congenital or chromosomal abnormalities were excluded.

Outcome

The primary outcome variables were HPA specificity, maternal/ neonatal demographic characteristics and clinical outcome. Cases affected by ICH were not extensively described; we refer to our previous published paper for characteristics of the ICH group.[7] In that study, both older and younger siblings with ICH were also identified and included, resulting in a cohort of 43 cases. In the current study we focus only on the ICH case that were originally included in the NOICH regisitry, explaining the lower number (n=23) described in this paper.

Secondary outcome variable were antenatal and postnatal interventions performed in pregnancies complicated by FNAIT. Outcomes of pregnant women with a previous child with ICH, considered as at high risk, are described separately.

To set up the database, approval was given by the Leiden University Medical Centre's Medical Ethics Committee (MEC PP04.203) and by each centre's respective Institutional Review Board.

Statistical analysis

Categorical data are summarized as actual numbers and percentages. Continuous data reported are presented as medians or means values with ranges. The analysis was done using independent samples t-test. A p value of 0.05 was considered statistically significant. Data analysis was generated using SPSS software (version 20; SPSS Inc., Chicago, IL, USA).

RESULTS

The NOICH registry database contained data on 615 pregnancies complicated by FNAIT; part of these were registered prospectively: 23 for inclusion into the NOICH trial[8], and 177 as a result of the screening study in Norway.[9]

Figure 9.1 shows the contribution of the participating centres, a major part of which are situated in Norway, Sweden, and the Netherlands. The HPA antibody specifics are outlined in table 9.1, with anti-HPA-1a being the most commonly implicated antibody (88%). The maternal and neonatal demographics are shown in table 9.2; the cohort comprised 100 (19%) primiparous and 499 (83%) multiparous women. Of the

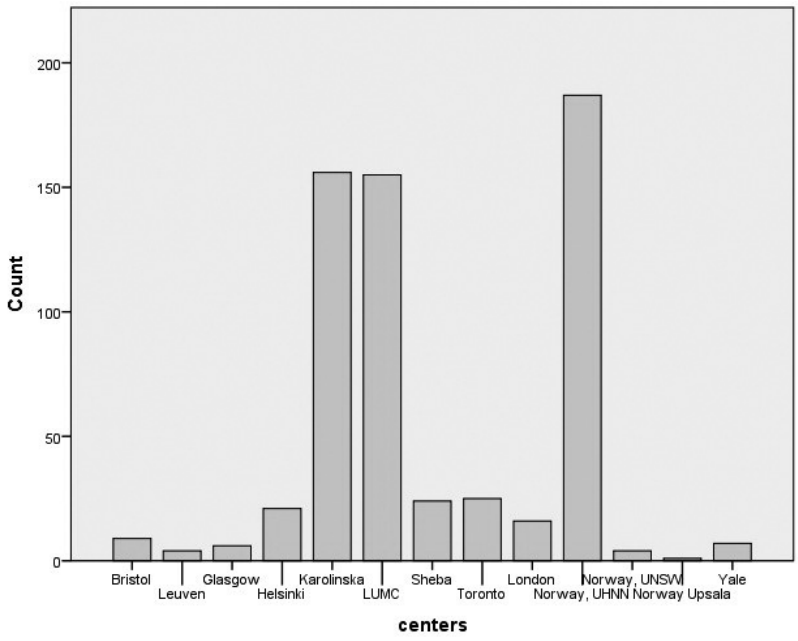

Figure 9.1
Participating centres. LUMC = Leiden University Medical Centre; UHNN = University Hospital North Norway; UHSW = University Hospital South Nor-way.

Table 9.1 HPA specifics

HPA type	Cases, n(%)	Mean PC $\times 10^9$/L	ICH, n
HPA-1a	544 (88)	105	19
HPA-5b	23 (3.6)	136	2
HPA-3a	7 (1.1)	147	
HPA-5a	4 (0.6)	184	
HPA-15a	5 (0.8)	200	
HPA-1a + -5b	18 (3)	94	2
HPA-1a + other*	5 (0.8)		
Negative	2 (0.03)		
Unknown	7 (1.1)		
Total	615		

HPA=Human platet antigen, PC= platelet count, ICH=intracranial hemorrhage

Table 9.2 *Maternal and neonatal demographic*

Pregnancy type	
Singleton	599 (97)
Multiple	16 (3)
Parity[a]	
Primiparous	100 (19)
Multiparous	499 (83)
History	
FNAIT[b]	343 (56)
IUFD	24 (7)
ICH	50 (15)
Mode of delivery[c]	
Vaginal	240 (39)
Instrumental	4 (0.7)
Elective Caesarean	289 (50)
Emergency Caesarean	46 (7.5)
Birthweight[d], g	
Mean (range)	2985 (400-4775)
Gestational Age, weeks	
mean (range)	37 (24-42)
Sex[e]	
Boys	229 (55)
Girls	188 (45)
Platelet count at birth[f]	
Mean (range) $\times$ 10^9/L	108 (1-104)
$<30 \times 10^9$/L	191 (31)
$< 50 \times 10^9$/L	235 (43)
50-100 $\times$ 10^9/L	78 (14)
100-150 $\times$ 10^9/L	59 (11)
$>150 \times 10^9$/L	172 (32)
Petechiae	100 (17)
Petechiae and PC $\times$ 10^9/L	81 (81)
ICH n(%)	23 (3.7)
IUFD n(%)	4 (0.7)

FNAIT= fetal and neonatal alloimmune thrombocytopenia, ICH=intracranial haemorrhage, IUFD= intrauterine fetal death
Values are presented as n (%) unless specified otherwise. a 17 cases missing. b 154 cases missing. c 36 cases missing. d 71 cases missing. e 198 cases missing. f 40 cases missing.

multiparous women, 343 were known to be at risk because of a previous history of FNAIT. This group contained 50 siblings with ICH (15%) and 23 cases of fetal demise (7%), which can be classified as high-risk pregnancies.

Almost all pregnancies were singleton pregnancies (97%). Of the 575 pregnancies with a known mode of delivery, 240 (39%) deliveries were by vaginal route, including 4 assisted by ventouse or forceps. Three hundred thirty-five (50%) caesarean sections were performed, of which 289 were elective. Most deliveries were after 32 weeks of gestation (98%). The mean birth weight was found to be 2985 g, with a mean gestational age of 37 weeks. The neonates were boys in 55% of the cases. When comparing boys and girls there was no significant difference in mean platelet count at birth (102×10^9/L in both groups) or in mean birth weights (2990 vs 2951 g). Severe thrombocytopenia ($<50\times10^9$/L) was found in 235 cases (43%) including 191 (31%) neonates with platelet counts of less than 30×10^9/L. Skin bleeding was reported in 94 (18%) cases, in most of these cases severe thrombocytopenia was found (94%).

Adverse Perinatal Outcomes

In the database 4 cases of intrauterine fetal death (IUFD) and 3 neonatal deaths were found, giving an overall perinatal mortality of 1.14%. In these cases no antenatal treatment had been given. Twenty-three neonates (4.5%) were affected by ICH, 9 were first-born children. Of the other 14 cases, 4 had a sibling affected by ICH due to FNAIT.

HPA-1a was the concerning antigen in the majority of cases (83%); in the other 4 cases, HPA-5a, HPA-5b, and a combination of HPA-1a/-5b was found. Nine of the neonates with ICH were treated during pregnancy; in 4 pregnancies treatment was started after ICH had been found. This is described in more detail in the next section.

A high proportion of the pregnancies complicated by ICH ended preterm: 12 of 23 children (52%) were born before 37 weeks, and 2 children were born before 28 weeks of gestation. Two children suffering from ICH died in utero and 1 post term. The neonates with ICH were male in the majority (76%) of cases. Furthermore 2 cases of intra-abdominal bleeding were found, 1 ending in IUFD at 24 weeks of gestation. In both pregnancies no antenatal treatment had been given.

Antenatal interventions

In 273 pregnancies some form of antenatal treatment was given, varying from cordocentesis with intrauterine platelet transfusions (IUPT) to maternal administration of intravenous immunoglobulins (IVIG), steroids or a combination of those (table 9.3). In most pregnancies (n=138) single treatment with IVIG was given, in 24 cases with 0.5 and in 102 cases with 1.0 g/kg per week (n=12 unknown). In 124 pregnancies invasive treatment was offered. The two groups were comparable according the definition of high-risk FNAIT. There was no difference in number of ICH or IUFD in previous sibling between the invasive and non-invasive group [26/124, 21% vs. 28/138 (20%), p=1.0].

The number of cordocentesis performed differed per centre. This ranged from 0% (0 per offered treatments in Scotland and Norway) to 100% (Canada). If we have a closer

Table 9.3 *Antenatal therapy*

	Cases n (%)	Mean PCat birth (range) $\times 10^9$/L	ICH n	IUFD n
FBS	21(3)	131 (5-302)	0	1
FBS + IVIG + steroids	20 (3)	144 (17-277)	2	0
FBS+IVIG	75 (12)	166 (12-391)	2	0
FBS +steroids	8 (1)	71 (4-166)	1	0
IVIG + steroids	8 (1)	70 (2-298)	0	0
IVIG	138 (22)	122 (4-354)	4	1
Steroids	3 (0.5)	270*	0	0
No treatment	338 (55)	86 (1-405)	14	2
Unknown	4			

FBS=fetal bloodsampling, IVIG= immunoglobulins, PC= platelet count, ICH= intracranial hemorrhage, IUFD=intrauterine fetal death
**= 1 case*

look at the major contributing centres in the database that performed cordocentesis during the study period (in the Netherlands and Sweden), a decline is seen in invasive procedures over the years (from 22% in 2005 to 0% in 2008, 2009 and 2010). One single centre performed cordocentesis up to 2009 (Canada; n=25).

There were 9 cases of ICH reported in the antenatally treated group. Looking at the data in more detail, 5 cases of ICH occurred in the invasive procedure group; in 4 patients it seems likely that invasive therapy with fetal blood sampling (FBS) and serial IUPT (with IVIG or steroids) had been started before the diagnosis of ICH was made during ultrasound examination. In 1 case ICH was found and subsequently invasive therapy was given.

The other 4 cases of ICH had been diagnosed before any antenatal treatment was given. In these pregnancies treatment with IVIG was started to prevent further worsening of bleeding. One of these pregnancies ended in IUFD 1 week after IVIG treatment had been started.

In the single FBS group, 1 IUFD was reported, seen 1 week after the first IUPT. It is unclear whether this was related to spontaneous fetal bleeding or to a complication of the procedure.

Outcomes of High-Risk Pregnancies

Th clinical outcomes of the high-risk pregnancies (n=73) are outlined in table 9.4. Fifty-six cases received antenatal treatmen (26 invasive versus 30 non-invasive). In this high-risk group, 3 cases of ICH were found (4.2%), 1 resulting in IUFD. In 1 of these pregnancies antenatal treatment was given (IVIG+ serial IUPT); in the other 2 cases IVIG was started after the diagnosis of ICH.

Table 9.4 *Clinical outcome in the high-risk group*

	Cases, n (%)	Median PC at birth (range) $\times 10^9$/L	ICH,n	IUFD, n
Total high-risk cases	73	96 (1 - 391)	3	1[a]
Platelet count < 50 X 10^9/L	18 (25)			
Platelet count < 30 X 10^9/L	16 (16)			
Antenatal treatment	**56**			
FBS+IVIG+steroids	4	70 (17 - 170)		
FBS+IVIG	21	181 (21 - 391)	1	
FBS	1	253		
IVIG+steroids	3	60 (2 - 296)		
IVIG	27	63 (10 - 340)	2	1[a]

FBS=fetal blood sampling, IVIG=immunoglobulins, ICH=intracranial hemorrhage, IUFD=intrauterine fetal death
[a] 1case suffering from ICH ended in IUFD

DISCUSSION

In this study we evaluated treatment and outcome of more than 600 cases of FNAIT, the largest cohort of cases of FNAIT published on thus far. The majority of cases were collected after a first affected pregnancy. The overall frequency of ICH in our study group is 3,7%, which is lower than previously reported.[10] This might well be explained by the several antenatal interventions performed in this group.

Antenatal treatment

Different treatment regimens were offered to avoid recurrence of any burden, divided into invasive (n=124) and non-invasive (n=138). An important observation was that, although rare, all bleeding complications (n=5) occurred in the invasively treated group (FBS+IUPT). No ICH cases were reported in the non-invasive group (maternal administration of IVIG). Although the exact cause of the adverse outcome in the invasively treated group could no be reliably assessed, at least we can conclude that there appears to be no benefit from invasive treatment over a non-invasive approach. Previous studies have calculated a cumulative risk of fetal loss per pregnancy of 6% directly related to complications of FBS and IUPT.[11,12]

Overall we can state that non-invasive antenatal treatment with maternal administration of IVIG appears successful in protecting fetuses and neonates from bleeding (138 cases treated, 0 cases of ICH reported). Furthermore, our study clearly indicates that over the years the invasive diagnostic and treatment approach in FNAIT has been almost completely replaced by safer non-invasive protocols.

Several reports have been published on IVIG treatment for FNAIT with a close to 100% success in preventing bleeding complications in fetuses and neonates.[13-18] Until now, the working mechanism of IVIG is not clear. Most likely, it acts on various levels,

i.e.in maternal serum, at the level of placental transfer of IgG and in the fetal blood, blocking Fc-receptors on macrophages.[19] Recent research by Yougbaré et al (2015) supports the hypothesis that IVIG may aid in protection against bleeding through a direct effect on endothelial cells, instead of merely causing a rise in platelets.[20] They showed that impairment of angiogenesis rather than thrombocytopenia is the critical cause of ICH in FNAIT. In their murine-model study, ICH only occurred in fetuses and neonates with anti–β3 integrin–mediated, but not anti-GPIbα–mediated FNAIT, despite similar levels of thrombocytopenia in both groups. Only anti–β3integrin–mediated FNAIT reduced brain and retina vessel density, impaired angiogenic signaling, and increased endothelial cell apoptosis. This might be an explanation for the phenomenon of 'non-responders', i.e. fetuses not responding to IVIG with platelet counts remaining below 50×10^9/L, reported to be around 20%.[21]

Outcomes in High-Risk Pregnancies

Most reports support the assumption that pregnant women with a previous child with an intracranial bleeding compose the highest-risk group; the ICH recurrence rate in subsequent pregnancies is reported to be around 79%.[22] Therefore, most clinicians caring for such pregnancies choose for a more aggressive approach as compared with the group in which the affected sibling did not have an ICH.

In our study, 73 FNAIT cases with a previous sibling with ICH or IUFD were reported; 56 of them received antenatal treatment with 1 case ending in ICH and finally IUFD. In this pregnancy, invasive therapy was given (combination FBS+serial IUPT with IVIG). In this high risk group 30 cases were treated completely non-invasively (all with IVIG, 3 in combination with steroids) and no ICH occurred.

FNAIT and Fetal gender

In our large data set of pregnancies affected by FNAIT, 55% of fetuses/neonates were male. Interestingly, in the group of children with ICH, the proportion of males was much higher (76%). A study by Tiller et al. showed a clear association between the levels of maternal HPA-1a antibodies and reduced birth weight in boys.[23] How the sex of the offspring is involved in FNAIT remains to be explored.

The strengths of our study are its large sample size,which is 3 times larger than the previously largest series[24], the input of data by multiple international centres and the variation in interventions performed. The results obviously need to be interpreted with care, given the limitation that a large part of the data was collected retrospectively, and a selection bias cannot be ruled out. More reliable data can only be obtained from prospective population screening studies.

CONCLUSION

Pregnancies affected by FNAIT, even with a severe history, now have an excellent prognosis. Our data confirm that there appears to be no benefit from invasive diagnostic or therapeutic procedures. Non-invasive management using IVIG with or without additional steroids prevents bleeding in the fetus or neonate in virtually all cases. Remaining issues for future research are timing and optimal dose of IVIG, the role of gender, and long-term neurologic outcome of the surviving affected children.

In several countries, preparations are being made to implement population screening studies of FNAIT.

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CHAPTER 10

LONG-TERM OUTCOME IN CHILDREN BORN WITH INTRACRANIAL HEMORRHAGE DUE TO FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA; OBSERVATIONAL COHORT STUDY

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ABSTRACT

Objective

To evaluate the long-term outcome in children with intracranial hemorrhage due to fetal and neonatal alloimmune thrombocytopenia (FNAIT).

Study design

All pregnancies with a fetus with intracranial hemorrhage caused by FNAIT between 1993 and 2015 were included in this observational cohort study. Neurological, motor and cognitive development was assessed at a minimum of one year of age. Primary outcome were perinatal death or severe neurodevelopmental impairment (NDI). Severe NDI was defined as any of the following: severe cerebral palsy (Gross Motor Function Classification System ≥ 2), bilateral deafness, blindness, severe motor and/or cognitive development delay (<-2 standard deviation). Moderate NDI was defined as cerebral palsy with gross motor function classification system <2, motor and/or moderate cognitive developmental delay (< -1 standard deviation).

Results

Eighteen pregnancies with a fetus with intracranial hemorrhage due to FNAIT were included in the study. Fetal or neonatal mortality rate was 8/18 (44%). Severe NDI and moderate NDI were diagnosed in 6/10 (60%) and 1/10 (10%) of the surviving children. Only 4/18 (22%) of fetuses survived without severe NDI.

Conclusions

The risk of perinatal death or severe NDI in children with intracranial hemorrhage due to FNAIT is high. Only screening and effective preventive treatment can avoid this burden.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but potentially serious condition . The most feared complication of FNAIT is intracranial hemorrhage (ICH) and its associated lifelong risks of handicaps and neurologic sequelae.[1,2] Human Platelet Antigen (HPA) allo-antibodies formed during pregnancy due to incompatibility of maternal and fetal/paternal antigen, can cross the placenta, and lead to fetal or neonatal thrombocytopenia.

Anti-HPA-1a is the platelet-specific antibody most commonly involved in FNAIT and this antibody is also responsible for the most severe cases of FNAIT.[1] It may cause bleeding complications such as ICH leading to brain damage and life-long handicaps or even death. One in 50 pregnancies is at risk for FNAIT. Antibodies are present in 1:350 pregnancies, leading to FNAIT-related perinatal death or ICH in at least 1:11.000 fetuses or newborns, and this is likely an underestimation.[2] Also, ICHs caused by FNAIT have a very high recurrence rate in subsequent pregnancies, up to 79%.[3] Therefore, in the absence of screening programs for FNAIT, current management is mainly focused on reducing the risk of recurrence of ICH in subsequent pregnancies.[4]

In view of implementation of such a screening, as well as in order to perform adequate counselling of parents, it is important to increase our knowledge on the implications of these ICHs. Overall the prognosis of ICH in neonates with FNAIT is often more severe compared to neonatal ICH from other causes, associated with a poor clinical outcome and a high mortality rate.[3,5,6] Unfortunately, due to the presumed underreporting and the relative rarity of the disease, little is known about the long term consequences and clinical outcome of these severe bleeding complications. The only available data on long-term follow-up in neonates with severe ICH due to FNAIT are based on two small case series with respectively three and six survivors.[5,6] All survivors had very poor neurodevelopmental outcome. However, these two studies were small and dated.

We therefore set up a study to evaluate the long-term neurodevelopmental outcome in a larger and more recent cohort of children with ICH due to FNAIT and clearly outline the burden of this disease in survivors in the current era of fetal medicine and neonatal intensive care treatment possibilities.

METHODS

Study population

Leiden University Medical Centre serves as the national centre of expertise for FNAIT in the Netherlands. Therefore women at risk for FNAIT with a history of a severe thrombocytopenic child (platelet count < 50×10^9 /L), a child with bleeding complications (ICH or other), or an intrauterine fetal demise due to FNAIT are referred to our center for counseling and treatment in their consecutive pregnancies. Other referral reasons include

newly diagnosed FNAIT during a current pregnancy, for example in case of fetal ICH. From 1993 onward all cases with ICH, as retrieved from medical files, due to FNAIT were identified and included in our study.

The study was approved by the institutional review board at the Leiden University Medical Centre (P11.190) and all parents gave written informed consent for the follow-up of their children.

A case was defined as FNAIT if incompatibility between maternal and paternal/fetal HPA type was confirmed and maternal anti-HPA antibodies were detected.

Outcomes

Primary outcome was perinatal death and/or severe neurodevelopmental impairment (NDI). Severe NDI was defined as any of the following: severe cerebral palsy (GMFCS ≥ 2), cognitive and/or motor test score of less than 70 (<-2 SD), bilateral blindness, or bilateral deafness requiring amplification.

Secondary outcome was moderate NDI defined as cerebral palsy with GMFCS <2, motor and/or mild-to-moderate cognitive developmental delay (< -1 SD and > -2 SD).

The following antenatal and neonatal data were recorded: antenatal treatment, gestational age at birth, mode of delivery, birth weight, platelet count at birth, clinical course, cerebral imaging. When available, neuroradiological images were reviewed by an experienced neonatologist (SS) to confirm the presence of ICH and to classify the type of bleeding. When images were obtained in another hospital and not available for review, written reports of the imaging evaluation by other experienced radiologists were obtained from the patient files. Hemorrhage was classified as subdural, subarachnoid, cerebellar, intraventricular or intraparenchymal with a separate notion for unilateral or bilateral occurrence and the extent of lobar involvement (frontal, parietal, occipital or temporal).[7]

Neurological, motor and cognitive development was assessed at a minimum of 1 year of age. Most children underwent neurodevelopmental follow-up and reports of tests were requested at the institution where the neurological examination and assessment of neuromotor and cognitive development had taken place. In cases were neurodevelopmental testing was not performed, children were asked to visit our outpatient clinic for a follow-up examination by our medical psychologist. This included neurological examination and assessment of cognitive and motor development using standardized psychometric tests appropriate for age (BSID-III: Bayley Scales of Infant and Toddler Development third edition, WPPSI-III: Wechsler Preschool Primary Scale of Intelligence third edition, WISC-III: Wechsler Intelligence Scale for Children third edition).[8-10] Bayley-III, WPPSI and WISC scores follow a normal distribution curve with a mean of 100 and a standard deviation (SD) of 15. A cognitive test score that is, a Bayley-III cognitive composite score, WPPSI Total IQ- or WISC Total IQ score below 70 (< -2 SD) indicates severe cognitive delay and scores below 85 (< -1 SD) indicate mild-to-moderate cognitive delay. Children with severe cognitive impairments (with scores below 50) or who were unable to participate in standardized testing due to severe cognitive impairment were assigned a score of 49 in the database.

Cerebral palsy (CP) was defined according to the European CP Network and classified as diplegia, hemiplegia, quadriplegia, dyskinetic, or mixed. Subsequently CP was scaled according the Gross Motor Function Classification System (GMFCS) in level I-V varying from decreased speed, balance and coordination at level I to impaired in all motor functions, cannot sit, stand, walk independently and has physical impairments that restrict voluntary control of movement and the ability to maintain head and neck position against gravity at level V.[11]

Statistical analysis

All data were analyzed with SPSS software (V.18.0 SPSS Inc, Chicago, Illinois, USA), using descriptive statistics. Categorical data are presented as numbers and percentages. Continuous variables are presented as median with Inter Quartile Range (IQR) or mean with standard deviation (SD).

RESULTS

A total of 20 children with severe bleeding complications due to FNAIT were identified. All children were born between 1993 and 2015 in different areas in the Netherlands. Two of these children were excluded because bleeding occurred in another organ, being a pulmonary and gastrointestinal hemorrhage. Of the 18 children with ICH, perinatal death occurred in eight cases (44%). Demise was due to fetal death at 22 weeks (n=1), fetal death during labour after drainage of post-hemorrhagic hydrocephalus (n=1) and neonatal demise within the first days after delivery due to severe ICH (n=6).

The median platelet count at birth was 11×10^9/L (IQR: 7.5-24.5$\times 10^9$/L). Maternal characteristics of the infants with ICH are given in table 10.1; clinical outcome characteristics are shown in table 10.2. Neurodevelopmental outcome was assessed in all ten surviving children.

Antenatal treatment

Antenatal treatment was administered in three pregnancies complicated by ICH. One mother (#15) had a previous child with FNAIT without ICH that led to the proposed plan of antenatal treatment with 0.5 g/kg/week intravenous immunoglobulins (IVIG) from 28 weeks of gestation onward in this subsequent pregnancy. Just before the start of treatment a hemorrhage was found during fetal cranial ultrasound. Fetal magnetic resonance imaging (MRI) confirmed parenchymal ICH in the left hemisphere. As planned IVIG was started, only in a higher dose of 1.0 g/kg/week. The second case concerned a dichorionic twin pregnancy (#14) of which one suffered from ICH. Maternal HPA-5b antibodies were found and amniocentesis showed HPA-5b positive status in the unaffected co-twin. IVIG was started to protect the co-twin from bleeding and to prevent worsening of bleeding of the affected fetus. In the third case ICH was diagnosed during routine ultrasound at 20 weeks of gestation, the mother had a previous child with ICH,

Table 10.1 Maternal characteristics of ICH infants

Maternal age in years median (IQR)	30 (28 - 31.5) [1]
Obstetrical history	
sibling with ICH	1
sibling with FNAIT without ICH	2
primigravida	4
firstborn	8
miscarriage	9
HPA type	
HPA 1a	13 [2]
HPA 5b	2
HPA 5a	2
Pregnancy type	
single	17
multiple	1
Antenatal treatment, IVIG 1 gr/kg/week after diagnose ICH	**3**
Delivery	
vaginal	6 [1]
ventouse	3
caesarean section	7

Data in [ ] indicate number of missing data points.

Table 10.2 Clinical outcome characteristics of the ICH infants

Gestational age at delivery, weeks median (IQR)	36 (35 - 38) [2]
Birth weight, grams mean (range)	2407 (1991 - 2942) [6]
Gender female/male (n)	3/11 [4]
Platelet count at birth, $x10^9$/L median (IQR)	11 (7.5- 24.5) [4]
Antenatal treatment, IVIG 1 g/ kg/wk after diagnosis ICH (n)	3

Data in [ ] indicate number of missing data points.

presumed to be caused by birth trauma (case #17 and #18). In this subsequent pregnancy HPA 5a antibodies were detected and FNAIT was diagnosed. Maternal administration with 1.0 g/kg/week IVIG followed from 28 weeks of gestation onward.

In sixteen cases ICH occurred antenatally. In the other two cases this was not reported.

Neuroimaging examinations of intracranial hemorrhage

Type and localization of ICH of the 18 infants with ICH is reported in table 10.3. From five children MRI images were available for review; the other thirteen could be classified using written reports. Seventeen children had intraparenchymal hemorrhage. In five cases there was also intraventricular, and in 1 case subarachnoidal hemorrhage. One child had solely an extensive subarachnoidal hemorrhage. Eight cases had bilateral hemorrhage. Eleven cases were complicated by post-hemorrhagic hydrocephalus, of which 6 developed a porencephalic cyst, resulting in five of these children requiring a ventricular peritoneal shunt.

Long-term neurodevelopmental outcome

In total, ten surviving children with ICH were included for long-term follow-up (table 10.3).

Neurodevelopment was already assessed elsewhere (rehabilitation clinic or pediatric department) in six cases, using developmental tests adapted to their cognitive, motor and/or visual impairments. Two children were evaluated by the medical psychologist at our centre. Two children could not be assessed with psychometric tests due to very severe cognitive and motor impairment and were assigned a score of 49. Children were tested at a median age of 7.5 years (IQR 5-14)

Severe NDI in the studied cohort was found in 6/10 cases (60%). Cerebral palsy was diagnosed in seven cases (70%). One child had moderate NDI due to spastic hemiparesis with a GMFC score of I (moderate NDI).Severe cognitive delay was detected in six children (60%) and severe motor delay in six children (60%). Three children were blind (30%). One child was diagnosed with attention deficit hyperactivity disorder, one child had problems with behavior and attention-regulation but was too young to diagnose ADHD.

DISCUSSION

FNAIT is a potentially hazardous disease associated with fetal thrombocytopenia and severe bleeding complications in the fetus and neonate. The most feared complication of FNAIT is ICH and its associated risk of lifelong handicap and neurologic sequelae.[12-14] This study shows that the risk of death or severe neurodevelopmental impairment in children with ICH due to FNAIT is high (14/18, 78%). Adverse outcome was due to perinatal mortality in 8/18 (44%) of cases and severe NDI in 6 of 10 survivors (60%). In two of four survivors without severe NDI, moderate abnormalities were detected including spastic hemiplegia (GMFC score I) and attention deficit disorders (ADHD), therefore only two of the ten survivors were free of neurodevelopmental sequelae. Remarkably 40% of the children had visual impairment. Our findings stress the severity and implications of major and permanent life-long handicaps associated with FNAIT, particularly in case of ICH.

Table 10.3 Long-term outcome of all infants with ICH

Child	Location ICH	Associated lesions	Age at evaluation	Cerebral palsy	Developmental test	Total IQ	Outcome Long term outcome	Severe NDI
1	extensive subarachnoidal and unilateral parenchymal frontal/temporal/occipital						neonatal death	
2	unilateral intraventricular and parenchymal	hydrocephalus					neonatal death	
3	bilateral parenchymal						neonatal death	
4	extensive bilateral parenchymal.						neonatal death	
5	extensive bilateral parenchymal	hydrocephalus					fetal death during labour	
6	bilateral parenchymal	hydrocephalus					fetal death at 22 wks	
7	extensive subarachnoidal						neonatal death	
8	bilateral intraventricular and parenchymal	hydrocephalus					neonatal death	
9	unilateral parenchymal, occipital		8 year	-	WISC	86	ADHD	no
10	unilateral parenchymal, temporal	hydrocephalus, VPD	2, 8 and 14 years	spastic tetraplegia GMFCS V	Bayley/BSID;Reynell-Zinkin;KID-N	49	bilateral blindness, severe cognitive and motor delay, epilepsy	yes
11	bilateral parenchymal, temporal	porencephalic cyst hydrocephalus, VPD	20 year	spastic tetraplegia GMFCS V	not tested due to severe impairment	49	bilateral blindness, severe cognitive and motor delay, epilepsy	yes
12	bilateral parenchymal, temporal and occipital	porencephalic cyst hydrocephalus, VPD	23 year	spastic tetraplegia GMFCS V	not tested due to severe impairment	49	bilateral blindness, hearing impairment, severe cognitive and motor delay	yes
13	extensive bilateral intraventricular, parenchymal and cerebellar haemorrhage	bilateral porencephalic cyst, cerebellar destruction hydrocephalus, VPD	3 year	spastic diplegia GMFCS IV	SON	60	severe cognitive and motor delay	yes
14	unilateral parenchymal, occipital and cerebellar		5 year	-	WPPSI	110		no
15	bilateral parenchymal, parietal, temporal and occipital	bilateral porencephalic cyst hydrocephalus, VPD	1 year	spastic hemiplegia GMFCS IV	KID-N	49	visual impairment, severe cognitive and motor delay, epilepsy	yes
16	unilateral parenchymal, fronto-temporal		7 year		WISC	112		no
17	unilateral parenchymal, intraventricular and bilateral cerebellar	unilateral porencephalic cyst	5 year	spastic hemiplegia GMFCS I	WPPSI	85	problems with behaviour and attention-regulation	no
18	bilateral frontal parenchymal and intraventricular	bilateral porencephalic cysts	8 year	spastic diplegia GMFCS II	SON	50	severe cognitive and motor delay, epilepsy	yes

Tests: WISC Wechsler intelligence scale for children, WPPSI: Wechsler Preschool and Primary Scale of Intelligence, BSID: Bayley Scales of Infant and Toddler Development Reynel Zinkin: Developmental Scales for Young Visually Handicapped 2 months – 4/5 years, KID-N: Kent Infant Development Scale, dutch version 0- 15 months/8 years, SON: Snijders-Oomen Non verbal Intelligence test

Most cases of ICH seem to occur antenatally, with the majority before the 28th week of gestation.[15] Which is in line with the results from our study. All ICHs occurred antenatal and were mostly parenchymal hemorrhages, with the majority complicated by hydrocephalus and/or porencephalic cysts.

Interestingly the proportion of males was much higher (11 male versus 3 female) An association between sex and ICH due to FNAIT was also found in previous publications. How the sex of the offspring is involved in FNAIT remains to be explored[16,17].

This study has some limitations. Cases could have been missed because only cases referred to our center were included. Furthermore the retrospective nature of this cohort study makes it susceptible to confounding and information bias and may have led to the inclusion of more severe ICH cases and therefore possibly cases with poorer outcome. However, it is not likely that cases with better developmental outcome were missed because the cases in this study were selected by ICH from all FNAIT cases referred to our own center and not by developmental problems. In addition due to this retrospective design, no conclusions on the prevalence of these ICHs compared to the total cases of FNAIT detected can be drawn. Cultural differences or legal restrictions in administration of intensive neonatal care may have influenced the outcome in this cohort. It is plausible that withholding or withdrawing neonatal intensive care treatment in cases with poor prognosis, may have led to a higher perinatal mortality and therefore to a lower number of survivors with poor neurodevelopmental outcome. Lastly, there is heterogeneity in developmental testing performed, adapted to the age as well as to the severity of impairment of the children included for follow-up. Importantly, standardized psychometric testing was not feasible in all children due to severe motor, cognitive and neurosensory impairments.

Nevertheless, despite these limitations, this is the first study that focuses on long-term outcome of ICH due to FNAIT and the largest study to describe actual long-term follow-up. Our findings show that FNAIT-related ICH is associated with severe developmental delay.

In absence of cohort studies assessing the long term outcome of ICH caused specifically and solely by FNAIT, data originate from cohort studies of ICH in term neonates with a variety of other causes (mainly birth trauma, asphyxia).[5,6,12-14]

The largest case series of intraventricular hemorrhage (IVH) in full term newborns is described by Mao et al.[5] They analyzed a total of 36 newborns and found a low mortality rate and generally favorable outcome, with 63% of all cases having no or only mild handicaps. In contrast, they found FNAIT to be the single most important cause of poor outcome. Out of nine cases three infants died and six were severely handicapped. Jocelyn et al studied a cohort of 15 IVH cases in full term newborns, of which three were caused by FNAIT.[6] Of these three, two survived and were both severely impairment. Both studies are limited by the small number of patients as well as by their selection of cases. Whereas both studies selected newborns with diagnosis of IVH, there might be an underrepresentation of (minor) intracranial hemorrhages that have been caused by FNAIT.

In the absence of screening programs for FNAIT the disease is mostly always detected after birth of an affected child and preventive measures with antenatal IVIG can only be taken in next pregnancies. Implementation of routine HPA-typing and antibody screening in the near future would strongly reduce the burden associated with this disease. Several groups have published calculations of costs and potential benefits of screening and intervention, all reaching the same conclusion that such programs are likely to be cost-effective.[2,18,19] The main reason for this cost-effectiveness, despite large-scale testing and, in case of IVIG, expensive treatment, are the disease burden and excessive costs for a child with life-long severe neurological sequelae.

As long as screening for FNAIT is not implemented in standard care, reliable information about the incidence of ICH among FNAIT cases cannot be given. Furthermore, prospective studies including general screening for FNAIT and follow-up are needed to learn more about the pathophysiology of this disease, including establishing if there is also a milder phenotype of ICH with discrete symptoms and better outcome.

CONCLUSION

This is the first study focusing and reporting on long-term neurodevelopmental outcome of children suffering from ICH caused by FNAIT. In the vast majority of cases, ICH leads to either perinatal death or, in survivors, severe impairment. These long-term sequelae can only be avoided by screening and effective preventive treatment.

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CHAPTER 11

GENERAL DISCUSSION

DISCUSSION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) was first described in 1950 as feto maternal allo immune thrombocytopenia (FMAIT).[1] Subsequently FNAIT has been known for decades as "neonatal alloimmune thrombocytopenia" (NAIT).[2,3] The usual presentation is a full-term neonate exhibiting petechiae or widespread purpura at birth, or a few hours after birth, born to a healthy primiparous mother. Subsequent laboratory testing then reveals isolated severe thrombocytopenia. The most serious complication is fetal or neonatal intracranial haemorrhage (ICH) leading to severe neurological sequellae or perinatal death.

Women at risk of having their fetus affected with FNAIT are usually identified because of a previous child with thrombocytopenia due to alloantibodies against platelets, in the worst cases with ICH. As long as pregnancies at risk for FNAIT will stay unrecognised due to the lack of screening programs, affected babies will keep being born with complications such as ICH. Over the last decade, the knowledge of FNAIT has markedly increased leading to improvement of care and management. However, still a lot of questions remain, and new questions arise.

The studies described in this thesis were designed to improve the current state of knowledge about incidence, burden and management of FNAIT. The goal was to contribute to the international debate whether it is time to implement universal screening programs for FNAIT. In this discussion section, the respective studies are summarised. Furthermore we provide speculations and ideas with regard to future development and research in this field.

Incidence of FNAIT

In chapter 2 and 3, systematic reviews of the literature on screening studies for FNAIT are given. In chapter 2, a systematic review of all screening studies on HPA typing, immunisation and perinatal outcome in pregnancies is provided. Chapter 3 illustrates the results of a review of screening studies in neonates to detect thrombocytopenia and estimates the incidence of FNAIT and related ICH. These prospective cohort studies provide a pooled estimate of the naive prevalence among pregnant women of human platelet antigen(HPA)-1a negativity, the risk of HPA-antibody formation, incidence of severe neonatal thrombocytopenia and risk of adverse outcome. Based on these two systematic reviews of the literature the expected incidence of FNAIT caused by HPA-1a immunisation is 1 in 366 pregnancies. In one third of these pregnancies, severe thrombocytopenia develops, and of those, 10% of neonates suffer from ICH, or 1 in 11,000 pregnancies. Older studies on risks for ICH in HPA-immunised pregnancies quoted an incidence ranging from 7 to 26%, or 3 to 10 per 100,000 pregnancies.[4,5]

The pooled data confirm that a significant proportion of severe disease already occurs in the first pregnancy, with the majority of bleedings originating in utero, before 36 weeks of gestation. However, none of the studies reported on the true natural history of the disease. Understandably, the investigators offered interventions to women

in whom they detected HPA antibodies, with the aim of reducing the incidence of the true clinical disease, which is fetal or neonatal bleeding. It seems safe to assume that the incidence of 31% severe fetal or neonatal thrombocytopenia in HPA-immunised women, with 10% severe adverse outcome, is an underestimation of the true risk in non-screened populations (chapter 3). This was confirmed by a study from Norway, where the authors compared two groups of infants with FNAIT, a non-screened versus a screened population of pregnant women. Their reported detection rate of FNAIT without screening was only 14% of the expected true rate.[6]

Our second systematic review (chapter 3), using data from postnatal screening studies, confirmed that attempts to assess the prevalence of neonatal FNAIT purely based on clinical evaluation of symptomatic bleeding leads to a significant underestimation of the prevalence. Better estimations are unavailable, only large prospective (non-intervention) screening studies can demonstrate the true numbers of FNAIT and the associated adverse perinatal outcome.

Finally, given the antenatal origin of most intracranial bleedings, the best option to reduce the associated mortality and morbidity seems to be screening all pregnant women for HPA alloimmunisation instead of screening neonates, so effective antenatal treatment can be offered.

Antenatal treatment

In Chapter 4 we critically evaluate the literature on preventive interventions for pregnancies known to be complicated with FNAIT. With the lack of screening programmes, focus is on how best to manage pregnancies with known FNAIT, thus those pregnant women with a previously affected child. Antenatal management is ultimately aimed at preventing bleeding complications.

There is still controversy on type and timing of various interventions in pregnancies with FNAIT.

Until 1984, the traditional management of subsequent pregnancies in women with a previous history of FNAIT consisted of an early elective caesarean section and transfusion of platelets after birth. Since the publication of Daffos et al[7], one of the pioneers of fetal blood sampling (FBS), several centres throughout the world started in a similar fashion to anaemia due to red cell alloimmunisation with serial intrauterine platelet transfusions for the treatment of fetal thrombocytopenia.[8-10] However, these procedures are associated with up to 6% fetal loss. This has led to the exploration of non-invasive maternal treatment. Bussel et al were the first to describe the clinical efficacy of immunoglobulins (IVIG), based on the experience with idiopathic thrombocytopenic purpura (ITP) patients.[11] Since Bussel's first report, the standard dose of IVIG in FNAIT has empirically been 1 g/kg given weekly, not based on any dose finding studies, starting anywhere between 20 and 32 weeks of gestation.[12,13] Several studies have been published supporting the safety and efficacy of non-invasive, IVIG-only treatment in FNAIT with a close to 100% success.[14-16] The rationale of using high dose IVIG is based on the assumption that it blocks FcR(fragment crystallizable region)-mediated trans-

placental transport of pathological anti HPA-1a antibodies and increases the clearance of anti-platelet antibodies. In a mouse model lacking expression of FcRs, treatment with IVIG did not increase anti-platelet antibodies clearance in comparison to mice with functioning Fc receptors. [17]

The treatment is usually stratified according to the presence or absence of ICH in the previous child and the timing of its occurrence, i.e., antenatal or postnatal. Several (small) trials have shown the effectiveness of preventing ICH in FNAIT, with no consensus about appropriate dose (varying from 0.5 to 2.0 g/kg maternal weight per week).[18,19] The use of any medication in pregnancy, in particular substances that cross the placenta, should be carefully considered, balancing perceived benefit against potential harm for mother and the developing fetus. IVIG is used off-label for a number of rare diseases, including FNAIT, only based on observational studies or small underpowered controlled trials.

In 2000, in collaboration with the Karolinska institute in Sweden and the University of Northern Norway, the NOICH (No Intra Cranial Haemorrhage) international registry was set up as a great potential to collect and share data on this rare disease. Many centres throughout the world entered patient data of FNAIT cases, what led to a total of 615 included cases.

Together with introduction of this registry a randomised clinical trial (RCT) was started to evaluate the effectiveness of IVIG at a lower dose of 0.5g/kg. Unfortunately the trial was stopped prematurely due to slow recruitment. Twenty-three women were randomised (low dose 11, standard dose 12). No ICH occurred and comparable platelet counts at birth were found. The lack of power to prove equivalence means that the data must be interpreted with care. However as argued by Kahn and Hills[20], results have to be taken as they stand and need to be shared with the medical community when trials are stopped early. This resulted in publication of the study (chapter 7) suggesting that our data at least do not show any benefit of a higher than 0.5 g/kg dose in the treatment of FNAIT.

Subsequently the NOICH registry led to another study (chapter 8). In view of lack of evidence for a particular dose, we decided to continue offering 0 .5 g IVIG/kg per week to FNAIT women with a previous affected child without ICH. We performed a cohort study including 109 pregnant women suffering from FNAIT with a previous affected child without ICH collected from the NOICH database, treated with either 0.5 and 1.0 g/kg IVIG per week. In equivalence with the RCT, the results (chapter 7) show no difference in platelet count (PC) at birth and incidence of severe thrombocytopenia ($<30x10^9$/L). Furthermore, no ICH occurred. The number of women included in this study is still limited. However with a cohort of 109 women treated with IVIG for FNAIT, it is still one of the largest studies evaluating treatment modalities in such a rare disease as FNAIT. Unless trials are done using screening of populations as a mean of subject selection, it seems unlikely that prospective studies will ever be significantly larger.

In chapter 9 we describe the overall outcome of the NOICH database consisting of 615 cases affected by FNAIT. Our most important observation was that overall antena-

tal treatment for FNAIT results in favourable perinatal outcome and it illustrates that in most centres, over time, treatment for FNAIT changed from an invasive to a complete non-invasive procedure.

Treatment for FNAIT is usually stratified according to the presence or absence of ICH in the previous child and the timing of its occurrence, i.e., antenatal or postnatal. The highest risk for FNAIT-related complications in subsequent pregnancies seems to be among those infants with siblings that experienced antenatal ICH, with a reported recurrence rate of 90%.[21,22] Therefore it is of upmost importance to demonstrate that antenatal treatment in this 'high-risk' group is effective. Kanhai et al[23] were the first to describe completely non-invasive management using IVIG only in this group. They treated seven pregnancies with IVIG 1.0 g/kg/week, no ICH occurred. Bussel et al reported a relatively large series of FNAIT pregnancies with a previous child with ICH.[24] The 37 cases were subdivided in three risk categories depending on the assumed timing of the ICH, and received IVIG 1.0 or 2.0 g/kg. However we cannot consider the antenatal treatment offered in this study to be solely non-invasive because fetal blood sampling was applied to discover failure of therapy. Failure of therapy was defined when fetal platelet count < 30×10^9/L. This led to intensification of therapy by increasing the dose of IVIG or adding prednisone. Only one pregnancy was treated with the use of serial IUPT. Three ICHs occurred, two in fetuses treated with IVIG 1.0 g/kg + prednisone 1 mg/kg/day, and one in a fetus treated with 1.0 g/kg IVIG alone. No clear advantage from one type of treatment over another could be observed.

In the NOICH data base 30 FNAIT pregnancies with a previous sibling with ICH were treated solely with immunoglobulins. In this group no ICH occurred.

Although these numbers are small and optimal management remains to be determined all studies confirm effectiveness of non-invasive IVIG treatment in preventing thrombocytopenia-related ICH, and although views on the optimal dose may differ, it appears clear that there is no place left for invasive treatment using platelet transfusions.

Burden of disease

The actual goal of treatment for FNAIT is preventing its most feared complication, namely ICH. Untreated newborns with FNAIT are reported to be affected by ICH in 7–26% of pregnancies (chapter 2 and 3), with a recurrence rate of ICH in subsequent pregnancies as high as 90%.[21,22] Surviving children with ICH suffer from severe neurological sequelae including mental retardation, cerebral palsy, cortical blindness and seizures. It is of clinical importance to detect neonates with FNAIT to offer effective and safe treatment to avoid this burden in subsequent pregnancies.

In chapter 5 we evaluate the rate and consequences of a late or missed diagnosis of fetal and neonatal alloimmune thrombocytopenia (FNAIT) by assessing the clinical presentation of first affected children, the timing of diagnosis of FNAIT and the outcomes of subsequent children.

In this cohort study of 26 women with FNAIT, delay of diagnosis was identified in four pregnancies (15%). Two of these four fetuses suffered from severe ICH. Several fac-

tors were presumed to have caused the low platelet count, ie. down syndrome, intrauterine growth restriction and birth trauma, and kept the clinicians from requesting the appropriate investigations.

These illustrative examples of delayed diagnosis show that missing the diagnosis of FNAIT can have devastating consequences for subsequent children, including ICH or perinatal death.

In chapter 6 we report the time of occurrence of ICH in pregnancies affected by fetal ICH. For this study the NOICH database was used to identify pregnancies complicated by ICH. This study, using clinical information together with radiographic imaging and autopsy reports, shows that the majority of ICH bleedings occurred by the end of the second trimester and that clinical outcome was devastating for most cases. The high frequency of bleedings occurring before 28 weeks indicates that the fetus may be severely affected already in the second trimester. Our antenatal review and earlier studies[22] have suggested the onset of bleedings to be in the third rather than the second trimester and are in variance with this study. Importantly, these studies reported the gestational age when the ICH was diagnosed, but could not reliably assess when the bleeding may have occurred. Bussel et al reported on gestational age at the time of ICH, in a study where antenatal management to prevent recurrence of ICH caused by FNAIT was studied. The results of their study population support our observation that many of ICH cases (8/37, 22%) occurred before 28 gestational weeks.[24]

Furthermore, we saw that fetal ICH due to FNAIT often occurred in the first child and even in the first pregnancy. These important findings of occurrence of ICH before the 28th week of gestation and high number of fist borns affected challenges the current management strategy where antenatal treatment is reserved for subsequent pregnancies after FNAIT has been diagnosed in the first child. Firstborns with FNAIT can only be treated when picked up in a screening program. Possible interventions to reduce risk of ICH need to be introduced before or at the latest at the 28th week of gestation. With the current limited data, cost-benefit studies or number-needed-to-treat analyses of various gestational age cut-offs are difficult to perform.

There were no confirmed cases of ICH occurring intrapartum in this study, and only two bleedings occurred after delivery. This suggests that mode of delivery may not be so important in the prevention of ICH. Whether or not delivery by caesarean section prevents ICH needs to be further addressed.[25]

In chapter 10 we describe the first study that focuses on long-term outcome of children born with ICH due to FNAIT. We found that the risk of death or severe neurodevelopmental impairment in children with ICH due to FNAIT is high (78%). Adverse outcome was due to perinatal mortality in 44% of cases and severe NDI in 6 of 10 survivors. Even in the majority of the remaining cases disabilities were found, such as attention deficit disorders (ADHD) and cerebral palsy grade I. These findings stress the severity and implications of major and permanent life-long handicaps associated with FNAIT.

The pathogenic mechanism of bleeding complications due to FNAIT is not fully understood, although recently published data suggest that impairment of angiogen-

esis rather than thrombocytopenia alone is the critical cause of ICH in FNAIT, possibly explaining the vulnerability of the fetal brain.[26]

The Kaplan group recently did an effort to determine non-invasive predictive factors of ICH in women with anti-HPA-1a FNAIT. Their findings implicate that the presence of certain HLA determinants, as immune response genes (DRB 30101 as a positive and DRB 40101 as a negative factor), might influence anti-HPA 1a production and affinity. A larger cohort is needed to validate these observations.[27]

Overall a lot of questions remain weather some fetuses bleed and others don't in the presence of severe alloimmune thrombocytopenia.

Conclusion and Future perspectives

For many years it has been discussed as to whether routine antenatal screening to identify women at risk of FNAIT should be implemented, but so far no country has embarked on this strategy. Along with other research and the studies described in this thesis, we achieved real progress in the diagnosis, management and outcome of FNAIT. Nowadays the benefits of an FNAIT screening program are generally recognised, but main limitations remain uncertainty about suitable means to identify severely affected fetuses in need of antenatal treatment.

Further studies must focus on improvements in antenatal management and laboratory tests to identify those fetuses at 'true 'risk for bleeding. Until procedures are found that predict which women will have an affected fetus, maternal screening has a low sensitivity, and will result in unnecessary anxiety and "over-treatment".

Only data gathered from prospective large screening studies will enable us to develop a screening platform for FNAIT, including data on the 'true history' of the disease and the opportunity to develop diagnostic assay(s) that enables us to identify fetuses at high risk for severe FNAIT.

In collaboration with Sanquin, our research team has obtained funding for a new research project called :'Towards Routine HPA-screening in Pregnancy to prevent FNAIT: Assessing Disease Burden and Optimising Risk Group Selection' In this observational cohort study the following issues will be studied:

- assessing the incidence of HPA-1a alloantibodies and the incidence of clinically relevant FNAIT in the Netherlands.
- developing a screening platform, including diagnostic assay(s) to identify fetuses at high risk; *The interaction of opsonized platelets with phagocytic cells; Fucosylation level of anti-HPA1a antibody;Interaction of anti-HPA-1a with FcRIIIa.;Effect on the integrity of endothelial layer; Effect on platelet aggregation; C-reactive protein (CRP) concentration in the maternal plasma.*
- To determine the number of pregnant women whose children would benefit from treatment and the number who will receive treatment unnecessarily.
- assessing costs and effectiveness of implementing a nationwide screening program for FNAIT.

Other Remaining questions for clinical research to answer are:

- Is there is also a milder phenotype of ICH with discrete symptoms and better outcome?
- Will IVIG protect the first born child from ICH?
- What is the optimal dose for treatment with IVIG and what it the best time to initiate treatment in pregnancy?
- What is the real working mechanism of IVIG?
- Is there a difference in type of HPA antigen in relation to severity of FNAIT?

In Conclusion

After years of study reflected in this thesis we can conclude that we first have to conduct a general screening program for pregnant women to provide the missing knowledge on essential aspects before implementation of a large-scale screening and intervention trial can be performed.

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CHAPTER 12

SUMMARY/SAMENVATTING

SUMMARY

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by an immunological process in which the mother produces an antibody-mediated response against a platelet-specific antigen (human platelet antigen, HPA) that she herself lacks but that is present on the fetal platelets, inherited from the father. The mother's antibodies [of the immunoglobulin G (IgG) type] can cross the placenta and destroy fetal platelets or inhibit their production. The major risk of FNAIT is severe bleeding, particularly intracranial haemorrhage, which can lead to severe neurological sequelae including mental retardation, cerebral palsy, cortical blindness, seizures or even death.

For several years, fetal blood sampling with intrauterine platelet transfusion was the standard treatment for FNAIT. However, in-utero platelet transfusion as invasive procedure carries a risk of fetal loss. Currently, administration of immunoglobulins (IVIG) to the pregnant mother is mainly offered to women affected by FNAIT. There is no consensus about the dosage of IVIG, varying from 0.5 to 2.0 g/kg per week.

With the current lack of screening programs, the diagnosis of FNAIT is usually only established following the birth of a clinically affected child with signs of bleeding or coincidentally when thrombocytopenia is found with laboratory test for other reasons. As a consequence, antenatal treatment modalities are nowadays only provided for women with a previously affected child.

The aim of the studies described in this thesis was to contribute to the knowledge of FNAIT, in particular to improve management and outcome of pregnancies affected by FNAIT to finally answer the question whether the time has come to implement a screening and intervention program for FNAIT.

In chapter 2 and 3, systematic reviews of the literature on screening studies for FNAIT are given.
In chapter 2, a systematic review of all screening studies on HPA typing, immunisation and perinatal outcome in pregnancies is provided. Chapter 3 illustrates the results of a review of screening studies in neonates to detect thrombocytopenia and estimates the incidence of FNAIT and related ICH. Based on these two systematic reviews of the literature, the expected incidence of FNAIT caused by HPA-1a immunisation is 1 in 366 pregnancies. In one third of these pregnancies, severe thrombocytopenia develops, and of those, 10% of neonates suffer from ICH, or 1 in 11,000 pregnancies. None of the studies reported on the true natural history of the disease. Understandably, the investigators offered interventions to women in whom they detected HPA antibodies, with the aim of reducing the incidence of the true clinical disease, which is fetal or neonatal bleeding. Therefore it seems safe to assume that the incidence of adverse outcome due to FNAIT is an underestimation of the true risk in non-screened populations. However

better estimations are unavailable, only large prospective (non-intervention) screening studies can demonstrate the true numbers of FNAIT and the associated adverse perinatal outcome.

In chapter 4 an extensive overview of FNAIT and the currently used preventive management options are evaluated. There is still controversy on type and timing of various interventions in pregnancies with FNAIT. Most centres have abandoned treatment with serial fetal blood sampling and platelet transfusions, because of a reported high rate of complications and the availability of quite effective non-invasive alternatives. In pregnancies with FNAIT and a previous affected child without ICH, weekly intravenous administration of immunoglobulins to the mother appears close to 100% effective to prevent fetal or neonatal ICH. Some centres add prednisone; this combination leads to slightly higher platelet counts at birth. In pregnant women with a previous child with ICH, the recurrence risk seems particularly high, and more aggressive maternal medical treatment is recommended, including starting earlier with immunoglobulins. Whether a higher intravenous immunoglobulin dose or the addition of prednisone is really necessary is unclear. What does seem generally accepted is that the use of fetal blood sampling should be minimised, possibly even abandoned completely.

In chapter 5 we evaluated the rate and consequences of a late or missed diagnosis of FNAIT by assessing the clinical presentation of first affected children, the timing of diagnosis and the outcomes of subsequent children. In this cohort study of 26 women with FNAIT, delay of diagnosis was identified in four pregnancies (15%). Two of the subsequent pregnancies resulted in children suffering from severe ICH. Several factors were presumed to have caused the low platelet count, i.e. Down syndrome, intrauterine growth restriction and birth trauma, and kept the clinicians from requesting the appropriate investigations.
These illustrative examples of delayed diagnosis show that missing the diagnosis of FNAIT can have devastating consequences for subsequent children, including ICH or perinatal death.

In chapter 6 we characterised pregnancies where the fetus or neonate suffered from ICH. This study, using clinical information together with radiographic imaging and autopsy reports, shows that the majority of ICH bleedings occurred mainly in first pregnancies and by the end of the second trimester. Clinical outcome was devastating for most cases. We concluded that possible interventions to reduce the risk of ICH need to be introduced before or at the latest at the 28th week of gestation and that firstborns with FNAIT can only be treated when identified in a screening program.

In chapter 7 the results of the NOICH trial are reported. This international randomised trial comparing a lower dose of IVIG of 0.5 gram per kg to the standard dose of 1 gram per kg showed no difference in frequency of neonatal ICH, platelet counts at birth, need

for neonatal treatment and levels of cord blood levels of IgG. Unfortunately this trial had to be stopped prematurely due to slow recruitment, resulting in insufficient power to prove equivalence of the lower dose to the standard dose.
After ending of the NOICH trial, we decided, in view of lack of evidence for a particular dose, to continue offering 0 .5 gram IVIG /kg per week to FNAIT women with an affected previous child without ICH. This resulted in a cohort study (chapter 8) where two dosage regimes of IVIG were evaluated; cases were treated with either 0.5 or 1.0 g/kg/wk. There was no difference in platelet count at birth; mean 112 versus 119, crude difference 7 (CI -37.4-23.7) and incidence of severe thrombocytopenia ($<30x10^9$/L); N= 7/46 versus N= 7/63 OR 1.43 (CI 0.46-4.42). Furthermore no ICH occurred. We suggested that a lower dose of IVIG might be as effective as the more standard higher dose of IVIG in preventing severe thrombocytopenia. Further prospective studies are needed to confirm this.

In chapter 9 we describe the overall outcome of the NOICH database consisting of 615 cases affected by FNAIT. Our most important observation was that antenatal treatment for FNAIT results in favourable perinatal outcome. In most centres, over time, treatment for FNAIT changed from an invasive to a complete non-invasive procedure.

In chapter 10 we describe the first study that focuses on long-term outcome of children born with ICH due to FNAIT. We found severe neurodevelopmental impairment in 60% and perinatal mortality in 42% of the children with ICH. Even in the majority of the remaining cases disabilities were found, such as attention deficit disorders (ADHD) and cerebral palsy grade I. These findings stress the severity and implications of major and permanent life-long handicaps associated with FNAIT.

In the general discussion in chapter 11, suggestions for future research are given.
Further studies must focus on improvements in antenatal management and laboratory tests to identify those fetuses at 'true' risk for bleeding. Until procedures are found that predict which women will have an affected fetus, maternal screening has a low sensitivity, and will result in unnecessary anxiety and "over-treatment".
In collaboration with Sanquin, our research team has obtained funding for a new research project called :'Towards Routine HPA-screening in Pregnancy to prevent FNAIT: Assessing Disease Burden and Optimising Risk Group Selection'. Hopefully this observational cohort study, the HIP-study, will teach us more about the true incidence of FNAIT, will lead to a screening platform, including diagnostic assay(s) to identify fetuses at high risk and will assess a costs and effectiveness analysis of a nationwide screening platform.

In Conclusion

After years of study reflected in this thesis we can conclude that we first have to conduct a general screening program for pregnant women to provide the missing knowledge on essential aspects before implementation of a large-scale screening and intervention trial can be performed.

SAMENVATTING

Foetale en neonatale allo-immuun trombocytopenie (FNAIT) wordt veroorzaakt door een immunologisch proces waarbij de moeder een antilichaam-gemedieerde respons produceert tegen een bloedplaatjes-specifiek antigeen (Human Platelet Antigen, HPA) dat ze zelf ontbeert, maar dat aanwezig is op de foetale bloedplaatjes, en geërfd is van de vader. Deze antistoffen [van het immunoglobuline G (IgG) type] kunnen de placenta passeren en foetale bloedplaatjes afbreken of de productie ervan remmen. De meest gevreesde complicatie van FNAIT is het ontstaan van bloedingen, voornamelijk hersenbloedingen met ernstige neurologische gevolgen zoals mentale retardatie, cerebrale parese, corticale blindheid, epileptische aanvallen, of sterfte voor of na de geboorte.

Herhaalde intra-uteriene foetale bloedafname met zo nodig bloedplaatjestransfusies was jarenlang de standaard behandeling voor FNAIT. Echter, deze invasieve procedure kan het verlies van de zwangerschap tot gevolg hebben. Tegenwoordig worden de meeste zwangere vrouwen met FNAIT behandeld met immunoglobulinen (IVIG). Er is tot op heden geen consensus over de dosering van IVIG, die in de wereld varieert van 0,5 tot 2,0 g / kg moederlijk lichaamsgewicht per week.

Tot op heden bestaan er geen routine screening programma's naar FNAIT, waardoor de diagnose vaak pas wordt gesteld na de geboorte van een klinisch aangedaan kind, door zichtbare tekenen van bloeding of wanneer trombocytopenie per toeval wordt ontdekt door laboratoriumtesten om andere redenen. Prenatale behandelingen voor vrouwen met FNAIT zijn daarom uitsluitend voorbehouden aan vrouwen met een eerder aangedaan kind.

Het doel van de studies beschreven in dit proefschrift was het verbeteren van de behandeling en uitkomsten van zwangerschappen gecompliceerd door FNAIT om uiteindelijk te kunnen concluderen of het tijd is voor implementatie van een screening- en interventieprogramma voor FNAIT.

In hoofdstuk 2 en 3 worden systematische reviews van de literatuur over screeningstudies naar FNAIT gegeven.
Hoofdstuk 2 geeft een overzicht weer van alle screeningstudies naar HPA-1a typering en immunisatie in zwangerschappen en hun perinatale uitkomsten. Hoofdstuk 3 illustreert de resultaten van screeningstudies in pasgeborenen naar het opsporen van neonatale trombocytopenie, de incidentie van FNAIT en aanverwante opgetreden hersenbloedingen.
Gebaseerd op deze twee systematische reviews van de literatuur is de verwachte incidentie van FNAIT veroorzaakt door HPA-1a immunisatie 1:366 zwangerschappen. In een derde van deze zwangerschappen, ontwikkelt zich ernstige trombocytopenie (trombocyten aantal < 50×10^9/L) en in 10% van deze zwangerschappen worden kinde-

ren geboren met een hersenbloeding, oftewel 1 op 11.000 zwangerschappen.
Aangezien, begrijpelijkerwijs, in nagenoeg al deze studies interventies werden aangeboden aan screen-positieve patiënten zijn er geen gegevens gerapporteerd over het natuurlijk beloop van FNAIT.
We concludeerden dat screening naar HPA-1a immunisatie ongeveer twee gevallen per 1000 zwangerschappen opspoort. Echter het berekende 10 % risico op perinatale ICH in zwangerschappen met een ernstige FNAIT lijkt een onderschatting, omdat studies zonder interventies ontbraken. Screenen van alle zwangerschappen in combinatie met een effectieve antenatale behandeling, zoals intraveneus immunoglobulines, zou het risico op mortaliteit en morbiditeit geassocieerd met FNAIT kunnen verlagen.

In hoofdstuk 4 wordt een uitgebreid overzicht van FNAIT en de prenatale opties voor het beleid in de zwangerschap gegeven. De pathofysiologie van FNAIT wordt beschreven en de momenteel gebruikte prenatale interventies worden uiteen gezet. Controverse bestaat over de beste interventie om de kans op de meest gevreesde complicatie, een hersenbloeding, te voorkomen. De meeste centra hebben invasieve behandeling met herhaalde foetale bloedafname en bloedplaatjestransfusies verlaten, mede door het complicatierisico en de beschikbaarheid van zeer effectieve niet-invasieve alternatieven. In zwangerschappen gecompliceerd door FNAIT en een eerder aangedaan kind zonder hersenbloeding is wekelijkse intraveneuze toediening van immunoglobulinen aan de moeder bijna 100% effectief gebleken in het voorkomen van een foetale of neonatale hersenbloeding. Sommige centra voegen hier prednison aan toe. Deze combinatie leidt mogelijk tot een iets hoger bloedplaatjes aantal bij de geboorte. Bij zwangere vrouwen met een eerder kind met een hersenbloeding lijkt het recidief risico bijzonder hoog, waardoor meer agressieve medische behandeling wordt aanbevolen, waaronder eerder starten met immunoglobulinen. Of een hogere dosis immunoglobulines of toevoeging van prednison zinnig is blijft onduidelijk. Wat wel kan worden geconcludeerd is dat het gebruik van invasieve methoden moet worden geminimaliseerd, en mogelijk zelfs geheel moet worden verlaten.

In hoofdstuk 5 wordt ingegaan op het aantal en de consequenties van een te late of gemiste diagnose van FNAIT door beoordeling van de klinische presentatie van eerst getroffen kinderen, de timing van de diagnose en de uitkomsten van de daarop volgende kinderen. In deze cohort studie van 26 vrouwen met FNAIT werd een vertraagde diagnose gesteld in vier zwangerschappen (15%). In twee daarop volgende, onbehandelde zwangerschappen werd ICH gediagnostiseerd. Verschillende factoren werden verondersteld het lage aantal bloedplaatjes te hebben veroorzaakt, waaronder het syndroom van Down, dysmaturiteit en geboortetrauma. Dit weerhield de clinici om gericht onderzoek in te zetten naar FNAIT.
Deze voorbeelden van een 'vertraagde' of gemiste diagnose van FNAIT illustreert dat dit verwoestende gevolgen kan hebben voor volgende zwangerschappen, waaronder het optreden van ICH of perinatale sterfte.

In hoofdstuk 6 worden zwangerschappen beschreven waarin de foetus of het pasgeboren kind lijdt aan een hersenbloeding als gevolg van FNAIT. Uit dit onderzoek, op basis van klinische gegevens, beeldvorming en autopsieverslagen, bleek dat het merendeel van de hersenbloedingen optrad in eerste zwangerschappen, tegen het einde van het tweede trimester. De klinische uitkomst was in de meeste gevallen zeer ernstig. We concludeerden dat interventies, bedoeld om het risico op hersenbloedingen te voorkomen, moeten worden gestart voor de 28ste week van de zwangerschap. Bovendien kunnen eerstgeborenen alleen worden behandeld als deze worden opgespoord door middel van een screeningsprogramma.

In hoofdstuk 7 worden de resultaten van NOICH-trial weergegeven. In deze gerandomiseerde studie, waarin een lagere dosering IVIG van 0,5 gram per kg wordt vergeleken met de standaard dosering van 1 gram per kg, werd geen verschil in de frequentie van neonatale ICH, aantal bloedplaatjes bij de geboorte, noodzaak tot neonatale behandeling of waardes van IgG in navelstrengbloed gevonden. Helaas moest deze studie door onvoldoende inclusies voortijdig worden beëindigd, waardoor we door onvoldoende power de gelijkwaardigheid van de lagere dosering tegenover de standaard dosering niet hebben kunnen aantonen.

Na het beëindigen van de NOICH-trial, besloten we, gezien het gebrek aan bewijs voor een bepaalde dosering, om 0,5 gram IVIG per kg per week te blijven aanbieden aan vrouwen met FNAIT met een eerder kind zonder ICH. We voerden een cohort studie uit (hoofdstuk 8) waarbij twee doseringsregimes van IVIG werden geëvalueerd, patiënten werden behandeld met 0,5 of 1,0 gram per kg per week. Er was geen verschil in het aantal bloedplaatjes bij de geboorte; gemiddeld 112 versus 119, (CI -37,4-23,7) en de incidentie van ernstige trombocytopenie ($<30x10^9$ /L); N = 7/46 versus N = 7/63 OR 1,43 (CI 0,46-4,42). Verder werd in geen van de beide groepen ICH gevonden. Wij denken dat een lagere dosis IVIG net zo effectief kan zijn in het voorkomen van ernstige trombocytopenie dan de meer standaard hogere dosis IVIG. Verdere prospectieve studies zijn nodig om dit te bevestigen.

In hoofdstuk 9 beschrijven we de algehele uitkomsten van de NOICH database bestaande uit 615 casussen met FNAIT. De belangrijkste observatie is dat over het algemeen prenatale behandeling van FNAIT resulteert in gunstige perinatale uitkomsten. Verder laat het zien dat in de meeste centra de behandeling van FNAIT in de jaren is veranderd van een invasieve in een volledig niet-invasieve procedure.

In hoofdstuk 10 beschrijven we de eerste studie die zich richt op de lange termijn uitkomsten van kinderen geboren met ICH als gevolg van FNAIT. We vonden ernstige neurologische stoornissen in 60% en perinatale sterfte in 42% van de kinderen met ICH. Zelfs in het merendeel van de overige gevallen werden beperkingen gevonden, zoals attention deficit stoornissen (ADHD) en cerebrale parese (graad I). Deze bevindingen

benadrukken de ernst en de gevolgen van ernstige en levenslange handicaps geassocieerd met FNAIT.

In de algemene discussie in hoofdstuk 11 worden suggesties voor onderzoek gegeven. Toekomstige studies moeten zich vooral richten op verbetering van antenatale behandelingen en ontwikkeling van laboratoriumtesten die foetussen kunnen identificeren die een daadwerkelijk verhoogd risico hebben op een bloeding. Totdat methodes zijn ontwikkeld die nauwkeurig kunnen voorspellen welke vrouwen een aangedane foetus krijgen heeft screening van alle zwangere vrouwen op FNAIT in de huidige praktijk een lage gevoeligheid en leidt dit tot onnodige angst en "over-behandeling".
In samenwerking met Sanquin heeft ons onderzoeksteam financiering voor een nieuw onderzoeksproject verkregen, genaamd :'Towards Routine HPA-screening in Pregnancy to prevent FNAIT: Assessing Disease Burden and Optimising Risk Group Selection' (de HIP-studie)
Hopelijk zal deze observationele cohort studie ons meer informatie geven over de werkelijke incidentie van FNAIT en hersenbloedingen. Een ander doel is de ontwikkeling van een screening platform, met inbegrip van nauwkeurige diagnostische test(s) om foetussen met een hoog risico op bloedingen te kunnen identificeren en zal er tenslotte een kosten en batenanalyse van een landelijke screening programma worden verricht.

Conclusie

Na jaren van studie weergegeven in dit proefschrift kunnen we concluderen dat we eerst een algemene screening naar FNAIT moeten uitvoeren onder zwangere vrouwen om ons te voorzien van de ontbrekende kennis over essentiële aspecten van deze ziekte voordat een grootschalig landelijk screening en interventie programma kan worden opgezet.

ADDENDUM

ABBREVIATIONS

LIST OF PUBLICATIONS

CURRICULUM VITAE

DANKWOORD

ABBREVIATIONS

BSID-III	bayley scales of infant and toddler development, third edition
CI	confidence interval
CP	cerebral palsy
CS	caesarean section
FNAIT	feto and neonatal alloimmune thrombocytopenia
FBS	fetal blood sampling
GA	gestational age
GMFCS	gross motor function classification system
HLA	human leukocyte antigen
HPA	human platelet antigen
ICH	intracranial haemorrhage
ITP	idiopathic thrombocytopenic purpura
IVIG	immunoglobulins
IQR	inter quartile range
IUPT	intra uterine platelet transfusion
LUMC	Leiden university medical centre
MRI	magnetic resonance imaging
NAIT	neonatal allo immune thrombocytopenia
NDI	neurodevelopmental impairment
NOICH	no intracranial haemorrhage
PC	platelet count
PLT	platelet
SD	standard deviation
TOP	termination of pregnancy
WPPSI-III	Wechsler preschool primary scale of intelligence, third edition

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CURRICULUM VITAE

Marije Kamphuis, author of this thesis was born on August 19th 1977 in the 'Heil der Kranken' hospital in Oldenzaal. She grew up in the east of the Netherlands together with her brother Wouter and sister Maaike. She graduated from the Thijcollege, Oldenzaal in 1995. After her graduation she moved to Utrecht to study Psychology. After a year she was admitted to the Erasmus Medical faculty and moved to Rotterdam.

During her study she became interested in Gynaecology and Obstetrics. In 2003 she obtained her medical degree (cum laude) and went to Papua New Guinea, to work with Drs. F. Garsen in a rural hospital situated in Aitape at the west coast.

After this unforgettable experience she returned in 2004 and started to work as a physician, (ANIOS) first at the department of Obstetrics and Gynaecology at the Leyenburg hospital and in 2005 at the Bronovo hospital in the Hague. Here is where her residency started under supervision of dr. C. Holleboom in 2006.

She continued her residency at the LUMC (mentor prof.dr.J.M.M van Lith), where she got the opportunity to join the fetal therapy team to start research on Fetal an neonatal alloimmune thrombocytopenia.

During her studies she was supervised by Prof.Dr.D. Oepkes from the department of Obstetrics and Prof.dr. E. Lopriore from the department of Neonatology. After finishing her residency in 2013 she started a fellowship on Perinatology at the LUMC.

Since September 2016 she is part of the staff of Gynaecology at the Onze Lieve Vrouwe Gasthuis in Amsterdam, with Obstetrics as her main focus.

Marije lives in Amsterdam together with Niels and their children Mik (2010), Goos (2012) and Saar (2015).

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Vrienden
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