Fetal thrombocytopenia : preventive strategies.
Akker, E. van den

Cover illustratie: Käthe Kollwitz, Bettelnde, um 1909, Schwarze Kreide auf gelblichem Ingres-Bütten, NT 522, Käthe Kollwitz Museum Köln - Kreissparkasse Köln © VG Bild-Kunst, Bonn. Foto: Lothar Schnepf

ISBN 978-90-9023078-8

Cover design: Jeroen Bosch, gebaseerd op het proefschrift van Dr. P.A.M. van den Akker, Niet-gehuwd moeder zijn… en dan? Katholieke Hogeschool Tilburg, 1977.

Lay out: Jeroen Bosch
Printed by: Drukkerij Bariet Ruinen

Fetal thrombocytopenia:

Preventive strategies

Eline van den Akker



Fetal thrombocytopenia:

Preventive strategies

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 19 juni 2008
klokke 16.15 uur

door

Eline van den Akker

geboren te Tilburg
in 1972

PROMOTIECOMMISSIE

Promotores:
Prof. Dr. H.H.H. Kanhai
Prof. Dr. A. Brand

Copromotor:
Dr. D. Oepkes

Referent:
Prof. Dr. A. Cameron (Queen Mother's Hospital, Glasgow)

Overige leden:
Prof. Dr. F.J. Walther
Prof. Dr. M. Westgren (Karolinska Institute, Huddinge, Sweden)
Prof. Dr. R.M. Egeler





Aan mijn lieve ouders
Voor Dick en Teun

Table of contents

Chapter 1

General introduction and outline of thesis

GENERAL INTRODUCTION

Intracranial haemorrhage (ICH) among term neonates is associated with neonatal death or lifelong disability[1-3]. Between all the proposed etiological mechanisms, including impairments in coagulation, hypoxic-ischemic injury and birth related trauma, thrombocytopenia seems to be the most important predictor of ICH among term neonates and is also associated with the most severe forms of haemorrhage[4,5].

Normal platelet counts in term neonates are in the same range as those of healthy older children and adults (150-450 x 10^9/L)[6]. Thrombocytopenia is defined as a platelet count < 150 x 10^9/L, although many otherwise healthy newborns may have counts between 100 and 150 x 10^9/L[6,7]. For severe thrombocytopenia, with a risk for bleeding problems, a cut-off level of 50 x 10^9/L is commonly used[4,8,9].

The incidence of thrombocytopenia (< 150 x 10^9/L) in all newborns is 1-4%[10-14]. However, due to absence of clinical signs, it is often not noted. This means that in the Dutch population every year an estimated 2000-8000 thrombocytopenic neonates are born.

In general, the etiology of thrombocytopenia can be classified into disorders associated with increased destruction, including consumption, or decreased production of platelets. In the table, the causes of fetal and early neonatal (<72 h old) thrombocytopenia are summarised. Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of thrombocytopenia, especially in otherwise healthy term newborns.

Most patients at risk for a fetal platelet disorder are identified only after a baby is born with a low platelet count. It is vital to identify the cause of the thrombocytopenia as quickly as possible, primarily to be able to start the correct treatment without delay. In addition establishing the cause of any neonatal thrombocytopenia is essential to institute proper management in the next pregnancy.

The Department of Obstetrics at the Leiden University Medical Centre is the national referral centre for the management of severe alloimmune pregnancy disorders. In 1965 the first intrauterine blood transfusion was performed in Leiden for Rhesus D alloimmunisation.

After the publication of Daffos *et al.* in 1984, fetal blood sampling with intrauterine platelet transfusion became, at least for several years, the standard treatment in FNAIT[15]. Consequently since then FNAIT cases were, in addition to the severe red cell alloimmunisation cases, also referred to the LUMC. Because of this centralisation with a single centre for a referral base of 16 million people, the

Leiden centre is one of the largest referral centres for patients with FNAIT in the world. This provides us with the opportunity, and the obligation, to contribute to scientific research to advance our understanding of this rare disease.

The studies described in this thesis were designed to further improve the outcome of pregnancies complicated by fetal thrombocytopenia.

Table: Causes of fetal and early neonatal (<72 h old) thrombocytopenia

FNAIT
Congenital infections (CMV, Syphilis, PARVO, Toxoplasmosis, Rubella, HIV)
Maternal autoimmune diseases (ITP, SLE)
Severe fetal haemolytic disease by red cell alloimmunisation
Placental insufficiency (pre-eclampsia, IUGR, diabetes)
Asphyxia
Perinatal infections (GBS, E. coli, Listeria)
Disseminated intravascular coagulation (DIC)
Thrombosis (renal vein, aortic)
Congenital syndromes (TAR, Kasabach-Meritt, Amegakaryocytosis, trisomies, triploidy)
Metabolic disorders
Hepatomegaly / splenomegaly

OUTLINE OF THIS THESIS

The aim of the studies described in this thesis was to contribute to improve the outcome of pregnancies complicated by fetal thrombocytopenia, caused by alloimmune thrombocytopenia, red cell alloimmunisation (Rhesus D and Kell) and Parvovirus B19 infection.

In *Chapter 2*, an extensive review of the literature is given on fetal and neonatal alloimmune thrombocytopenia (FNAIT), which is the most common cause of thrombocytopenia in term neonates.

In *Chapter 3*, we describe the outcome of pregnancies with FNAIT treated in our centre, in relation to the invasiveness of the management protocol.

In *Chapter 4*, we report our less invasive treatment strategy in FNAIT in cases at

high risk for intracranial haemorrhage (ICH). After balancing the risk for serious complications from cordocentesis for fetal blood sampling on one hand and ICH on the other, we designed a protocol to further reduce invasive procedures in these patients.

In *Chapter 5*, we report our experience with the safety of vaginal delivery in FNAIT pregnancies without ICH in a previous child.

In *Chapter 6*, the results from the NOICH study are reported. In this randomised trial the hypothesis was tested that intravenous immunoglobulin (IVIG) in a low dose of 0.5 g/kg/wk was at least as effective as the standard dose of 1.0 g/kg/wk in preventing ICH and severe fetal thrombocytopenia in pregnancies at risk for FNAIT.

The calculated sample size was 2 arms of 106 patients. After almost three years of recruitment, a total of only 23 pregnancies had been randomised, which led to the decision by the steering committee to prematurely end the recruitment.

In *Chapter 7* we evaluated the clinical significance of fetal thrombocytopenia in RhesusD alloimmunised pregnancies.

In *Chapter 8* we report that in contrast to hydropic fetuses with Rhesus D haemolytic disease, we found that fetuses with severe anaemia due to Kell alloimmunisation are generally not at risk for substantial thrombocytopenia.

In *Chapter 9* we evaluated the significance of thrombocytopenia in hydropic anaemic fetuses with congenital Parvovirus B19 infection.

In *Chapter 10*, a discussion of the overall results is presented. In a flowchart, the Leiden management protocol of FNAIT is given. Finally, future perspectives and proposals for future research are given.

REFERENCES

1. Fenichel GM, Webster DL, Wong WK. Intra cranial hemorrhage in the term newborn. *Arch Neurol* 1984; 41: 30-34.

2. Selzer SC, Lindgren SD, Blackman JA. Longterm neuropsychological outcome of high risk infants with intracranial hemorrhage. 1992; 17: 407-422.

3. Hanigan WC, Powell FC, Miller TC, Wright RM. Symptomatic intracranial hemorrhage in full-term infants. *Childs Nerv Syst* 1995; 11: 698-707.

4. Jhawar BS, Ranger A, Steven D, et al. Risk factors for intracranial hemorrhage among full-term infants: a case-control study. *Neurosurgery* 2003; 52: 581-590.

5. Bussel JB, Zacharoulis S, Kramer K, McFarland JG, Pauliny J, Kaplan C. Clinical and diagnostic comparison of neonatal alloimmune thrombocytopenia to non-immune cases of thrombocytopenia. *Pediatr Blood Cancer* 2005; 45: 176-183.

6. Sola MC, Del Vecchio A, Rimsza LM. Evaluation and treatment of thrombocytopenia in the neonatal intensive care unit. *Clin Perinatal* 2000; 27: 655.

7. Andrew M, Kelton J. Neonatal thrombocytopenia. *Clin Perinatol* 1984; 11: 359.

8. Segal M, Manning FA, Harman CR, Menticoglou S. Bleeding after intravascular transfusion: experimental and clinical observations. *Am J Obstet Gynecol* 1991; 165: 1414-1418.

9. Hohlfeld P, Forestier F, Kaplan C, Tissot JD, Daffos F. Fetal thrombocytopenia: a retrospective survey of 5,194 fetal blood samplings. *Blood* 1994; 84: 1851-1856.

10. Burrows RF, Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N Engl Med* 1993; 329: 1463–1466.

11. Dreyfus M, Kaplan C, Verdy E, et al. Immune Thrombocytopenia Working Group. Frequency of immune thrombocytopenia in newborns: a prospective study. *Blood* 1997; 89: 4402–4406.

12. Roberts I, Murray NA. Neonatal thrombocytopenia: causes and management. *Arch Dis Child Fetal Neonatal Ed.* 2003; 88: F359-364.

13. Sainio S, Jarvenpaa AL, Renlund M, *et al.* Thrombocytopenia in term infants: a population-based study. *Obstet Gynecol* 2000; 95: 441-446.

14. de Moerloose P, Boehlen F, Extermann P, *et al.* Neonatal thrombocytopenia: incidence and characterization of maternal antiplatelet antibodies by MAIPA assay. *Br J Haematol* 1998; 100: 735–740.

15. Daffos F, Forestier F, Muller JY, Reznikoff-Etievant M, Habbi B, Capella-Pavslovsky M. Prenatal treatment of alloimmune thrombocytopenia. *Lancet* 1984; 2: 632.

Chapter 2

Fetal and neonatal alloimmune thrombocytopenia

Van den Akker ESA, Oepkes D. Fetal and neonatal alloimmune thrombocytopenia. *Best Practice & Research Clinical Obstetrics and Gynaecology* 2008; 22: 3-14.

ABSTRACT

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is one of the major causes of both severe thrombocytopenia and intracranial haemorrhage in fetuses and term neonates. The incidence of FNAIT is estimated to be one in 1000–2000 births. FNAIT is caused by maternal immunoglobulin G alloantibodies, which cross the placenta and are directed against human platelet antigens (HPA) on fetal platelets. In Caucasian individuals, the immunodominant antigen is HPA-1a, which is responsible for approximately 85% of FNAIT cases. The most feared complication of a low platelet count in the fetus or the neonate is intracranial haemorrhage and subsequent neurological handicaps. Over the last 15 years, there has been a gradual change in antenatal treatment, from an invasive management protocol to a less invasive management protocol to a completely non-invasive approach. However, controversy still exists over the optimal antenatal management strategy.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is one of the major causes of both severe thrombocytopenia and intracranial haemorrhage (ICH) in fetuses and term neonates.[1,2] The incidence of thrombocytopenia (<150 x 10^9/L) in all newborns is 1–4%; however, due to the absence of clinical signs, it is often not noted. Thrombocytopenia with an immunological origin is encountered in 0.3% of the newborns.[2–6] FNAIT and idiopathic thrombocytopenic purpura (ITP) are the most important immune-mediated thrombocytopenias. In this chapter we focus on the FNAIT.

The diagnosis is made (rarely) during pregnancy when ICH occurs as a consequence of severe fetal thrombocytopenia, or within the first days after delivery because of neonatal bleeding manifestation or, most often, because of a coincidental finding of neonatal thrombocytopenia. Therefore, testing for this disorder should be performed for any fetus or neonate with an unexplained ICH and for any neonate with unexplained thrombocytopenia, with and without bleeding symptoms, both for proper treatment as for future pregnancies.

FNAIT is caused by maternal immunoglobulin G (IgG) alloantibodies, which cross the placenta and are directed against human platelet antigens (HPA) on fetal platelets. The mechanism is the platelet equivalent of Rhesus disease but, unlike Rhesus disease, it can occur in a severe form in the first pregnancy. As routine screening programs for HPA antibodies is not (yet) done, it invariably occurs unexpectedly. Like Rhesus disease, FNAIT seems to worsen in subsequent pregnancies.[3,7,8]

INCIDENCE, NATURAL HISTORY AND PATHOPHYSIOLOGY

FNAIT occurs in approximately 1: 1500 random fetuses/newborns.[9–15] It is the result of 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 specific HPAs identified so far are all known to be able to cause FNAIT and are shown in Table 1. This table lists also the glycoproteins (GP) on which the antigens are located, the position of the genetic single nucleotide polymorphism and the amino acid change.[16]

The immunodominant antigen in Caucasian individuals is the HPA-1a, which is responsible for approximately 85% of FNAIT cases.[17,18] Two percent of pregnant

Caucasian women are HPA-1a negative.[11,13,19,20] The proportion of individuals belonging to a particular platelet antigen type varies according to the race involved. Some of these differences in frequencies of HPA alloantigens in different populations are shown in Table 2.[21–26]

Untreated newborns with FNAIT are reported to be affected by ICH in 7–26% of cases.[17,18,27–33] There is surprisingly little information about both the pathophysiology and natural history of FNAIT. FNAIT is considered the platelet equivalent of red cell alloimmunisation or haemolytic disease of the newborn. However, in contrast to red cell alloimmunisation, FNAIT occurs in the first pregnancy in over 50% of cases.[17]

Table 1: Human Platelet Antigens[16]

System	Antigen	Original Names	Glycoprotein	Nucleotide change	Amino acid change	CD
HPA-1	HPA-1a	Zwa, PlA1	GPIIIa	T176	Leu33	CD61
	HPA-1b	Zwb, PlA2		C176	Pro33	
HPA-2	HPA-2a	Kob	GPIbα	C482	Thr145	CD42b
	HPA-2b	Koa, Siba		T482	Met145	
HPA-3	HPA-3a	Baka, Leka	GPIIb	T2621	Ile843	CD41
	HPA-3b	Bakb		G2621	Ser843	
HPA-4	HPA-4a	Yukb, Pena	GPIIIa	G506	Arg143	CD61
	HPA-4b	Yuka, Penb		A506	Gln143	
HPA-5	HPA-5a	Brb, Zavb	GPIa	G1600	Glu505	CD49b
	HPA-5b	Bra, Zava, Hca		A1600	Lys505	
	HPA-6bw	Caa, Tua	GPIIIa	1544G>A	Gln489Arg	CD61
	HPA-7bw	Moa	GPIIIa	1297C>G	Ala407Pro	CD61
	HPA-8bw	Sra	GPIIIa	1984C>T	Cys636Arg	CD61
	HPA-9bw	Maxa	GPIIb	2602G>A	Met837Val	CD41
	HPA-10bw	Laa	GPIIIa	263G>A	Gln62Arg	CD61
	HPA-11bw	Groa	GPIIIa	1976G>A	His633Arg	CD61
	HPA-12bw	Iya	GPIbα	119G>A	Glu15Gly	CD42c
	HPA-13bw	Sita	GPIa	2483C>T	Met799Thr	CD49b
	HPA-14bw	Oea	GPIIIa	1909_1911 Del AAG	Del Lys611	CD61
HPA-15	HPA-15a	Govb	CD109	C2108	Ser703	CD109
	HPA-15b	Gova		A2108	Tyr703	
	HPA-16bw	Duva	GPIIIa	497C>T	Thr140Ile	CD61

HPA antigens are already expressed on fetal platelets in the first trimester. Once the mother has produced HPA antibodies, these specific IgG antibodies are able to cross the placenta and cause platelet destruction in the fetus. Unfortunately, there is a lack of a reliable, clinically useful correlation between the maternal antibody levels and the severity of FNAIT[34–36], although some studies showed a higher risk of severe FNAIT with high antibody levels.[37,38]

Although thrombocytopenia is commonly defined as a platelet count below 150 x 10^9/L, clinical symptoms are only likely to occur when the platelet count drops to below 50 x 10^9/L.[39] The most feared complication of a low platelet count in the fetus or the neonate is ICH, with its subsequent neurological handicaps. In a literature review by Spencer and Burrows, ICH was reported to occur in 74/281 (26%) of cases of FNAIT.[18] Mortality related to ICH is estimated to occur in 7% of cases.[18,31] In a study by Bussel *et al.* an incidence of ICH of 11% was found in a series of 110 cases of FNAIT.[1]

Table 2: Human platelet alloantigen frequencies [21-26]

Antigens	Percentage frequency						
	Caucasian	Japanese	Korean	African-American	Indian	Indonesian	Han Chinese
HPA-1a	97.9	>99.9	99.5	99.9	99.9	>99.4	>99.9
HPA-1b	28.6	3.7	2.0	16.0	n.t.	n.t.	1.2
HPA-2a	>99.9	n.t.	99.0	97.0	n.t.	n.t.	99.9
HPA-2b	13.2	25.4	14.0	33.0	n.t.	n.t.	9.6
HPA-3a	80.9	78.9	82.5	85.0	89.3	72.9	83.1
HPA-3b	69.8	70.7	71.5	60.0	n.t.	80.7	64.2
HPA-4a	>99.9	99.9	>99.9	100.0	99.9	>99.4	>99.9
HPA-4b	0.0	1.7	2.0	0.0	0.9	0.6	0.9
HPA-5a	99.0	n.t.	>99.9	96.0	n.t.	>99.4	99.9
HPA-5b	19.7	n.t.	4.5	38.0	4.9	9.3	2.7

n.t. not tested

ANALYSIS IN THE NEXT PREGNANCY

Maternal–fetal HPA incompatibility has to be confirmed for all patients by paternal HPA typing. In cases where the father is homozygous for the specific HPA antigen, one can assume that the fetus is at risk. In cases where the father is heterozygous for the HPA antigen, amniocentesis is currently used for fetal HPA typing. Methods are being developed to assess the fetal HPA-type using free fetal DNA in maternal plasma. Unfortunately, quantifying and serial monitoring anti-HPA antibodies does not accurately predict the severity of fetal thrombocytopenia.[34,35,38]

Therefore, all pregnancies in which the mother carries HPA antibodies and the fetus is positive for the corresponding HPA antigen must be regarded as at risk for low fetal and neonatal platelet counts and bleeding complications. The only distinction made in the at-risk group is based on whether the previous affected child had asymptomatic low platelet counts or suffered from actual bleeding problems especially ICH. The latter group is regarded as a higher-risk group although, as stated before, very little is known about the natural history.

ANTENATAL TREATMENT

As most countries do not have a screening program, women are identified as at risk only after a previous child with FNAIT. The goal of antenatal treatment is to prevent severe thrombocytopenia and the concomitant risk for ICH and its sequelae, including death (which can occur either antenatally or after birth) or severe disability. Several treatment options are available, depending on the severity of the illness of the previous sibling.

Before 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.

Antenatal treatment: fetal blood sampling and intrauterine platelet transfusion

In 1984, Daffos *et al.* published the successful use of fetal blood sampling (FBS) in obtaining fetal platelet count at 34 weeks, followed by an intrauterine platelet transfusion (IUPT) at 37 weeks, followed by a caesarean section.[40] Since then, FBS with and without IUPT became standard treatment, in different regimes: from a weekly to only a predelivery one. However, although this seemed to be a

method to keep platelet counts at a safe level, it became more and more clear that this was a hazardous procedure, especially for fetuses with thrombocytopenia. Based on a review of the literature, the complication rate of FBS and IUPT in FNAIT pregnancies was calculated as 1.6% fetal loss and 2.4% other complications.[41] Data from three recent studies combined showed a 6% fetal loss rate directly related to FBS.[42–44]

Antenatal treatment: maternal treatment

Driven by the risks of invasive treatment in FNAIT, maternal treatment was explored. In 1984, Daffos *et al.* reported the successful use of corticosteroids[40], but in later publications they found that this treatment did not raise fetal platelet count.[31]

Bussel *et al.*[29] were the first to report the effects of maternal administration of intravenous gammaglobulin (IVIG) in the treatment of FNAIT. In all seven cases reported, the fetal platelet count increased substantially after treatment with IVIG 1.0 g/kg/week. Many centres since have adopted this policy. Later studies found that not all fetuses show a substantial increase in platelet count with this treatment. The reported response rate in the literature varies between 30% and 85% (unpublished data). In addition, observational studies have suggested that IVIG reduced the risk of ICH even in non-responders to IVIG.[32,45,46] One randomised, placebo-controlled trial was published in 1996 by Bussel *et al.*, in which no effect of adding dexamethasone to the administered IVIG was observed.[32]

The mechanism of action of IVIG in FNAIT is still unclear. Three possible explanations are cited in the literature. First, in the maternal circulation the IVIG will dilute the anti-HPA antibodies, resulting in a lower proportion anti-HPA antibodies among the IgG transferred via the Fc-receptors in the placenta. Second, in the placenta, IVIG can block the placenta receptor (Fc-R) and decrease the placental transmission of maternal antibodies including anti-HPA-antibodies. Third, in the fetus, IVIG may block the Fc-receptors on the macrophages and prohibit the destruction of antibody-covered cells.[47] We found evidence for the first mechanism.[48] However, other effects of IVIG, such as anti-idiotypic neutralisation of anti-HPA antibodies or suppression of antibody producing B cells, cannot be excluded.

The long-term side-effects for mother and child are still unclear. A recent 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.[48]

As IVIG is known for its immunomodulating characteristics, there are concerns about long-time side effects for the mother and child.

IVIG is widely used in other diseases, such as in prophylaxis and therapy of complications after stem-cell transplantation[49], autoimmune thrombocytopenic purpura (ITP)[50] and dermatological and neurological diseases. The dose of 1.0 g/kg/week has been commonly used since Bussel *et al.*'s first publication.[29] In FNAIT, no lower doses of IVIG are published and no dose–effect studies have yet been done. Results of a recent study suggest that placental antibody transfer is not further increased despite high IgG concentrations in the mother as a result of IVIG treatment.[47] In other immune platelet disorders, the optimal dose of IVIG is also still unclear. For example, in treating ITP, an effective dose of IVIG appears to be between 0.5 and 1.0 g/kg per day, commonly for five days.[50] If no response is observed, increased doses are suggested to a maximum of 2.0 g/kg per day.

The results of a recent study suggest that placental antibody transfer is not further increased despite high IgG concentrations in the mother as a result from IVIG treatment. This suggests a limitation of the placental Fc-receptor.[47] When maternal titres of anti-HPA antibodies are low, a lower dose of IVIG might be sufficient to reduce transmission of pathogenic HPA-antibodies leading to thrombocytopenia.

Based on the lack of rationale for the dose of 1 g/kg/week, the cost of IVIG and the long-term effects of IVIG on the infants are unknown, an international multicentre study is currently being performed. This study compares the preventive effect of IVIG 0.5 and 1.0 g/kg/week on FNAIT and ICH in patients with FNAIT and a low risk for ICH. More information can be obtained from the website for the study (www.noich.org).

Antenatal treatment: present situation

Over the last 15 years, there has been a gradual change in antenatal treatment, from an invasive management protocol to a less invasive management protocol to a completely non-invasive approach. However, there is still controversy over the optimal antenatal treatment, especially the safety of the completely non-invasive policy.

The recent study by Berkowitz *et al.*[51] states that FBS still has a place in treatment with or without platelet transfusion therapy. Van den Akker *et al.* have published their treatment experience over the last 16 years, in which period the transition occurred from an invasive strategy, via a minimally invasive to an ultimately completely non-invasive strategy. The completely non-invasive approach

resulted in an excellent outcome for all 49 non-invasively treated patients, without any loss or complications of FBS.[52] The non-invasive strategy was supported when these results were compared with two recently published series in which more invasive management protocols were used.

Birchall *et al.* reported on an observational study from 12 European centres, with a total of 50 women with 55 pregnancies and 56 fetuses, all with HPA-1a alloimmunisation treated between 1988 and 2001.[43] Multiple management options were described, all after an initial FBS. ICH occurred in 5% of the children (3/56). FBS-related adverse outcomes occurred in 18% of the fetuses (10/56), with two fetal losses and eight deliveries before 34 weeks' gestation. In addition, in 9% (5/56) an emergency delivery after 34 weeks' gestation had to be performed following FBS. Maternal treatment with IVIG was used in 18 patients, combined with one or more FBSs. Four of these 18 neonates were born before 34 weeks, one fetal loss occurred and one emergency caesarean section was performed, both associated with FBS. The mean platelet count at birth in this group was 80 x 10^9/L with six of the 18 neonates having a platelet count < 50 x 10^9/L. None of their cases were treated completely non-invasively.

Berkowitz *et al.* performed a randomised, multicentre study stratifying 79 pregnancies in a high-risk and a low-risk arm.[44] All women underwent initial FBS at 20 weeks' gestation. High risk cases (n = 40) were defined as either a sibling with peripartum ICH or an initial platelet count < 20 x 10^9/L. Randomisation was between IVIG and prednisone, or IVIG only. Low-risk cases were randomly assigned to IVIG or prednisone. A second FBS was used to adapt the medication dose in non-responders. Platelet transfusions were given in an unknown number of cases. ICH occurred in three cases (4%). Emergency deliveries related to FBS were required in 13% (10/79) of the pregnancies and 24% (19/79) of the neonates were born before 34 weeks. One fetus died due to a complication of FBS; two pregnancies ended in unexplained fetal demise. A total of 175 FBSs were done, with serious complications occurring in 6%. Mean platelet count at birth in the high-risk group was 99 x 10^9/L in the IVIG group and 69 x 10^9/L in the IVIG combined with steroids group. In the low-risk group, 15% (6/39) of fetuses had a platelet count < 50 x 10^9/L.

In Table 3, the LUMC cases (only the non-invasive treated cases) are compared to the IVIG treated cases described by Birchall and those described by Berkowitz. Van den Akker *et al.* concluded that the considerable number of complications and adverse outcomes associated with FBS described by Birchall and Berkowitz could be acceptable if the invasive management would result in a better overall

Table 3: Outcomes of antenatal treatment of FNAIT in three different studies.

	LUMC[53,a]		**Birchall *et al.***[43b]		**Berkowitz *et al.***[44]			
					high risk [d]		standard risk [e]	
Sibling	ICH	no ICH	ICH	no ICH	7x ICH, 33x no ICH		no ICH	
Treatment	IVIG	IVIG	IVIG	IVIG	IVIG	IVIG+steroids	IVIG	Steroids
	n=5	*n*=48[c]	*n*=6	*n*=12	*n*=21	*n*=19	n=19	n=20
Mean platelet count sibling	20.7(1 na)	15	7 (5 na)	37.9	28.4	14.4	23	25.7
ICH in sibling	5	0	6	0	4	3	0	0
Mean GA at first treatment	16	32	25	28	24	25	24	25
Delivery mode: vaginal delivery	0/5	31/48 (65%)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Mean platelet count at birth	55.4	137	78.3	82.7	99.4	68.9	n.a.	n.a.
Platelet count ≤ 50 x 10^9/l	4/5 (80%)	6/48 (13%)	2/6 (33%)	4/12 (33%)	n.a.	n.a.	6/39 (15%)	
ICH	0	0	1/6 (17%)[g]	0	1	0	2/39 (5%)[f]	
Unexplained fetal demise	0	0	0	0	0	0	1	1
Loss rate due to FBS	0	0	1/6 (17%)[g]	0/12	1/79 (1.3%)			
Emergency delivery due to FBS	0	0	1/6 (17%)[g]	2/12 (17%)	10/79 (13%)			
Delivery before 34 weeks	0	0	4/6 (67%)	0	19/79 (24%)			
Neonatal survival	100%		17/18 (94%)		76/79 (96%)			

[a] subgroup from total studygroup, only noninvasive treated cases

[b] subgroup from total studygroup, only IVIG treated cases

[c] 48 neonates, resulting from 47 pregnancies

[d] women in the high risk arm: either a previous child with a peripartum ICH and/or an initial platelet count < 20 x 10^9/l

[e] women in the standard risk: prior child without ICH and initial platelet counts > 20 x 109/l

[f] possibly not related to FNAIT

[g] Emergency CS at 24+2 weeks due to premature labour caused by infection introduced by cordocentesis

FBS, fetal blood sampling; GA, gestational age; ICH, intracerebral haemorrhage; IVIG, intravenous gammaglobulin; LUMC, Leiden University medical centre; n.a., not available;

outcome when compared with a completely non-invasive approach. But there seems to be no advantage to the use of FBSs in the management of pregnancies complicated by FNAIT. Adherence to the principle of *primum non nocere* means, in our view, that potentially hazardous diagnostic procedures should be employed only when proven to do more good than harm.[52]

After cost-effectiveness analysis by Thung *et al.*, which compared non-invasive empiric intravenous immunoglobulin with FBS-based treatment, non-invasive IVIG was found to be a cost-effective strategy when the rate of perinatal ICH is less than 28%.[53]

DELIVERY

Caesarean section is often routinely employed for delivery in pregnancies with FNAIT. Practice guidelines advise vaginal delivery as an option in case of a platelet count > 50 x 10^9/L established by FBS with or without an IUPT.[8,32,54] Spencer and Burrows estimated that the bleeding occurs (long) before labour in 80% of neonates with ICH.[18] As we estimate the ICH risk to be 7% in a subsequent pregnancy after a previous child with thrombocytopenia but without ICH, this implies that the chance of developing ICH during labour or postpartum is approximately 1.4% in this group. Van den Akker *et al.* did an evaluation on the safety of vaginal delivery in pregnancies with FNAIT by studying 32 pregnancies with FNAIT with a sibling with thrombocytopenia but without an ICH. They found that vaginal delivery was not associated with neonatal intracranial bleeding.[55]

SCREENING

The pros and cons for FNAIT screening have been discussed for several years.[10,11,15,19,38,56] Williamson *et al.* showed that 1 in 450 random pregnant women produce HPA-1a antibodies.[11] Based on literature, the incidence of new cases of FNAIT is 1: 1200. Severe FNAIT (<50 x 10^9 platelets/L) is seen in 1:1700 random newborns resulting in neonatal ICH in 1: 37,000.[9–15] Durand-Zaleski *et al.* compared the costs and clinical outcomes of screening primiparous women with screening all neonates. They found that neonatal screening was the more cost-effective approach.[10] There is no clear approach to antenatal therapy for the first affected pregnancy with FNAIT.[19] However, Davoren *et al.* argue that antenatal screening can identify those fetuses at risk for FNAIT and, even if the optimal antenatal management has not yet been established, high-risk pregnancies can be

identified and at least early postnatal treatment can be started.[13]

As well as screening for HPA antibodies in pregnancy, female relatives of affected women could be tested for their HPAstatus and, if found to be negative for the sameHPAtype, serial antibody screening during their pregnancies could be done. In addition, these sisters could be tested for HLA-DRw52a. If the affected patient is HLA-DRw52a positive and the female relative is HLA-DRw52a negative, the chance that FNAITwill occur is very low, even if there is a parental antigen mismatch with the relative and her partner.[57,58]

THE FUTURE

At present, the optimal treatment strategy for pregnancies complicated by FNAIT is still not clear. We hope that it will be possible to abandon the invasive procedures with their inherent risks in the future. As in Rhesus alloimmunisation, in which the diagnosis of fetal anaemia relied for many years on invasive testing and reliable non-invasive tests only recently became available, it would be a great advantage if fetal platelet counts could be measured non-invasively. A development expected soon is reliable assessment of the fetal HPA status using free fetal DNA in maternal plasma instead of amniocentesis. Improved laboratory methods might show a more useful predictive value of antibody levels or antibody function. The use of IVIG seems a relatively 'crude' method to influence immunological processes, and more specific treatment might become available. Again, using the comparison with Rhesus disease, a prophylactic drug similar to anti-D might even be developed.

SUMMARY

FNAIT is one of the major causes of both severe thrombocytopenia and ICH in fetuses and term neonates. The incidence of FNAIT is estimated to be one in 1000–2000 births. Testing for this disorder should be performed on any fetus or neonate with an unexplained ICH and any neonate with unexplained thrombocytopenia, with and without bleeding symptoms.

FNAIT is caused by maternal IgG alloantibodies against HPA on fetal platelets; these alloantibodies cross the placenta. In Caucasians, the immunodominant antigen is the HPA-1a, which is responsible for approximately 85% of FNAIT cases.

The most feared complication of a low platelet count in the fetus or the neo-

nate is ICH and subsequent neurological handicaps.

Over the last 15 years there has been a gradual change in antenatal treatment, from an invasive management protocol to a less invasive management protocol to a completely non-invasive approach. However, there is still controversy over the optimal medical treatment regimen and the role of diagnostic invasive procedures in the management of FNAIT.

PRACTICE POINTS

- The incidence of FNAIT is estimated to be one in 1000–2000 births.
- Testing for FNAIT should be performed for any fetus or neonate with an unexplained ICH and for any neonate with unexplained thrombocytopenia, with and without bleeding symptoms.
- In Caucasians, the immunodominant antigen is the HPA-1a antigen, responsible for approximately 85% of FNAIT cases.
- Pregnancies complicated by FNAIT are best treated with weekly intravenous immunoglobulin. There is no evidence that FBS improves outcome.

RESEARCH AGENDA

- Optimal treatment strategy is not clear.
- Non-invasive management seems to be safe but larger series are needed.
- The mechanisms of action of IVIG in FNAIT need to be elucidated.
- The optimal dose of IVIG is unclear, a multicentre trial (the NOICH study) is ongoing.
- Routine screening of the HPA status of pregnant women needs to be evaluated prospectively for cost-benefit assessment.
- A non-invasive method to predict fetal thrombocytopenia would greatly benefit the management.

REFERENCES

1. Bussel JB, Zacharoulis S, Kramer K et al. Clinical and diagnostic comparison of neonatal alloimmune thrombocytopenia to non-immune cases of thrombocytopenia. *Pediatr Blood Cancer* 2005; 45(2): 176–183.

2. Dreyfus M, Kaplan C, Verdy E et al. Frequency of immune thrombocytopenia in newborns: a prospective study. *Blood* 1997; 89(12): 4402–4406.

3. Burrows RF & Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N Engl J Med* 1993; 329(20): 1463–1466.

4. Roberts I & Murray NA. Neonatal thrombocytopenia: causes and management. *Arch Dis Child Fetal Neonatal Ed* 2003; 88(5): F359–F364.

5. Sainio S, Jarvenpaa AL, Renlund M *et al.* Thrombocytopenia in term infants: a population-based study. *Obstet Gynecol* 2000; 95(3): 441–446.

6. de Moerloose P, Boehlen F, Extermann P et al. Neonatal thrombocytopenia: incidence and characterization of maternal antiplatelet antibodies by MAIPA assay. *Br J Haematol* 1998; 100: 735–740.

7. Bussel JB, Zabusky MR, Berkowitz RL *et al.* Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997; 337: 22–24.

8. Kaplan C, Murphy MF, Kroll H *et al.* Feto-maternal alloimmune thrombocytopenia: antenatal therapy with IvIgG and steroids–more questions than answers. European Working Group on FMAIT. *Br J Haematol* 1998; 100: 62–65.

9. Blanchette VS, Chen L, de Friedberg ZS *et al.* Alloimmunization to the PlA1 platelet antigen: results of a prospective study. *Br J Haematol* 1990; 74(2): 209–215.

10. Durand-Zaleski I, Schlegel N, Blum-Boisgard C *et al.* Screening primiparous women and newborns for fetal/neonatal alloimmune thrombocytopenia: a prospective comparison of effectiveness and costs. *Am J Perinatol* 1996; 13(7): 423–431.

11. Williamson IM, Hackett GA, Rennie JM *et al.* The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PLA1, Zwa) as determined by antenatal screening. *Blood* 1995; 92: 2280–2287.

12. Maslanka K, Guz K & Zupanska B. Antenatal screening of unselected pregnant women for HPA-1a antigen, antibody and alloimmune hrombocytopenia. *Vox Sang* 2003; 85(4): 326–327.

13. Davoren A, McParland P, Crowley J *et al.* Antenatal screening for human platelet antigen-1a: results of a prospective study at a large maternity hospital in Ireland. *BJOG* 2003; 110(5): 492–496.

14. Kjeldsen-Kraghl J, Killie MK, Aune B, *et al.* An intervention program for reducing morbidity and mortality associated with neonatal alloimmune thrombocytopenic purpura. 8th European Symposium on platelet and Granulocyte Immunobiology; 2004, Rust.

15. Turner ML, Bessos H, Fagge T *et al.* Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. *Transfusion* 2005; 45(12): 1945–1956.

16. Metcalfe P, Watkins NA, Ouwehand WH *et al.* Nomenclature of human platelet antigens. *Vox Sang* 2003; 85(3): 240–245.

17. Mueller-Eckhardt C, Kiefel V, Grubert A *et al.* 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989; 1: 363–366.

18. Spencer JA & Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust N Z J Obstet Gynaecol* 2001; 41: 45–55.

19. Murphy MF, Williamson LM & Urbaniak SJ. Antenatal screening for fetomaternal alloimmune thrombocytopenia: should we be doing it? *Vox Sang* 2002; 83(Suppl. 1): 409–416.

20. Mueller-Eckhardt C, Mueller-Eckhardt G, Willen Ohff H *et al.* Immunogenicity of an immune response to human platelet antigen Zw (a) is strongly associated with HLA-B8 and DR3. *Tissue Antigens* 1985; 26: 71–76.

21. Williamson LM, Lubenko A, Hadfield R *et al.* Large-scale genotyping of platelet donors for the plateletspecific alloantigens HPA-1, 2 and 3. *Br J Haematol* 1993; 84: 7.

22. Kim HO, Jin Y, Kickler TS *et al.* Gene frequencies of the five major human platelet antigens in African American, white, and Korean populations. *Transfusion* 1995; 35: 863–883.

23. Urwijitaroon Y, Barusrux S, Romphruk A *et al.* Frequency of human platelet antigens among blood donors in northeastern Thailand. *Transfusion* 1995; 35: 868–870.

24. Simsek S, Faber NM, Bleeker PM *et al.* Determination of human platelet antigen frequencies in the Dutch population by immunophenotyping and DNA (allele-specific restriction enzyme) analysis. *Blood* 1993; 81: 835–840.

25. Feng ML, Liu DZ, Shen W *et al.* establishment of an HPA-1- to -16-typed platelet donor registry in China. *Transfus Med* 2006; 16(5): 369–374.

26. Seo DH, Park SS, Kim DW *et al.* Gene frequencies of eight human platelet-specific antigens in Koreans. *Transfus Med* 1998; 8(2): 129–132.

27. Kuijpers RWAM. Neonatal alloimmune thrombocytopenia: relation between serological parameters and clinical picture. Thesis: Maastricht University, 1993.

28. Herman JH, Jumbelic MI & Ancona RJ. In utero cerebral hemorrhage in alloimmune thrombocytopenia. *Am J Pedaitr Hemotol Oncol* 1986; 8: 312–317.

29. Bussel JB, Richard MD, Berkowitz L *et al.* Antenatal treatment of neonatal alloimmune thrombocytopenia. *N Engl J Med* 1988; 319: 1374–1378.

30. Muller JY, Reznikoff-Etievant MF, Patereau C *et al.* Thrombopénies néo-natales allo-immunes étude clinique et biologique de 84 cas. *La Presse Médicale* 1985; 14: 83–86.

31. Kaplan C, Morel-Kopp MC, Kroll H *et al.* HPA-5b (Bra) neonatal alloimmune throbocytopenia: clinical and immunological analysis of 39 cases. *Br J Haematol* 1991; 78: 425–429.

32. Bussel JB, Berkowitz RL, Lynch L *et al.* Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: a randomised trial of the addition of low-dose steroid to intravenous gamma-globulin. *Am J Obstet Gynecol* 1996; 174: 1414–1423.

33. Sharif U & Kuban K. Prenatal intracranial hemorrhage and neurologic complications in alloimmune thrombocytopenia. *J Child Neurol* 2001; 16(11): 838–842.

34. Kaplan C, Daffos F, Forestier F *et al.* Management of alloimmune thrombocytopenia: antenatal diagnosis

and in utero transfusion of maternal platelets. *Blood* 1988; 72: 340–343.

35. Proulx C, Filion MM, Goldman M *et al.* Analysis of immunoglobulin class IgG subclass and titre of HPA-1a antibodies in alloimmunised mothers giving birth to babies with or without neonatal alloimmunethrombocytopenia. *Br J Hematol* 1994; 87: 813–817.

36. Metcalfe P, Allen D, Chapman J *et al.* Interlaboratory variation in the detection of clinically significant alloantibodies against human platelet alloantigens. *Br J Hematol* 1997; 97(1): 204–207.

37. Jaegtvik S, Husebekk A, Aune B *et al.* Neonatal alloimmune thrombocytopenia due to anti-HPA 1a antibodies; the level of maternal antibodies predicts the severity of thrombocytopenia in the newborn. *BJOG* 2000; 107(5): 691–694.

38. Bertrand C, Martageix C, Jallu V *et al.* Predictive value of sequential anti-HPA-1a antibody concentrations for the severity of fetal alloimmune thrombocytopenia. *J Thromb Haemost* 2006; 4(3): 628–637.

39. Jhawar BS, Ranger A, Steven D *et al.* Risk factors for intracranial hemorrhage among full-term infants: a case-control study. *Neurosurgery* 2003; 52(3): 581–590.

40. Daffos F, Forestier F, Muller JY *et al.* Prenatal treatment of alloimmune thrombocytopenia. *Lancet* 1984; 2: 632.

41. Radder CM, Brand A & Kanhai HHH. Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? *Vox Sang* 2003; 84: 318–325.

42. Overton TG, Duncan KR, Jolly M *et al.* Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. *Am J Obstet Gynecol* 2002; 186: 826–831.

43. Birchall JE, Murphy MF, Kaplan C *et al.* European collaborative study of the antenatal management of fetomaternal alloimmune thrombocytopenia. *Br J Haematol* 2003; 122: 275–288.

44. Berkowitz RL, Kolb EA, McFarland JG *et al.* Parallel randomized trials of risk-based therapy for fetal alloimmune thrombocytopenia. *Obstet Gynecol* 2006; 107: 91–96.

45. Wenstrom KD, Weiner CP & Williamson RA. Antenatal treatment of fetal alloimmune thrombocytopenia. *Obstet Gynecol* 1992; 80: 433–435.

46. Radder CM, Brand A & Kanhai HHH. A less invasive treatment strategy to prevent intracranial hemorrhage in fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2001; 185: 683–688.

47. Radder CM, Kanhai HH & Brand A. On the mechanism of high dose maternal intravenous immunoglobulin (IVIG) in alloimmune thrombocytopenia. In: *Management of fetal alloimmune thrombocytopenia*. Amsterdam: Print Partners Ipskamp, 2004, pp. 69–81.

48. Radder CM, de Haan MJJ, Brand A *et al.* Follow up of children after antenatal treatment for alloimmunethrombocytopenia. Early Hum Dev 2004; 80: 65–76.

49. Cordonnier C, Chevret S, Legrand M *et al.* Should immunoglobulin therapy be used in allogeneic stemcell transplantation? A randomized, double-blind, dose effect, placebo-controlled, multicenter trial. *Ann Intern Med* 2003; 139: 8–18.

50. Godeau B, Caulier MT, Decuypere L *et al.* Intravenous immunoglobulin for adults with autoimmune thrombocytopenic purpura: results of a randomized trial comparing 0.5 and 1 g/kg b.w. *Br J Haematol* 1999; 107: 716–719.

51. Berkowitz RL, Bussel JB & McFarland JG. Alloimmune thrombocytopenia: state of the art 2006. *Am J Obstet Gynecol* 2006; 195: 907–913.

52. Van den Akker ESA, Oepkes D, Lopriore E *et al.* Noninvasive antenatal management of fetal and neonatal alloimmune thrombocytopenia: safe and effective. *BJOG* 2007; 114: 469-473.

53. Thung SF & GrobmanWA. The cost effectiveness of empiric intravenous immunoglobulin for the antepartum treatment of fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2005; 193(3 Pt 2): 1094–1099.

54. Reznikoff-Etievant MF. Management of alloimmune neonatal and antenatal thrombocytopenia. *Vox Sang* 1988; 55: 193–201.

55. Van den Akker ESA, Oepkes D, Brand A *et al.* Vaginal delivery for fetuses at risk of alloimmune thrombocytopenia? *BJOG* 2006; 113: 781–783.

56. Killie MK, Husebekk A, Kaplan C *et al.* Maternal human platelet antigen-1a antibody level correlates with the platelet count in the newborns: a retrospective study. *Transfusion* 2007; 47(1): 55–58.

57. Skupski DW & Bussel JB. Alloimmune thrombocytopenia. *Clin Obstet Gynecol* 1999; 42: 335–348.

58. Kaplan C. Alloimmune thrombocytopenia of the fetus and the newborn. *Blood Rev* 2002; 16: 69–72.

Chapter 3

Noninvasive antenatal management of FNAIT: safe and effective

Van den Akker ESA, Oepkes D, Lopriore E, Brand A, Kanhai HHH. Noninvasive antenatal management of fetal and neonatal alloimmune thrombocytopenia: safe and effective *British Journal of Obstetrics and Gynaecology* 2007; 114: 469-473.

ABSTRACT

Objective To describe the outcome of pregnancies with fetal and neonatal alloimmune thrombocytopenia (FNAIT) in relation to the invasiveness of the management protocol.

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

Setting Leiden University Medical Centre, the national centre for management of severe red cell and platelet alloimmunisation in pregnancy.

Population Ninety-eight pregnancies in 85 women with FNAIT having a previous child with thrombocytopenia with (n = 16) or without (n = 82) an intracranial haemorrhage (ICH).

Methods Our management protocol evolved over time from (1) serial fetal blood samplings (FBS) and platelet transfusion (n = 13) via (2) combined FBS with maternal intravenous immunoglobulins (n = 33) to (3) completely noninvasive treatment with immunoglobulins only (n = 52 pregnancies, resulting in 53 neonates). Perinatal outcome was assessed according to the three types of management.

Main outcome measures Occurrence of ICH, perinatal survival, gestational age at birth and complications of FBS.

Results All but one of 98 pregnancies ended in a live birth; none of the neonates had an ICH. The median gestational age at birth was 37 weeks (range 32–40). In groups 1 and 2, three emergency caesarean sections were performed after complicated FBS, resulting in two healthy babies and one neonatal death.

Conclusion Noninvasive antenatal management of pregnancies complicated by FNAIT appears to be both effective and safe.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by maternal antibodies against human platelet antigens (HPA) on fetal platelets. The incidence of FNAIT is estimated to be one in 1000–2000 births.[1–3] The major complication of severe fetal thrombocytopenia is intracranial haemorrhage (ICH), occurring in 7–26% of untreated pregnancies with FNAIT.[4–6] In the absence of screening programs, the diagnosis is almost always established after birth of a symptomatic child. To prevent recurrence of FNAIT in a subsequent pregnancy, several interventions have been used. At first, we and others used serial fetal blood sampling (FBS) with often weekly platelet transfusions. After the empirical observation by Bussel *et al.*[7] in 1988 that antenatal maternal treatment with high-dose intravenous immunoglobulins (IVIG) seemed to prevent ICH in high-risk pregnancies, IVIG became the cornerstone of FNAIT treatment. Several centres in both Europe and the USA advocate the use of FBS for verification of fetal platelet count before and during maternal treatment. Controversy exists whether FBS, with its inherent risks of bleeding, boosting of antibody levels, emergency (preterm) caesarean section and fetal loss, should remain part of the management of FNAIT.[8] In the past 16 years, we gradually changed our management strategy towards a completely noninvasive approach for FNAIT. The aim of this study was to describe our experience with the transition from an invasive strategy via a minimally invasive to an ultimately completely noninvasive strategy.

METHODS

The Department of Obstetrics at the Leiden University Medical Centre is the national referral centre for pregnancies complicated by FNAIT in the Netherlands.

For this study we extracted data on pregnancy, delivery and neonatal course of all FNAIT pregnancies treated at our centre between March 1989 and December 2005. Maternal and fetal HPA incompatibility was confirmed for all patients by paternal HPA typing. In cases where the father was homozygous for the specific HPA, we assumed that the fetus would be at risk. Where the father was heterozygous for the HPA, amniocentesis was performed for fetal HPA typing.

We divided these pregnancies into three groups, according to the invasiveness of the management protocol used. The first group was managed with FBS and subsequent intrauterine transfusion in case of low platelet count, without the use of IVIG. The second group was treated with IVIG combined with FBS with in-

trauterine transfusion if needed. In all cases of FBS, matched platelets were available for immediate transfusion. Our threshold for platelet transfusion in group 1 was a fetal platelet count < 100 x 10^9/l. In group 2, the nonresponders, defined as fetuses with a platelet count < 50 x 10^9/l after at least 4 weeks of IVIG treatment, received platelet transfusions. In case of predelivery FBS, a threshold of 100 x 10^9/l was used for platelet transfusion. The third group was treated completely noninvasively with IVIG only. Groups 1, 2 and 3 were further subdivided into those pregnancies with a sibling with an ICH and those with a sibling without an ICH.

If the sibling had an ICH, in the second (invasive) group, IVIG was started 4–6 weeks before the estimated time of occurrence of the sibling's ICH. In the third (noninvasive) group, IVIG was started at 16 weeks of gestation if the sibling did have an ICH and at 32 weeks of gestation if the sibling did not.

In all cases, IVIG was given weekly in a dose of 1 g/kg maternal weight. Further details on our management protocols have been described previously.[9,10]

If the previous sibling had an ICH, a planned caesarean section was performed around 36 weeks of gestation. In a few cases, in group 2, with an easily accessible cord insertion, predelivery FBS was carried out, with platelet transfusion when needed, followed by induction of labour and vaginal delivery.

If the previous sibling did not have an ICH, IVIG was continued until induction of labour at 38 weeks of gestation, with a caesarean section only for obstetric reasons.

In all groups, serial ultrasounds of the fetal brain were performed. Platelet count at birth was assessed from umbilical cord blood. Neonatal cranial ultrasound was carried out in all children within 24 hours after birth.

The noninvasive management protocol was approved by our institution's medical ethics committee.

From all pregnancies, we collected the clinically relevant outcome variables: neonatal survival, occurrence of ICH, complications of FBS and gestational age at birth. We consider gestational age at birth as a relevant parameter because a complication during FBS at a viable gestational age is often followed by an emergency caesarean section, resulting in a preterm birth.

RESULTS

Ninety-nine fetuses from 98 pregnancies in 85 women were treated at our centre during the study period. HPA-1a antibodies were the leading cause of FNAIT,

Table 1: Characteristics and outcome of 98 pregnancies (99 fetuses) treated for FNAIT at Leiden University Medical Centre from March 1989 till December 2005

	Sibling with ICH		Sibling without ICH		
Treatment	Group 2 IVIG+ FBS± transfusion (invasive)	Group 3 IVIG without FBS (non-invasive)	Group 1 FBS± transfusion (invasive)	Group 2 IVIG+ FBS± transfusion (invasive)	Group 3 IVIG without FBS (non-invasive)
	n=11	*n*=5	*n*=13	*n*=22	*n*=48*
In utero					
Median platelet count sibling (range) x 10^9/l	20 (2-36)	12 (6-12)	37 (15-58)	24 (2-60)	15 (3-134)
Median GA at first IVIG treatment (weeks,range)	27 (12-30)	16 (16)	-	31 (22-35)	32 (30-36)
Median number of IVIG treatments (range)	11 (5-24)	20 (19-21)	-	6 (2-10)	5 (2-9)
Median number of FBS (range)	2 (1-9)	-	1 (1-3)	2 (1-5)	-
Median platelet count at first FBS (range) x 10^9/l	26 (2-125)	-	86 (3-188)	36 (0-245)	-
Median number of platelet transfusions (range)	2 (0-9)	-	1 (1-3)	1 (0-5)	-
At delivery					
Vaginal delivery (*n*,%)	7 (64%)	0	10 (77%)	14 (64%)	31 (65%)
Median platelet count at birth (range) x 10^9/l	180 (55-377)#	15 (10-199)	145 (3-302)#	171 (60-348)#	137 (4-259)
Platelet count at birth ≤ 50 x 10^9/l (*n*,%)	0	4 (80%)	3 (23%)	0	6 (13%)
Emergency delivery due to FBS (*n*,%)	0	-	0	3 (14%)	-
Neonatal death secondary to FBS (*n*,%)	0	-	0	1 (5%)	-
Delivery before 34 weeks (*n*,%)	1 (9%)	0	0	0	0
Median GA at delivery (weeks, range)	36 (32-38)	37 (34-37)	38 (34-40)	37 (34-40)	38 (35-40)

*48 neonates, 47 pregnancies (1 twin)

after intrauterine transfusion

FNAIT: fetal and neonatal alloimmune thrombocytopenia

FBS: fetal blood sampling

GA: gestational age

present in 76 of 85 women (89%); the remaining patients had HPA-5b (n = 7) or HPA-3a (n = 2) antibodies. In six pregnancies, a combination of HPA-1a with HPA-3a or HPA-5b was found. In 22 pregnancies, fetal HPA typing by amniocentesis showed that the fetus was positive for the HPA. Overall perinatal survival was 99% (98 of 99 fetuses). None of the neonates had an ICH. Median gestational age at birth was 37 weeks (range 32–40). Three emergency deliveries because of fetal distress after FBS occurred at 38, 37 and 34 weeks of gestation, two with good outcomes. One fetus died immediately after the emergency delivery at 38 weeks of gestation with an umbilical arterial pH of 6.88. Sixty-two of the 98 pregnancies ended in a vaginal delivery.

Thirteen pregnancies (group 1) were managed with FBS and platelet transfusions if needed, without the use of IVIG, of which none had a sibling with ICH. Thirty-three pregnancies (group 2) were treated with IVIG combined with FBS and intrauterine transfusions if needed. Fifty-two pregnancies (group 3), resulting in 53 neonates, were treated completely noninvasively, of which five had a sibling with an ICH.

In Table 1, the characteristics and the outcome of all 98 pregnancies are given, with the three groups subdivided in having a sibling with or without an ICH.

DISCUSSION

In this cohort study of a relatively large series of consecutive pregnancies complicated by FNAIT, the gradual change over time from an invasive management protocol to a completely noninvasive approach resulted in excellent outcome for all noninvasively treated patients. These findings encourage us to continue, and recommend to others, the noninvasive strategy that we have suggested in previous publications.[9,10]

To support this view further, the clinically important outcome data from our series compare favourably with two recently published studies describing results from more invasive management protocols. Birchall *et al.* reported on an observational study from 12 European centres, with a total of 50 women with 55 pregnancies and 56 fetuses, all with HPA-1a alloimmunisation treated between 1988 and 2001.[11] Multiple management options were described, all after an initial FBS. ICH occurred in 5% of the children (3/56). FBS-related adverse outcome occurred in 18% of the fetuses (10/56), with two fetal losses and eight deliveries before 34 weeks of gestation. In addition, in 9% (5/56) an emergency delivery after 34 weeks of gestation had to be performed following FBS. Maternal treat-

ment with IVIG was used in 18 patients, combined with one or more FBS. Four of these 18 neonates were born before 34 weeks, one fetal loss occurred and one emergency caesarean section was performed, both associated with FBS. The mean platelet count at birth in this group was 80 x 10^9/l with six of the 18 neonates having a platelet count < 50 x 10^9/l. None of their cases were treated completely noninvasively.

Berkowitz *et al.* performed a randomised multicentre study stratifying 79 pregnancies into high-risk and low-risk arms.[12] All women underwent initial FBS at 20 weeks of gestation. High-risk cases (n = 40) were defined as either a sibling with peripartum ICH or an initial platelet count < 20 x 10^9/l. Randomisation was between IVIG and prednisone, or IVIG only. Low-risk cases were randomly assigned to IVIG or prednisone. A second FBS was used to adapt the medication dose in nonresponders. Platelet transfusions were given in an unknown number of cases. ICH occurred in three cases (4%). In 13% (10/79) of the pregnancies, emergency deliveries related to FBS were required and 24% (19/79) of the neonates were born before 34 weeks. One fetus died because of a complication of FBS and two pregnancies ended in unexplained fetal demise. A total of 175 FBS were carried out, with serious complications occurring in 6%. Mean platelet count at birth in the high-risk group was 99 x 10^9/l in the IVIG group and 69 x 10^9/l in the IVIG combined with steroids group. In the low-risk group, 15% (6/39) of fetuses had a platelet count < 50 x 10^9/l.

In conclusion, the studies by Birchall *et al.* and Berkowitz *et al.* describe a considerable number of complications and adverse outcomes associated with FBS. However, such risks could be acceptable if the invasive management would result in a better overall outcome when compared with a completely noninvasive approach. This, as our data suggest, does not seem to be the case. For none of the clinically relevant outcome parameters, including survival, ICH and gestational ageat birth, our noninvasively managed series shows worse results than in the other studies. Platelet counts at birth, although arguably a surrogate measure, were similar in our group.

Our data as well as our comparison with the other two studies should be interpreted with some care. All three studies described fewer than 100 patients, over a considerable period of time, managed with different protocols. Patient populations in the three studies differed slightly, although the possible bias this could introduce would not weaken our main conclusions. In the study by Berkowitz *et al.*, patients with the most severe forms of FNAIT (sibling with ICH or platelet count < 20 x 10^9/l) were excluded, while we included all patients referred to us.

As far as can be ascertained, the severity of the disease in patients in our series was at least comparable with the other two studies. The level of evidence for the suggestion that FBS can be avoided in all pregnancies with FNAIT would obviously be stronger with properly controlled study designs. However, given the rarity of this disease, the data presented here represent the best possible evidence currently available. In our view, these limited data suggest that there seems to be no benefit in the use of diagnostic FBS in addition to treatment with IVIG in the management of FNAIT, irrespective of the severity of the disease.

The perceived advantages of FBS are to optimise the indication for IVIG treatment and identify nonresponders for treatment adjustment, and in case of predelivery sampling, to select patients that might safely deliver vaginally. The serious and, in this particular disease, even greater risks of FBS[13,14] have to be balanced against unnecessary IVIG treatment. Omitting FBS means starting IVIG treatment 'blindly', based only on the history of the disease in a previous child. With noninvasive management, adaptation of the dose, or adding steroids in case of insufficient response is obviously not possible. Compelling evidence that such adaptations lead to improved outcome however is lacking. Moreover, highdose prednisone is known to cause serious maternal adverse effects.[5] Because IVIG in a dose of 1 g/kg/week has been shown to have minimal, if any, adverse effects both for mother and fetus,[15,16] and excellent outcome, the risk of superfluous treatment in some pregnancies by starting IVIG blindly may well outweigh the risks of pretreatment FBS.

Apart from the immediate risks of exsanguination or haematoma formation during FBS, a more long-term negative effect might be a boosting of antibodies especially with transplacental procedures.[17] We speculate that this mechanism could have contributed to the lower platelet counts at birth in the patients with a sibling without an ICH described by Birchall *et al.* compared with our noninvasively managed patients. In future studies, changes in antibody titres could be monitored in order to address this important issue.[18]

Predelivery FBS to allow vaginal birth in case of a sufficient platelet count would be a logical intervention if caesarean section is considered safer in fetuses with low platelet counts, an assumption not based on any evidence. In a recent study we found no peripartum ICH in any neonate with FNAIT born vaginally.[19]

Although we do realise that the absolute number of patients treated completely noninvasively is still limited, we conclude that based on our data and the currently available literature, there seems to be no advantage to the use of

FBS in the management of pregnancies complicated by FNAIT. For clinically relevant endpoints, the noninvasive management strategy using IVIG without pretreatment or confirmatory FBS seems both effective and safe.[20] Adherence to the principle of *primum non nocere* means, in our view, that potentially hazardous diagnostic procedures should only be employed when proven to do more good than harm.

Further improvement of treatment strategies is certainly warranted, as we still observed fetuses and neonates with low platelet counts at risk for ICH. Future studies could be directed at the optimal time to start treatment and optimising the dose, possibly stratifying patients according to antibody levels or functional bioassays and obstetric history. Because FNAIT is a potentially devastating but rare disease, rapid advances in our insights to improve management can only be made by multicentre collaboration. We would therefore like to encourage all colleagues caring for these patients to consider participating in international trials and registries. Information on one of these initiatives can be found on www.noich.org.

REFERENCES

1 Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Decary F. Alloimmunization to the PlA1 platelet antigen: results of a prospective study. *Br J Haematol* 1990;74:209–15.

2 Dreyfus M, Kaplan C, Verdy E, Schlegel N, Durand-Zaleski I, Tchernia G, *et al.* Frequency of immune thrombocytopenia in newborns: a prospective study. *Blood* 1997;89:4402–6.

3 Williamson LM, Hackett G, Rennie J, Palmer CR, Maciver C, Hadfield R, *et al.* The natural history of feto-maternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening. *Br J Haematol* 1998;92:2280–7.

4 Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, *et al.* 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989;1:363–6.

5 Bussel JB, Berkowitz RL, Lynch L, Lesser ML, Paidas MJ, Huang CL, *et al.* Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: a randomized trial of the addition of lowdose steroid to intravenous gamma-globulin. *Am J Obstet Gynecol* 1996;174:1414–23.

6 Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust N Z J Obstet Gynaecol* 2001;41:45–5.

7 Bussel JB, Berkowitz RL, McFarland JG, Lynch L, Chitkara U. Antenatal treatment of neonatal alloimmune thrombocytopenia. *N Engl J Med* 1988;319:1374–8.

8 Berkowitz RL, Bussel JB, McFarland JG. Alloimmune thrombocytopenia: state of the art 2006. *Am J Obstet Gynecol* 2006;195:907–13.

9 Radder CM, Brand A, Kanhai HHH. A less invasive treatment strategy to prevent intracranial hemorrhage in fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2001;185:683–8.

10 Kanhai HHH, van den Akker ESA, Walther FJ, Brand A. Intravenous immunoglobulins without initial and follow-up cordocentesis in alloimmune fetal and neonatal thrombocytopenia at high risk for intracranial hemorrhage. *Fetal Diagn Ther* 2006;21:55–60.

11 Birchall JE, Murphy MF, Kaplan C, Kroll H. European collaborative study of the antenatal management of feto-maternal alloimmune thrombocytopenia. *Br J Haematol* 2003;122:275–88.

12 Berkowitz RL, Kolb EA, McFarland JG, Wissert M, Primani A, Lesser M, *et al*. Parallel randomized trials of risk-based therapy for fetal alloimmune thrombocytopenia. *Obstet Gynecol* 2006;107:91–6.

13 Paidas MJ, Berkowitz RL, Lynch L, Lockwood CJ, Lapinski R, McFarland, *et al*. Alloimmune thrombocytopenia: fetal and neonatal losses related to cordocentesis. *Am J Obstet Gynecol* 1995;172:475–9.

14 Overton TG, Duncan KR, Jolly M, Letsky E, Fisk NM. Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. *Am J Obstet Gynecol* 2002; 186:826–31.

15 Radder CM, Roelen DL, Van de Meer-Prins EM, Claas FHJ, Kanhai HHH, Brand A. The immunologic profile of infants born after maternal immunoglobulin treatment and intrauterine platelet transfusions for fetal/neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2004; 191:815–20.

16 Radder CM, de Haan MJJ, Brand A, Stoelhorst GMSJ, Veen S, Kanhai HHH. Follow up of children after antenatal treatment for alloimmune thrombocytopenia. *Early Hum Dev* 2004;80:65–76.

17 Nicolini U, Kochenour NK, Greco P, Letsky EA, Johnson RD, Contreras M, et al. Consequences of fetomaternal haemorrhage after intrauterinetransfusion. *BMJ* 1988;297:1379–81.

18 Bertrand C, Martageix C, Jallu V, Vitry F, Kaplan C. Predictive value of sequential anti-HPA-1a antibody concentrations for the severity of fetal alloimmune thrombocytopenia. *J Thromb Haemost* 2006;4:628–37.

19 van den Akker ESA, Oepkes D, Brand A, Kanhai HHH. Vaginal delivery for fetuses at risk of alloimmune thrombocytopenia? *BJOG* 2006; 113:781–3.

20 Radder CM, Brand A, Kanhai HHH. Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? *Vox Sang* 2003;84:318–25.

Chapter 4

IVIG without initial and follow-up cordocentesis in FNAIT at high risk for intracranial hemorrhage

Kanhai HHH, Van den Akker ESA, Walther FJ, Brand A. Intravenous immunoglobulins without initial and follow-up cordocentesis in alloimmune fetal and neonatal thrombocytopenia at high risk for intracranial hemorrhage *Fetal Diagnosis and Therapy* 2006; 21: 55-60.

ABSTRACT

Objective To report on a less invasive treatment strategy in alloimmune fetal and neonatal thrombocytopenia (FNAIT) at high risk for either in utero or neonatal intracranial hemorrhage (ICH).

Methods In seven pregnancies, with a history of ICH in the older sibling, weekly intravenous immunoglobulin (IVIG) therapy to the mother (1 g/kg) without initial cordocentesis was started at a median gestational age of 16 weeks.

Results In four pregnancies cordocentesis was avoided. One predelivery cordocentesis with platelet transfusion was performed in three further cases. Although none of the cases had a platelet count of > 50 x 10^9 /l at cordocentesis, predelivery or birth, no ICHs were observed. The neonatal periods of the infants were uncomplicated.

Conclusion IVIG treatment alone might be considered in patients with both severe platelet alloimmunization and an increased risk for morbidity and mortality at cordocentesis.

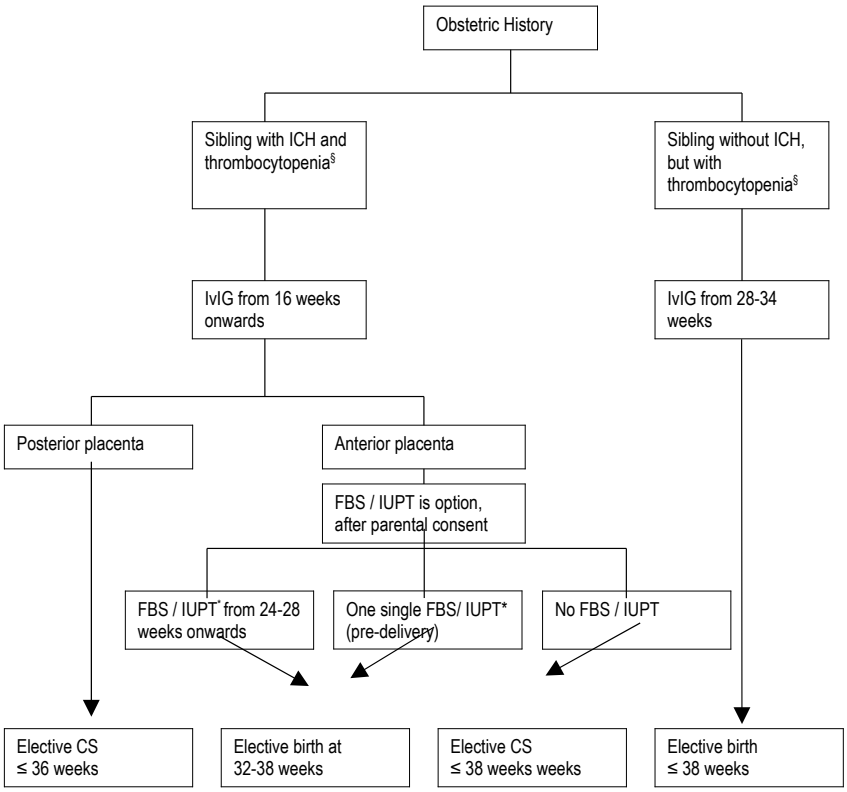
INTRODUCTION

Platelet alloimmunization, resulting in severe fetal or neonatal thrombocytopenia (FNAIT), is a rare but potentially devastating condition. The major complication of severe thrombocytopenia in the fetus or newborn is intracranial hemorrhage (ICH), resulting in either perinatal mortality or serious morbidity. In Caucasians, the immunodominant antigen is the human platelet antigen (HPA) 1a, responsible for approximately 85% of FNAIT cases[1, 2]. Two percent of pregnant Caucasian women are HPA-1a negative[3–6]. Severe thrombocytopenia is estimated to occur in 6–12% of the HPA-1a-negative mothers with antibodies[4, 7, 8]. The reported frequency of ICH in FNAIT varies from 7 to 26%[2,9], of which the large majority is presumed to occur in utero[2, 10]. After the birth of a child with ICH, the recurrence rate in the subsequent offspring carrying the offending antigen is estimated to be as high as 79%[10–13].

The accepted treatment to reduce the risk of both severe thrombocytopenia and ICH is maternal intravenous immunoglobulin (IVIG) administration and serial cordocentesis from 20 weeks onwards, and if thrombocytopenia persists, intrauterine platelet transfusions (IUPT)[9, 11, 14]. It is recognized that cordocentesis is a hazardous procedure, especially for patients with thrombocytopenia. Based on a review of the literature, the complication rate of cordocentesis in FNAIT pregnancies was calculated as 1.6% fetal loss and 2.4% other complications[10]. According to two recent studies, 6% of pregnancies were lost because of serial cordocentesis[15, 16].

Application of high-dose maternal immunoglobulins (1 g/kg body weight/week) has become increasingly important in the management of alloimmune thrombocytopenia of pregnancy. In addition to increasing fetal platelet counts, reported in 70% of the cases, the treatment seems to reduce the incidence of ICH, even when severe thrombocytopenia persists[17, 18]. In alloimmunized women with an older infant with severe thrombocytopenia, but without ICH, we have shown that weekly administration of immunoglobulins without cordocentesis is safe[18]. Balancing the risk for serious complications from cordocentesis on one hand and ICH on the other, we designed a protocol to further reduce invasive procedures in patients with the highest risk for fetal ICH. We describe the protocol and the results of the first eight consecutive pregnancies thus treated.

Figure 1: Flow chart of the management protocol in HPA-1a immunized women, used at Leiden University Medical Centre.



§ = Defined as a neonatal platelet count of < 100 x 10^9/l;
* = in case of a fetal platelet count < 100 x 10^9/l;

ICH = intracranial hemorrhage;
IVIG = intravenous immunoglobulins;
FBS = fetal blood sampling;
IUPT = intrauterine platelet transfusion;
CS = cesarean section.

PATIENTS AND METHODS

Between June 1998 and December 2003, six HPA-1a alloimmunized women with eight pregnancies with a prior child with ICH were treated at the Leiden University Medical Center (LUMC). The LUMC is the Dutch national referral centre for the management of severe alloimmune pregnancy disorders. One patient opted for follow-up cordocentesis, based on her experience in a previous pregnancy. This case will be excluded in this report. Therefore, seven cases without initial and follow-up cordocentesis will be described here. The six partners were homozygous HPA 1a/1a. The characteristics of the pregnancies are depicted in table 1. In all but one of the six siblings, ICH occurred in utero and before labor. In the elder sibling of cases 3 and 4 (table 1), a massive subarachnoid bleeding was diagnosed and the growth-retarded neonate died early in the neonatal period. Two of the six siblings survived, both with severe physical and mental handicaps. Case 3 (tables 1, 2) has been previously published, in the series described by Radder *et al.*[18].

Before the current pregnancy, all the couples were counseled extensively about the known risks of a subsequent pregnancy. They were informed about the treatment thus far [18] and the pros and cons of a management strategy, including repeated cordocentesis. The couples were also informed about the new management protocol (fig. 1). All of the couples decided to attempt a new pregnancy and agreed to follow the protocol.

During the current pregnancy, IVIG was started early in the second trimester (table 2) and continued until delivery. Detailed ultrasound examinations of the fetal brain were performed weekly. Cordocentesis with IUPT was performed once in three pregnancies, just before a planned delivery. In the other four pregnancies, cordocentesis was avoided. The local medical ethical commission approved the protocol.

RESULTS

Serial ultrasound examinations during pregnancy revealed no ICH in the fetus. The median gestational age at the start of IVIG was 16 weeks (range 16–29 weeks) based on the estimated gestational age when ICH occurred in the sibling. In two pregnancies of the same woman (cases 3 and 4 in table 2), IVIG treatment was initiated later, because the older sibling had a subarachnoid bleeding instead of ICH and was growth retarded. The total number of weekly IVIG infusions ranged from eight to twenty-one (table 2).

Table 1: Characteristics of the pregnancies

Mother	Case no.	Timing of ICH in sib (weeks GA)	Sib died? When?	PLC of sib (x 10^9/l)	BW sib (gram)
1	1	< 33	yes, 1 day pp	33	2750
	2	< 33	yes, 1 day pp	33	2750
2	3	33	yes, directly pp*	11	1200
	4	33	yes, directly pp*	11	1200
3	5	< 36	no, multiple handicaps	6	2200
4	6	< 33	no, multiple handicaps	11	2390
5	7	< 35	yes, antenatal	20	2730

* Extensive subarachnoid bleeding, uncertain when occurred, with severe intrauterine growth retardation

ICH: intracranial hemorrhage
Sib: sibling
GA: gestational age
pp: postpartum
PLC: platelet count
BW: birth weight

Table 2: Characteristics of the treatment with immunoglobulins, placenta localization and birth outcome

Mother	Case no.	First IVIG (GA) (weeks)	IVIG (*n*)	Placenta localisation	Birth (GA) (weeks)	PLC at birth (x 10^9/l)
1	1	16	20	Posterior	36	39
	2	16	21	Anterior	37	14
2	3	28	10	Fundal	38	26*
	4	29	8	Anterior	38	41*
3	5	18	19	Posterior	37	15
4	6	16	19	Posterior	34§	10
5	7	19	19	Anterior	37	49*

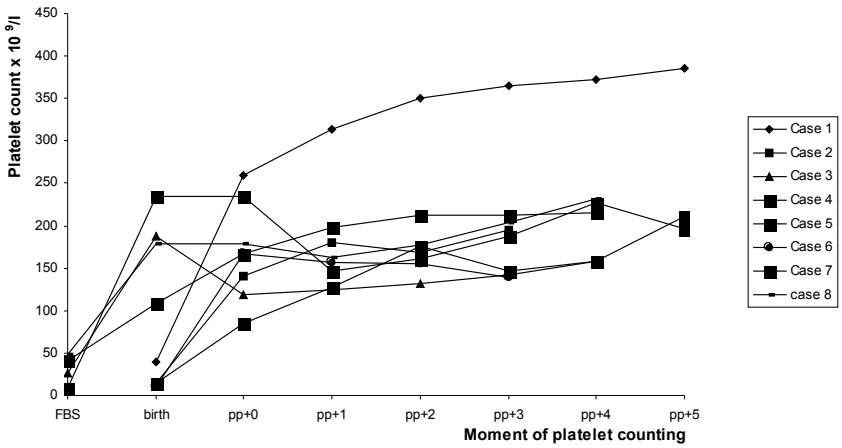
* Platelet count at fetal blood sampling predelivery, before intrauterine platelet transfusion
§ Elective cesarean section because of hydrothorax and fear for internal bleeding; after birth: infant with Down syndrome without signs of internal bleeding

IVIG: intravenous immunoglobulins
GA: gestational age
PLC: platelet count

Elective cesarean section was performed in four pregnancies between a gestational age of 34 and 37 weeks. In the other three pregnancies, labor was induced between 37 and 38 weeks, within three days after IUPT.

Although none of the cases had a platelet count of > 50 x 10^9/l, after several weeks of IVIG treatment (table 2), no ICH was diagnosed in antenatal or neonatal ultrasound examinations. Four neonates were given one platelet transfusion shortly after birth. None of the neonates received IVIG. The neonatal platelet counts for the first five days are depicted in figure 2. Strikingly, although some neonates experienced an initial thrombocytopenia, recurrence was not observed subsequently (fig. 2). At follow-up three months after birth, all of the infants were doing well.

Figure 2: Fetal and neonatal platelet counts



DISCUSSION

The present series describes the use of weekly immunoglobulins to the mother without initial or confirmatory cordocentesis in FNAIT with an older sibling with ICH. In seven alloimmunized pregnancies with an older sibling with severe internal bleeding, serial IUPT were avoided. With the less invasive approach, ICH did not recur in any of the cases. The cornerstone of therapy was maternal administration of immunoglobulins (1 g/kg bodyweight/week). Cordocentesis was only considered when the procedure did not pose an unacceptable risk. In our current protocol, women are given the option of cordocentesis after at least four weeks of IVIG treatment, when the placenta is anteriorly located. Although the placenta was anteriorly located in three of the cases, IVIG was administered as the sole treatment, and this was continued until term.

In general, procedure-related complications of cordocentesis are associated with factors such as experience of the operator, gestational age, indication for the procedure, and location of the placenta[19]. In fetuses with severe thrombocytopenia, cordocentesis is a procedure that poses a high risk for bleeding[10, 20]. Especially, puncture of a free loop or posteriorly located umbilical cord insertion may lead to either excessive fetal bleeding or cord hematoma.

Three patients delivered vaginally after cordocentesis and platelet transfusion. The other four underwent an elective cesarean section. The rationale to perform elective cesarean section in these patients was that we did not dare to attempt vaginal delivery in these high-risk cases for bleeding, without knowledge of the platelet counts. Although all infants had platelet counts of $< 50 \times 10^9$/l at birth or before a predelivery IUPT, no ICH was observed in the neonates. In addition, four newborns needed a single platelet transfusion shortly after birth and all had normal and stable platelet counts after birth. This observation is in concordance with earlier reports that IVIG therapy might prevent ICH in nonresponders to IVIG[17, 18, 21]. The reported recurrence risk of ICH in the next pregnancy of women with an older affected child is 79% (CI: 61–97%)[10]. Given this risk, approximately six cases of ICH would be expected in our series. This suggests a beneficial effect of IVIG on the recurrence of ICH, despite the presence of low platelet counts in utero. This may be the result of a specific protective effect of IVIG for bleeding in severe alloimmune thrombocytopenia. Potential disadvantages of IVIG are the generally mild side effects and the high costs of the treatment. Although the longterm side effects for mother and child treated with high dose IVIG are still unclear, reports thus far are satisfactory[11, 22]. At present cordocentesis, with or

without pretreatment with IVIG, and, in case of persisting thrombocytopenia, IUPT, given weekly, is the treatment of first choice in patients at high risk for in utero ICH. This approach is effective but has a significant risk, even in experienced hands. In a recent publication of the European Fetomaternal Alloimmune Thrombocytopenia Study Group[16], five problems occurred in the 33 fetuses that were treated with serial IUPT. Two fetuses died, one from exsanguination during cordocentesis and one on the day after IUPT. In the remaining three cases, delivery occurred before 32 weeks' gestation due to cordocentesis-related complications.

The results of the present series show that the effectiveness of maternally administered IVIG allows a further avoidance of cordocentesis in the most severe cases of FNAIT. However, we acknowledge that our series is small and that in the group of alloimmunized women at extreme high risk for ICH, IVIG therapy alone may not entirely eliminate the risk of ICH. There are anecdotal reports in the literature of cases where IVIG treatment failed to prevent ICH[23, 24]. It is, therefore, extremely important to initiate a collaborative database of cases of FNAIT to investigate the risk of invasive versus noninvasive treatment.

REFERENCES

1 Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, Mueller-Eckhardt G, Santoso S: 348 cases of suspected neonatal alloimmune thrombocytopenia. *Lancet* 1989; 1: 363–366.

2 Spencer JA, Burrows RF: Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust NZ J Obstet Gynecol* 2001; 41: 45–55.

3 Murphy MF, Williamson LM, Urbaniak SJ: Antenatal screening for fetomaternal alloimmune thrombocytopenia: should we be doing it? *Vox Sang* 2002; 83(suppl 1):409–416.

4 Mueller-Eckhardt C, Mueller-Eckhardt G, Willen-Ohff H, Horz A, Kuenzlen E, O'Neill GJ, Schendel DJ: Immunogenicity of and immune response to the human platelet antigen Zwa is strongly associated with HLA-B8 and DR3. *Tissue Antigens* 1985; 26: 71–76.

5 Williamson LM, Hackett G, Rennie J, Palmer CR, Maciver C, Hadfi eld R, Hughes D, Jobson S, Ouwehand WH: The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening. *Blood* 1998; 92: 2280–2287.

6 Davoren A, McParland P, Crowley J, Barnes A, Kelly G, Murphy WG: Antenatal screening for human platelet antigen-1a: results of a prospective study at a large maternity hospital in Ireland. *Br J Obstet Gynaecol* 2003; 110: 492–496.

7 Levy JA, Murphy LD. Thrombocytopenia in pregnancy. J Am Board Fam Pract 2002; 15: 290–297. 8 Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Decary F: Alloimmunization to the PlA1 platelet antigen: results of a prospective study. *Br J Haematol* 1990; 74: 209–215.

9 Kaplan C, Daffos F, Forestier F, Morel MC, Chesnel N, Tchernia G: Current trends in neonatal alloimmune thrombocytopenia: diagnosis and therapy, in Kaplan-Gouet C, Schlegel N, Salmon C, McGregor J (eds): Platelet Immunology: Fundamental and Clinical Aspects. Paris, Colloque INSERM/John Libbey Eurotext, 1991; 206: 267–278.

10 Radder CM, Brand A, Kanhai HH: Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? *Vox Sang* 2003; 84: 318–325.

11 Bussel JB, Zabusky MR, Berkowitz RL, Mc-Farland JG: Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997; 337: 22–26.

12 Kaplan C, Murphy MF, Kroll H, Waters AH: Feto-maternal alloimmune thrombocytopenia: antenatal therapy with IvIgG and steroids – more questions than answers. EuropeanWorking Group on FMAIT. *Br J Haematol* 1998; 100: 62–65.

13 Reznikoff-Etievant MF: Management of alloimmune neonatal and antenatal thrombocytopenia. *Vox Sang* 1988; 55: 193–201.

14 Bussel JB, Berkowitz RL, McFarland JG, Lynch L, Chitkara U: Antenatal treatment of neonatal alloimmune thrombocytopenia. *N Engl J Med* 1988; 319: 1374–1378.

15 Overton TG, Duncan KR, Jolly M, Letsky E, Fisk NM: Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. *Am J Obstet Gynecol* 2002; 186: 826–831.

16 Birchall JE, Murphy MF, KaplanC, Kroll H: European collaborative study of the antenatal management of feto-maternal alloimmune thrombocytopenia. *Br J Haematol* 2003; 122: 275–288.

17 Bussel JB, Berkowitz RL, Lynch L, Lesser ML, Paidas MJ, Huang CL, McFarland JG: Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: a randomized trial of the addition of low-dose steroid to intravenous gamma-globulin. *Am J Obstet Gynecol* 1996; 174: 1414–1423.

18 Radder CM, Brand A, Kanhai HH: A less invasive treatment strategy to prevent intracranial hemorrhage in fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2001; 185: 683–688.

19 Tongsong T, Wanapirak C, Kunavikatikul C, Sirirchotiyakul S, Piyamongkol W, Chanprapaph P: Fetal loss rate associated with cordocentesis at midgestation. *Am J Obstet Gynecol* 2001; 184: 719–723.

20 Paidas MJ, Berkowitz RL, Lynch L, Lockwood CJ, Lapinski R, McFarland JG, Bussel JB: Alloimmune thrombocytopenia: fetal and neonatal losses related to cordocentesis. *Am J Obstet Gynecol* 1995; 172: 475–479.

21 Wenstrom KD, Weiner CP, Williamson RA: Antenatal treatment of fetal alloimmune thrombocytopenia. *Obstet Gynecol* 1992; 80: 433–435.

22 Radder CM, Haan MJJ de, Brand A, Stoelhorst GMSJ, Veen S, Kanhai HHH: Follow up of children after antenatal treatment for alloimmune thrombocytopenia. *Early Hum Dev* 2004; 80: 65–76.

23 Kroll H, Kiefel V, Giers G, Bald R, Hoch J, Hanfl and P, Hansmann M, Mueller-Eckhardt C: Maternal intravenous immunoglobulin treatment does not prevent intracranial haemorrhage in fetal alloimmune thrombocytopenia. *Transfus Med* 1994; 4: 293–296.

24 Murphy MF, Metcalfe P, Waters AH, Ord J, Hambley H, Nicolaides K: Antenatal management of severe feto-maternal alloimmune thrombocytopenia: HLA incompatibility may affect response to fetal platelet transfusions. *Blood* 1993; 81: 2174–2179.

Chapter 5

Vaginal delivery for fetuses at risk of alloimmune thrombocytopenia?

Van den Akker ESA, Oepkes D, Brand A, Kanhai HHH. Vaginal delivery for fetuses at risk of alloimmune thrombocytopenia? *British Journal of Obstetrics and Gynaecology* 2006; 113: 781-783.

ABSTRACT

Objectives To evaluate the safety of vaginal delivery in pregnancies with fetal and neonatal alloimmune thrombocytopenia (FNAIT).

Design Prospective data collection.

Setting Leiden University Medical Centre, the national centre for management of severe red cell and platelet alloimmunisation.

Population Thirty-two pregnancies with FNAIT, with a sibling with thrombocytopenia but without an intracranial haemorrhage (ICH).

Methods The mode of delivery, platelet count in cord blood and neonatal outcome were analysed. All women received weekly intravenous immunoglobulin from 32 to 38 weeks of gestation. Head ultrasound scan was performed in all neonates.

Main outcome measures Signs of ICH or other bleeding in the neonates.

Results Twenty-three women delivered vaginally. Nine caesarean sections were performed, all for obstetric reasons. Median platelet count at birth was 142 x 10^9/l (range, 4–252 x 10^9/l), with severe thrombocytopenia (< 50 x 10^9/l) in four neonates, of which three were born vaginally. None of the neonates showed signs of ICH or other bleeding.

Conclusions In pregnancies with FNAIT and a thrombocytopenic sibling without ICH, vaginal delivery was not associated with neonatal intracranial bleeding. These initial results support our noninvasive management of these pregnancies with FNAIT.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but potentially devastating disease. It is the most common cause of thrombocytopenia in term neonates. In contrast to haemolytic disease due to rhesus D antibodies, the index pregnancy is often affected. Consequently, treatment is only possible in the next pregnancy. Many controversies still exist about the management, including mode of delivery.

The recurrence rate of severe thrombocytopenia and the risk to develop an intracranial haemorrhage (ICH) in the absence of ICH in a previous child is not known. Radder *et al.*[1] estimated the ICH risk to be 7% in a subsequent untreated pregnancy after a previous child with thrombocytopenia but without ICH.

Moreover, in 80% of neonates with ICH, this bleeding would occur before labour,[2] implying that, during labour or postpartum, the chance to develop ICH is approximately 1.4% in this group. In these milder FNAIT cases, the estimated 2.8% risk of complications associated with fetal blood sampling (FBS) and the 1.6% risk of fetal loss per procedure may not be justified when balanced against the risk of ICH.[1] Caesarean section is often routinely employed for delivery in these cases. Practice guidelines advise vaginal delivery as an option in case of a platelet count > 50 x 10^9/l established by FBS, with or without an intrauterine platelet transfusion.[3–5]

However, there is no evidence that a vaginal delivery poses the fetus with a platelet count < 50 x 10^9/l at higher risk for ICH than caesarean section. We report our experience with the safety of vaginal delivery in FNAIT pregnancies without ICH in a previous child.

METHODS

The Department of Obstetrics at the Leiden University Medical Centre is the national referral centre for pregnancies complicated by FNAIT in the Netherlands.

Our less invasive treatment strategy in women who are at risk for FNAIT and who have an index child with severe thrombocytopenia (< 50 x 10^9/l) but without an ICH has been described previously.[6] We prospectively collected all data from pregnancies complicated by FNAIT referred to us between March 1989 and August 2004. For this study, we selected all women with an index child with thrombocytopenia due to FNAIT but without an ICH. All women received weekly 1 g/kg bodyweight of intravenous immunoglobulins (IVIG) from 32 to 38

weeks followed by induction of labour. Head ultrasound scan was performed in all fetuses before starting the IVIG treatment and after birth. No diagnostic FBSs were performed. Assisted vaginal delivery was considered contraindicated. Caesarean section was only performed for obstetric reasons. The mode of delivery, the platelet count in cord blood and neonatal outcome were analysed.

RESULTS

Between March 1989 and August 2004, 29 women with 32 pregnancies met the inclusion criteria. The characteristics of the study group are given in Table 1. Twenty-three neonates from the 29 untreated siblings had a platelet count < 50 x 10^9/l. Four neonates were delivered by assisted vaginal delivery, having a platelet count of 11, 14, 21 and 30 x 10^9/l, respectively.

In Table 2, the characteristics of the neonates are given. Twenty-three deliveries were by vaginal route. Nine caesarean sections were performed, four because of breech presentation, one for transverse presentation and two because of an earlier caesarean section. Two secondary caesarean sections were performed for failure to progress, one breech and one vertex presentation.

Median platelet count in cord blood at birth was 145 x 10^9/l (range, 4–252 x 10^9/l). Median platelet count in the caesarean section group was 144 x 10^9/l (range, 4–231 x 10^9/l), with severe thrombocytopenia (4 x 10^9/l) in one neonate. Median platelet count in the vaginal group was 146 x 10^9/l (range, 12–252 x 10^9/l), with three neonates being severely thrombocytopenic (12, 40 and 41 x 10^9/l, respectively). Nine neonates needed treatment for thrombocytopenia; three received platelet transfusions combined with IVIG. Three others needed only platelet transfusions and three neonates received only IVIG after birth. None of the neonates had signs of ICH at ultrasound examination. In Figure 1, the relationship between the platelet count at birth in the index group and the platelet count at birth in the treated group is shown.

Table 1: Characteristics of the study group

Mothers (*n*=29)	
Median age (range)	33 (29-40)
Children involved, *n*	32
Previous pregnancy (*n*=29)	
Median platelet count (range)	30 x 10^9/l (4-134)
Platelet count < 50 x 10^9/l, *n*	23/29 (79%)
Delivery mode; Caesarean section	4/29 (14%)
Delivery mode; Instrumental delivery	4/29 (14%)
Current pregnancy (n=32)	
HPA-1a, *n*	28
Mean IVIG (weeks), *n* (range)	5 (3-7)
Preterm, *n*	1#

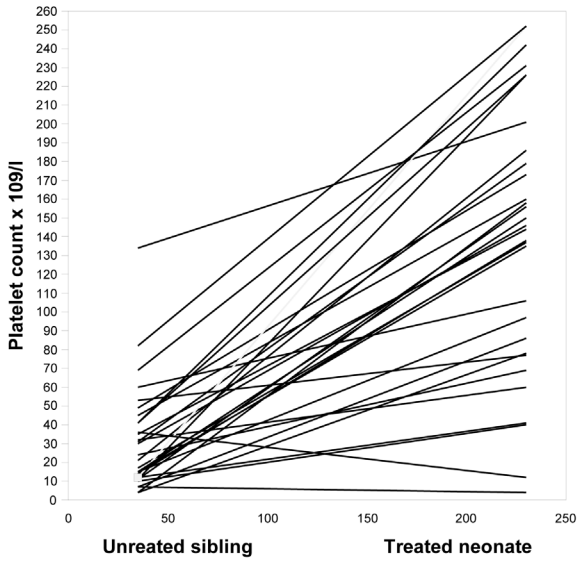
Delivered at 35+4 weeks

Table 2: Data on delivery, neonatal outcome and treatment

	Spontaneous vaginal delivery (*n*=23)	**Caesarean section (*n*=9)**
Intracranial haemorrhage, *n*	0	0
Median platelet count (range)	146 x 10^9/l (12-252)	144 x 10^9/l (4-231)
Platelet count < 50 x10^9/l, *n*	3	1
Neonatal treatment	6	3
IVIG only, *n*	3	0
Platelet transfusion only, *n*	0	3
Combination of IVIG and donor platelet transfusion, *n*	3	0

There were no significant differences between the two groups for all parameters.

Figure 1: Relationship between the platelet count at birth in the index group and the platelet count in the treated group.



DISCUSSION AND CONCLUSION

In our study group of pregnancies complicated by FNAIT, we achieved a 72% vaginal delivery rate. None of the 32 neonates developed an ICH, although in four neonates, the platelet count was less than 50 x 10^9/l. Three of these four children were safely born vaginally. The relatively high number of malpresentations could be explained by contraindication to external cephalic version in this group.

For the mother, the benefits of a vaginal delivery against a caesarean section are obvious. Although a caesarean section is safer then ever before, risks are not negligible. Maternal mortality, even with a planned section, is three times higher than with a vaginal birth[7] and maternal morbidity - thrombosis, hysterectomy, infections, extended hospital stay and chance of rehospitalisation - is also higher.[8,9] Uterine scar is also associated with increased risks in future pregnancies. [10,11]

Predelivery FBS is not without risk, especially for fetuses with thrombocytopenia. Mortality associated with FBS performed in a low-risk population is estimated to be 1.5% per procedure.[12,13] Based on a review in the literature, the complication rate of FBS in FNAIT pregnancies was calculated as 1.6% fetal loss and 2.8% other complications.[1]

In the group of FNAIT pregnancies with a sibling without ICH, not treated with IVIG, the risk to develop ICH is estimated to be 7%.[1] IVIG strongly reduces ICH in pregnancies with a sibling with ICH, but not completely, and at least four cases of recurrent ICH have been reported despite maternal IVIG treatment.[14–17] There is only one case report of an ICH after maternal IVIG treatment in a pregnancy in which the previous child had a platelet count of 8 x 10^9/l but no ICH.[18] We can therefore assume that the risk to develop an ICH after optimal IVIG treatment in the group without a previous ICH is low, even in fetuses or neonates without an adequate response to IVIG.

Although our study group of 32 pregnancies is relatively large for such a rare disease, the number of patients is still too low to sustain our results statistically. In the vaginal delivery group of 23 children, only three children had severe thrombocytopenia (<50 x 10^9/l). The fact that none of these three children had an ICH may be due to chance. Nevertheless, our experience supports that the mode of delivery in FNAIT without a history of ICH should be further investigated.

To achieve this, an international registry (www.noich.org) has been developed, in which all FNAIT cases from participating centres will be collected both retrospectively and prospectively. We expect to find more conclusive evidence through

this data collection to optimise management of pregnancies with FNAIT.

REFERENCES

1 Radder CM, Brand A, Kanhai HHH. Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? *Vox Sang* 2003;84:318–25.

2 Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust N Z J Obstet Gynaecol* 2001;41:45–55.

3 Bussel JB, Berkowitz RL, Lynch L, Lesser ML, Paidas MJ, Huang CL, et al. Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: a randomized trial of the addition of low-dose steroid to intravenous gamma-globulin. *Am J Obstet Gynecol* 1996;174:1414–23.

4 Kaplan C, Murphy MF, Kroll H, Waters AH. Feto-maternal alloimmune thrombocytopenia: antenatal therapy with IvIgG and steroids—more questions than answers. European Working Group on FMAIT. *Br J Haematol* 1998;100:62–5.

5 Reznikoff-Etievant MF. Management of alloimmune neonatal and antenatal thrombocytopenia. *Vox Sang* 1988;55:193–201.

6 Radder CM, Brand A, Kanhai HHH. A less invasive treatment strategy to prevent intracranial hemorrhage in fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2001;185:683–8.

7 Schuitemaker N, van Roosmalen J, Dekker G, van Dongen P, van Geijn H, et al. Maternal mortality after cesarean section in The Netherlands. *Acta Obstet Gynecol Scand* 1997;76:332–4.

8 Lydon-Rochelle M, Holt VL, Martin DP, Easterling TR. Association between method of delivery and maternal rehospitalization. *JAMA* 2000;283:2411–16.

9 Ham van MAPC, Dongen van PWJ, Mulder J. Maternal consequences of caesarean section: a retrospective study of intraoperative and postoperative maternal complications of caesarean section during a 10-year period. *Eur J Obstet Gynecol Reprod Biol* 1997;74:1–6.

10 Greene MF. Vaginal delivery after cesarean section—is the risk acceptable? *N Engl J Med* 2001;345:54–5.

11 Miller DA, Chollet JA, Goodwin TM. Clinical risk factors for placenta previa-placenta accreta. *Am J Obstet Gynecol* 1997;177:210–14.

12 Tongsong T, Wanapirak C, Kunavikatikul C, Sirirchotiyakul S, Piyamongkol W, Chanprapaph P. Fetal loss rate associated with cordocentesis at midgestation. *Am J Obstet Gynecol* 2001;184:719–23.

13 Van Kamp IL, Klumper FJ, Oepkes D, Meerman RH, Scherjon SA, Vandenbussche FP, *et al*. Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red-cell alloimmunization. *Am J Obstet Gynecol* 2005;192:171–7.

14 Bussel JB, Zabusky MR, Berkowitz RL, McFarland JG. Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997;337:22–6.

15 Murphy MF, Metcalfe P, Waters AH, Ord J, Hambley H, Nicolaides K. Antenatal management of severe feto/maternal alloimmune thrombocytopenia ± HLA incompatibility may affect responses to fetal thrombocyte

transfusions. *Blood* 1993;81:2174–9.

16 Murphy MF, Waters AH, Doughty A, Hambley H, Mibashan S,Nicolaides K, *et al*. Antenatal management of fetomaternal alloimmune thrombocytopenia— report of 15 affected pregnancies. *Transfus Med* 1994;4:281–92.

17 Sainio S, Teramo K, Kekomäki R. Prenatal treatment of severe fetomaternal alloimmune thrombocytopenia. *Transfus Med* 1999;9:321–30.

18 Kroll H, Kiefel V, Giers O, Bald R, Hoch J, Hanfland P, *et al*. Maternal intravenous immunoglobulin treatment does not prevent intracranial haemorrhage in fetal alloimmune thrombocytopenia. *Transfus Med* 1994;4:293–6.

Chapter 6

IVIG for pregnancies at risk for FNAIT: an uncompleted randomised trial comparing 0.5 and 1.0 g/kg bodyweight

Van den Akker ESA, Westgren M, Husebekk A, Kanhai HHH, Oepkes D, for the NOICH-study group. Intravenous immunoglobulin for pregnancies at risk for fetal and neonatal alloimmune thrombocytopenia: an uncompleted randomised trial comparing 0.5 and 1.0 g/kg bodyweight. *Submitted for publication.*

ABSTRACT

Objective To test the hypothesis that Intravenous immunoglobulin (IVIG) in a low dose of 0.5 g/kg/wk is at least as effective as the standard dose of 1.0 g/kg/wk in preventing intracranial haemorrhage (ICH) and severe fetal thrombocytopenia in pregnancies at risk for fetal and neonatal alloimmune thrombocytopenia (FNAIT).

Design Two dose open-label randomised international multicentre trial.

Setting Four tertiary care centres in Sweden, The Netherlands and Australia.

Population Pregnant women with alloantibodies against human platelet antigen (HPA) and a previous child with FNAIT, without an ICH.

Methods Pregnant women were randomised to low dose (*n*=12) or standard dose (*n*=11) IVIG.

Main outcome measures Primary outcome was ICH in the fetus or neonate, detected by cranial ultrasound. Secondary outcomes included fetal platelet count in cord blood at birth, the nadir of the platelet count, the IgG levels during pregnancy, type of neonatal treatment needed and signs of bleeding other than ICH.

Results The Trial Steering Committee recommended the study to be stopped early. After two years of recruitment, only 23 patients of the calculated sample size of two arms of 106 patients had been randomised. For this rare disease, the participation of at least ten other referral centres was needed and anticipated, but could not be realised.

All but one of the patients received the allocated dose of IVIG. Survival was 100%. None of the neonates had an ICH, a difference of 0% (95% CI: -25.2 to 23.6%). The upper limit of the 95% CI of the difference was not less than the prespecified 5%, therefore it remains uncertain if there is equivalence. The median platelet count at birth was 81 x 10^9/L (range 8-269) in the low dose group and 110 x 10^9/L (range 11-279) in the standard dose group (P = 0.644).

Conclusion Whether the effectiveness of a low dose of IVIG is equivalent to the standard dose in the treatment of FNAIT remains uncertain. The trial can be

regarded as a successful pilot, showing feasibility and acceptability of the study design both for patients and clinicians, a possible basis for a larger randomised trial. We regard the use of 0.5 g/kg/wk IVIG in pregnant women with FNAIT and a previous child without ICH still an option, only advised however in the setting of prospective studies.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by platelet destruction due to specific maternal IgG antiplatelet antibodies crossing the placenta. These antibodies are produced after exposure of the antigen-negative mother to the paternally inherited antigens on the fetal platelets during pregnancy. In Caucasians, FNAIT is most often caused by human platelet antigen HPA-1a. Two percent of the Caucasian population is HPA-1a negative (HPA-1bb). Sensitisation occurs in 6-12% of HPA-1bb mothers. Unlike Rhesus disease, the first pregnancy is frequently affected.

Prospective studies have shown that HPA-1a immunisation occurs in 1 in 365 pregnancies. The major complication of the resulting thrombocytopenia is intracranial hemorrhage (ICH) in the fetus or newborn, with an estimated incidence of 1 in 10,000 to 20,000 pregnancies[1-3].

Until recently, repeated fetal blood sampling and intrauterine platelet transfusions were the first choice of treatment of fetuses with alloimmune thrombocytopenia. Bussel *et al.* were the first to report the use of maternal administration of IVIG in the treatment of FNAIT[4]. In all seven cases reported, the fetal platelet count increased substantially after a weekly dose of 1 gram per kg maternal bodyweight. Several recent studies have shown that non-invasive management using IVIG alone is the safest and most effective option currently available[5,6].

The mechanism of action of IVIG in FNAIT is still unclear. Possible explanations are dilution of anti-HPA antibodies in maternal serum, blocking of the placenta receptor (Fc-R) and blocking of the Fc-receptors on the fetal macrophages[7].

IVIG is an immunomodulatory drug, produced from multiple donor blood transfusions. Potential risks therefore are transmission of viral diseases such as hepatitis B, C and HIV, although donor screening and viral inactivation measures make these risks in Western countries low. Headaches and fever, as well as renal and cardiovascular dysfunction have been described[8]. The long-term side effects for mother and child are still unclear. One study on short term follow-up, found

an increase of IgE in children after maternal IVIG administration. At present, no clinically apparent adverse effects in early childhood have been demonstrated[9]. As with any drug, especially in pregnancy, the lowest effective dose should be used since long-term safety is difficult to prove. Apart from the safety issue, IVIG is and will remain an expensive drug.

The empiric dose of 1.0 g/kg/wk has been commonly used since the first publication of Bussel *et al.*[4]. In FNAIT, no dose-effect studies have been done. Results of a recent study suggest that placental IgG is not further increased despite high IgG concentrations in the mother as a result from IVIG treatment. This suggests a saturation of the placental Fc-receptor and consequently a reduction in passage of antibodies to the fetus[7]. Based on these data, a lower dose of IVIG is possibly as effective, likely to be safer and certainly less expensive.

The aim of our study was to determine whether 0.5 g/kg/wk of IVIG is as effective as 1.0 g/kg/wk, in the prevention of ICH in FNAIT.

METHODS

Study design

This was a prospective, open label randomised study of two treatment arms, performed in four tertiary care centres in three countries.

Patients

Women with a singleton pregnancy who previously gave birth to a child with a platelet count < 150 x 10^9/L in the first week of life due to HPA alloimmunisation. HPA alloimmunisation was confirmed by the presence of maternal anti-HPA antibodies and the offending HPA antigen in the fetus or homozygous father. In the case of a heterozygous father the platelet antigen genotype of the fetus was proven positive by testing amniocytes before 28 weeks. Gestational age at inclusion was between 12 and 28 weeks. The study was performed according to the principles described in the Declaration of Helsinki, and each patient gave written informed consent to participate in the study. Each local Institutional Review Board (IRB) approved the protocol.

Exclusion criteria included: women with autoimmune thrombocytopenia, multiple pregnancies, fetuses and neonates with major congenital anomalies or chromosomal abnormalities and women with a previous children with FNAIT and an ICH. Patients with immunoglobulin-A deficiency were only excluded if

they had a severe allergic constitution and patients who ever had an allergic reaction to blood products due to anti-IgA antibodies.

Randomisation

Randomisation was performed between 26 and 28 weeks' gestation, after stratification for centre and for HPA-1a and non-HPA-1a, by the Central Internet Database Service (www.medscinet.net), through the study website (www.noich.org). The patient was randomised to either the low dose group, IVIG 0.5 gram per kg maternal bodyweight at the time of injection per week, or the standard dose group, IVIG 1.0 g/kg/wk.

Used medication

The IVIG used in this study was immunoglobulin from the company the clinicians were used to work with was given weekly, in a day-care setting, starting at 28 weeks and continued until delivery. Products used were Freeze-dried Immunoglobulin IV (CLB Sanquin Amsterdam) and Gammagard (Baxter). The IVIG infusions were administered over a period of 3 to 6 hours, according to the tolerance. Side effects and complications were recorded in the MedSciNet database.

Management protocol

Before the first IVIG treatment, and every two weeks thereafter, fetal ultrasound examination was done to rule out ICH. The total IgG levels were measured predelivery in maternal serum and postpartum in the umbilical cord blood.

The choice for timing and mode of delivery, elective Caesarean section or intended vaginal birth was left to the discretion of the obstetrician obviously with consent from the patient. Standard recommendations at vaginal delivery were not to use fetal scalp electrodes or scalp blood samplings and to refrain from ventouse or forceps application.

Immediately after birth the platelet count in the umbilical cord blood was measured in the local laboratory, first automatically and, in case of a platelet count ≤ 100 x 10^9/L, a manual count. In all centers where the deliveries took place, HPA compatible platelets were required to be available within 12 hours after delivery. A neonatologist examined all neonates directly after birth. Within the first days after birth, an ultrasound of the neonatal cerebrum was performed. Signs of minor and major bleedings were recorded. Neonatal management was left to the discretion of the neonatologist. The course of the neonatal platelet count was noted, together with any form of treatment for thrombocytopenia.

Laboratory investigations

After centrifugion, maternal and cord serum samples were stored at -80 degrees.. Maternal and cordblood sera were assayed for their total IgG levels by nephelometry (Behring Diagnostics Ltd, Ubridge, Middlesex, UK).

Outcomes

The primary outcome measure was fetal or neonatal ICH. Secondary outcome measures were platelet count in the umbilical cord blood at birth, the total IgG levels in maternal serum, the total IgG levels in cord blood, the occurrence of other signs of bleeding in the neonate and type of neonatal treatment. Any maternal and neonatal adverse event possibly associated with IVIG treatment was recorded.

Sample size calculation and statistical analysis

The required number of patients to prove that low dose IVIG is not associated with a worse outcome as compared to high dose, depends on the expected frequencies of adverse outcome in both groups. The study was set up as an equivalence trial. The null hypothesis was that the standard dose (1.0 g) is superior. We wanted to test if the lower dose, (0.5 g), was not inferior. We assumed that the probability of failure (occurrence of ICH) was 1%, if both groups were equal. For sample size calculation, we assumed that the low and the standard doses had the same risk of failure. 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 group 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 is less than 5%[12]. The confidence interval for the difference in proportion was calculated using the quasi-exact method by Chen[13].

Analysis was performed on the intention-to-treat principle, which comprised all randomised patients who received at least one dose of study medication. The Kruskal-Wallis test was used to compare continuous variables. Categorical variables were assessed with Fisher's exact test. All data are expressed as mean (SD) or median (range), and a P value < 0.05 was considered significant. Calculations were performed using SPSS 15.0 for Windows statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Recruitment for the study started on January 1, 2005. The first patient was randomised on January 10. At the start of the study, a total of 15 centers in eight countries agreed to the protocol and gave verbal assurance to their participation. We estimated that the inclusion of the required 212 patients would take three years. We had estimated that the specialised centers in The Netherlands, Germany, England and Canada, should be able to include 30 patients each, Sweden, Scotland and Switzerland were expected to include 20 patients each and Norway and Denmark 15 patients each.

Despite regular contact between the trial steering committee and the responsible investigators-to-be in these centres, only four centers, in the Netherlands, Sweden and Australia, managed to actually recruit patients for the study. In September 2007 a total of only 23 pregnancies had been randomised, which led to the decision by the steering committee to advise on prematurely ending the recruitment. Some of the reasons mentioned for not participating were inability to obtain IRB approval, an even lower number of eligible patients seen leading to a loss of interest in the study, and being too busy with projects of higher priority.

The last patient that was randomised delivered in December 2007. A total of 26 patients had been eligible for the study in the four participating centers. All but one of the randomised patients received the assigned treatment. One patient requested to change from 0.5 g to 1.0 gram per kg per week in week 34. The number of patients in each group with details of the flow through the stages of the trial is given in figure 1.

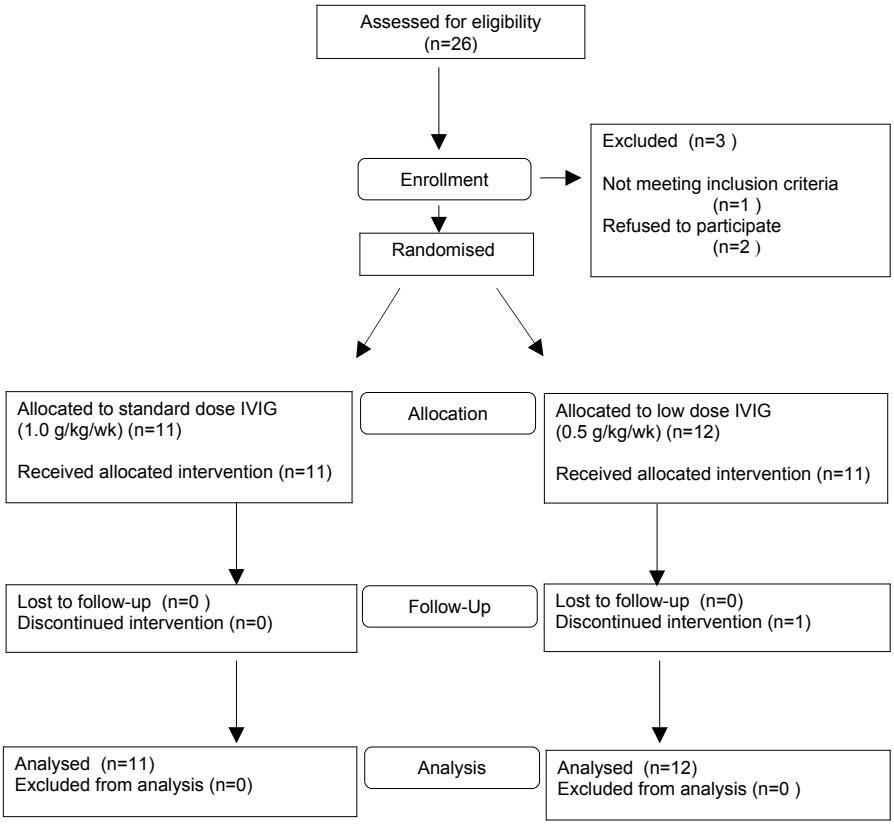
The baseline characteristics of all randomised patients are given in table 1, showing no statistically significant differences between the two groups.

Table 1: Patient characteristics

	IVIG 0.5 g/kg/wk	IVIG 1.0 g/kg/wk	*P-value*
	n=12	*n*=11	
Maternal age	31 (29-39)	32 (24-43)	0.597
Parity	1 (1-2)	1 (1-3)	0.810
Number of doses IVIG	10 (7-11)	11 (7-12)	0.436
Caucasian	12	11	1.0
HPA-1a	11	11	0.338
Platelet count (nadir) of sibling	17 (5-70)	11 (2-49)	0.532

Data shown as number or median (range)
IVIG: Intravenous Immunoglobulin

Figure 1: Flow diagram, showing the progress through the study of pregnancies at risk of FNAIT, treated with low or standard dose IVIG.



In table 2 the primary and secondary outcomes (ICH, platelet count at birth and nadir in first week, neonatal survival, neonatal treatment, maternal and fetal side effects and mode of delivery) are given. Perinatal survival was 100%, no fetal or neonatal intracranial haemorrhages were observed. The difference in primary outcome between the two groups was therefore 0%, with a 95% confidence interval of – 25.2% to 23.6%.

Table 2: Outcome of pregnancies with FNAIT after low or standard dose IVIG treatment

	IVIG 0.5 g/kg/wk	IVIG 1.0 g/kg/wk	*P-value*
	n=12	*n*=11	
Fetal ICH	0	0	1.0
Neonatal ICH	0	0	1.0
Platelet count at birth	81 (8-269)	110 (11-279)	0.644
Nadir of platelet count in 1st week	71 (8-266)	110 (9-202)	0.943
Signs of 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 or neonatal adverse events	0	0	1.0
Gestational age 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 caesarean section	4 (33%)	1 (9%)	0.168
Emergency caesarean section	1 (8%)	0	0.338
Birth weight (gram)	3087 (1940-3650)	3420 (2605-3750)	0.049
Platelet count < 30 x 10^9/L	1 (8%)	2 (18%)	0.493
Platelet count < 50 x 10^9/L	3 (25%)	4 (36%)	0.563
Platelet count < 150 x 10^9/L	9 (75%)	7 (64%)	0.563

Data shown as number (%), or median (range)
ICH: intracranial hemorrhage; IVIG: Intravenous Immunoglobulin

Table 3 shows the maternal and cord blood IgG concentrations at birth for the two groups, compared to the levels in the normal population[14]. Maternal IgG levels, as expected, were higher than in untreated pregnant women, no differences were observed between the two treatment regimens. Cord blood IgG levels were similar in the three groups.

Table 3: IgG concentrations (medians and ranges, g/L) at delivery in maternal and cord blood sera, in pregnancies with FNAIT treated with low (0.5 g/kg/wk) or standard dose (1.0 g/kg/wk) IVIG, compared with published reference ranges in normal pregnancies[14].

	Type of treatment		
Total IgG concentration	Low dose IVIG	Standard dose IVIG	Reference range
Cord blood	16.0 (14.4-21.1)	14.1 (12.9-18.4)	11.6 (7.5-15.9)
Maternal serum	19.4 (17.8-24.1)	26.2 (17.4-36.3)	8.0 (5.3-13.1)
Cordblood / Maternal serum ratio	0.82 (0.63-1.08)	0.61 (0.36-0.84)	1.4 (0.9-2.0)

DISCUSSION

The purpose of our study was to compare the effectiveness of a low dose of intravenous immunoglobulin, 0.5 g/kg maternal weight per week with the commonly used dose of 1.0 g/kg/wk, to prevent fetal or neonatal ICH in pregnant women with HPA antibodies. An equally effective lower dose of this expensive multidonor blood product in pregnancy would likely be safer and certainly less costly.

The trial however, had to be ended prematurely after three years, in which we managed to randomise only 23 of the planned 206 pregnant women. The main reason was a much lower than anticipated number of participating centres. Since the decision to end the trial prematurely was taken purely for administrative reasons, we agree with Kahn and Hills that the results have to be taken as they stand, and need to be shared honestly with the scientific community[15].

The trial design and workflow seemed both feasible and acceptable to all collaborating clinicians in the participating centres. Almost all eligible patients agreed to participate and completed the assigned intervention. These facts, together with the absence of any difference in primary or secondary outcomes of the study, lead us to suggest that this study might be regarded as a successful pilot study, a possible basis for a larger randomised trial. We regard the use of

0.5 g/kg/wk IVIG in pregnant women with FNAIT and a previous child without ICH still an option, only advised however in the setting of prospective studies. We obviously will refrain from any speculation on possible outcome of the trial in case of completion.

None of the children in our study suffered from ICH. Only one other trial has been published using this clinically most relevant parameter as the primary outcome measure[16]. They randomised 73 patients in five years, in 36 different centres, comparing IVIG 2.0 g/kg/wk to IVIG 1.0 g/kg/wk plus prednisone. Power calculations were not given. One child in each group had a mild ICH, apparently unrelated to FNAIT since both children had a platelet count > 100 x 10^9/L at birth. Apart from more maternal side-effects in the prednisone group, no significant differences between the treatment regimens were found.

The commonly quoted increased severity of FNAIT in subsequent pregnancies is based on a few small case-series[17-19]. The true risk for ICH in case of an previous pregnancy with FNAIT but without a fetal or neonatal ICH is unknown, and possibly very low. Since only fetuses with platelet counts < 50 x 10^9/L or < 30 x 10^9/L are supposed to be at risk for ICH, the use of the fetal or cord blood platelet count as surrogate outcome measure seems a logical choice. In our study, 8% in the low dose group and 18% in the standard dose group had platelet counts < 30 x 10^9/L at birth, a nonsignificant difference. The first published randomised trial comparing different treatment protocols for FNAIT used platelet counts as primary outcome[20]. They compared IVIG 1.0 g/kg per week with or without dexamethasone in 54 patients randomised in three years in 18 different centres. Dexamethasone appeared not to add to the effect of IVIG, with 5/26 versus 6/28 patients with cord platelet counts < 30 x 10^9/L. A Cochrane review concluded that their sample size was inappropriately calculated, leading to insufficient power to determine any significance in difference between the groups[21].

The same group published a second study describing two parallel trials, one with 40 patients and one with 39 patients, taking seven years and participation of 42 centres to complete[22]. Sample size calculations were not given. In the first trial, IVIG plus prednisone appeared to result in a higher platelet count at second sampling than IVIG alone. In the second trial, with patients comparable to our group, no differences in platelet counts between the treatment groups were found, with a total of 6/39 patients with cord platelet counts < 50 x 10^9/L.

We found that the maternal IgG levels were twice as high after IVIG treatment, independent of the dose, compared to published IgG levels in normal pregnancies. The IgG levels in cord blood were also similar in both treatment groups, however they were within the same range as published IgG levels in untreated controls. These results support the assumption that fetal IgG levels do not increase with higher IgG levels in the mother. The proposed working mechanism of IVIG by saturating the placental Fc-receptor as described by Urbaniak might already work at the low dose of 0.5 g/kg/wk[23].

Several lessons can be learned from the attempts, including our own, to perform randomised trials in this rare disease. Firstly, the only clinically relevant outcome measure is fetal or neonatal ICH, for which the risk in subsequent, treated pregnancies is not well known and possibly much lower than generally assumed. The few and consistently underpowered published trials all suggested absence of difference between the various compared treatments. No studies incorporating a placebo group have been published. Therefore in our view, none of the currently recommended management protocols are based on adequate evidence.

Secondly, although the true incidence of ICH in untreated pregnancies with a history of FNAIT but without a sibling with ICH is unknown, the use of IVIG seems very effective in preventing ICH. Therefore, although a dose-finding trial should ideally include a placebo-arm, it is unlikely that this would be acceptable for both patients and clinicians. Given the rarity of the occurrence of ICH in this group of patients, the use of this parameter as primary outcome measure results in sample sizes that seem very difficult to obtain. The surrogate outcome of a low platelet count may be more practical, with an incidence of around 20% in most studies published thusfar. Such a design however cannot address the suggested protective effect of IVIG on fetal endothelium, reducing the likelihood of ICH even in case of very low platelet counts, as suggested by Radder et al[24].

Thirdly, international multicentre studies running for long periods of time are likely the only option to complete adequately powered trials. This requires commitment from colleagues who may only deal with a handful of FNAIT pregnancies annually, and for whom this particular disease is not a main research subject. These and other large multicentre trials on the management of rare fetal disorders may benefit from international organisations such as the International Society for Fetal Medicine and Surgery (IFMSS), the North-American Fetal Therapy Network (NAFTNET) and the Eurofoetus initiative. Such networks may arrange or support web-based data-sharing facilities and funding to be used for jointly

by researchers studying rare diseases, helping eachother to complete large scale projects.

In conclusion, our trial failed to show equivalence of the two doses according to our prespecified criteria, thus it provides no support for the recommendation to lower the standard dose of IVIG of 1.0 g/kg/wk. However, there is also a lack of evidence for using the 1.0 g/kg/wk. Both based on previous in vitro studies and the current trial results, it is certainly possible that 0.5 g/kg/wk is as effective as 1.0 g/kg/wk. Taking safety and costs into account, treating new patients at risk for FNAIT without a sibling with an ICH with the low dose may be an option, although we would strongly recommend to restrict this to patients who give informed consent to participate in a formal prospective study.

REFERENCES

1 Williamson LM, Hackett G, Rennie J, Palmer CR, Maciver C, Hadfield R et al. The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening. *Br J Haematol* 1998; 92: 2280-2287.

2 Jaegtvik S, Husebekk A, Aune B, Oian P, Dahl LB, Skogen B. Neonatal alloimmune thrombocytopenia due to anti-HPA 1a antibodies; the level of maternal antibodies predicts the severity of thrombocytopenia in the newborn. *BJOG* 2000; 107: 691-694.

3 Turner ML, Bessos H, Fagge T, et al. Prospective epidemiologic study of the outcome and cost-effectiveness of antenatal screening to detect neonatal alloimmune thrombocytopenia due to anti-HPA-1a. *Transfusion* 2005; 45: 1945-1956.

4 Bussel JB, Richard MD, Berkowitz L, et al. Antenatal treatment of neonatal alloimmune thrombocytopenia. *N Engl J Med* 1988; 319: 1374–1378.

5 Van den Akker E, Oepkes D, Lopriore E, et al. Noninvasive antenatal management of fetal and neonatal alloimmune thrombocytopenia: safe and effective. *BJOG* 2007; 114: 469-473.

6 Ghevaert C, Campbell K, Walton J, Smith GA, Allen D, Williamson LM, Ouwehand WH, Ranasinghe E. Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. *Transfusion* 2007; 47: 901-910.

7 Radder CM, Kanhai HH, Brand A. On the mechanism of high dose maternal intravenous immunoglobulin (IVIG) in alloimmune thrombocytopenia. In: Management of fetal alloimmune thrombocytopenia. Amsterdam: Print Partners Ipskamp; 2004. p69-81.

8 Cantú TG, Hoehn-Saric EW, Burgess KM, Racusen L, Scheel PJ. Acute renal failure associated with immunoglobulin therapy. *Am J Kidney Dis*. 1995; 25: 228-234.

9 Radder CM, de Haan MJJ, Brand A, Stoelhorst GMSJ, Veen S, Kanhai HHH. Follow up of children after antenatal treatment for alloimmune thrombocytopenia. *Early Hum Dev* 2004; 80: 65-76.

10 Kiefel V, Santoso S, Weisheit M. Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): A new tool for the identification of platelet reactive antibodies. *Blood* 1987; 70: 1722–1726.

11 Bertrand G, Jallu V, Gouet M, Kjaer KM, Lambin P, Husebekk A, Kaplan C. Quantification of human platelet antigen-1a antibodies with the monoclonal antibody immobilization of platelet antigens procedure. *Transfusion* 2005; 45: 1319-1323.

12 Ware JH, Antman EM. Equivalence trials. *N Engl J Med* 1997; 337: 1159-1161.

13 Chen X. A quasi-exact method for the confidence intervals of the difference of two independent binomial proportions in small sample cases. *Stat Med* 2002; 21: 943-956.

14 Black CM, Plikaytis BD, Wells TW, Ramirez RM, Carlone GM, Chilmonczyk BA, Reimer CB. Two-site immunoenzymometrc assays for serum IgG subclass infant/maternal ratios at full-term. *J Immunol Methods* 1988; 21: 71-81.

15 Kahn KS, Hills R. Can we trust the results of trials that are stopped early? *BJOG* 2006; 113: 766– 768.

16 Berkowitz RL, Lesser ML, McFarland JG, Wissert M, Primiani A, Hung C, Bussel, JB. Antepartum treatment without early cordocentesis for standard-risk alloimmune thrombocytopenia. A randomized controlled trial. *Obstet Gynecol* 2007; 110: 249-255.

17 Herman JH, Jumbelic MI, Ancona RJ, et al. In utero cerebral hemorrhage in alloimmune thrombocytopenia. *Am J Pedaitr Hemotol Oncol* 1986; 8: 312-317.

18 Bussel JB, Zabusky MR, Berkowitz RL, et al. Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997; 337: 22–24.

19 Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust N Z J Obstet Gynaecol* 2001; 41: 45-45.

20 Bussel JB, Berkowitz RL, Lynch L et al. Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: A randomized trial of the addition of low-dose steroid to intravenous gammaglobulin. *Am J Obstet Gynecol* 1996; 174: 1414–1423.

21 Ryment, R., Brunskill, S.J., Stanworth, S., Soothill, P.W., Roberts, D.J. Murphy, M.F. Antenatal interventions for fetomaternal alloimmune thrombocytopenia. *Cochrane Database of Systematic Reviews* 2005; 25, CD004226.

22 Berkowitz RL, Kolb EA, McFarland JG, et al. Parallel Randomized Trials of Risk-Based Therapy for Fetal Alloimmune Thrombocytopenia. *Obstet Gynecol* 2006; 107: 91–96.

23 Urbaniak SJ, Duncan JI, Armstrong-Fisher SS, Abramovich DR, Page KR. Variable inhibition of placental IgG transfer in vitro with commercial IVgG preparations. *Br J Haematol* 1999; 107: 815-817.

24 Radder CM, Beekhuizen H, Kanhai HHH, Brand A. Effect of maternal anti-HPA-1a antibodies and polyclonal IVIG on the activation status of vascular endothelial cells. *Clin Exp Immunol* 2004; 137: 216-222.

25 Cederqvist LL, Caprio RE, Rappaport I, Litwin SD, Abdel-Latif N, Bhalla RB. Correlation between maternal and fetal immunoglobulin E levels. *Obstet Gynecol* 1984; 63: 674-676.

Chapter 7

Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies

Van den Akker ESA, De Haan TR, Lopriore E, Brand, Kanhai HHH, Oepkes D. Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies. *Submitted for publication*.

ABSTRACT

Objective To evaluate the clinical significance of fetal thrombocytopenia in Rhesus D alloimmunized pregnancies.

Study design Fetal platelet counts were measured before 914 intrauterine blood transfusions in 318 Rhesus D alloimmunized pregnancies, and correlated with the presence and severity of hydrops, hemorrhagic complications and perinatal outcome.

Results Severe thrombocytopenia (platelet count < 50 x 10^9/L) was found in 25/914 (3%) of all fetal blood samplings (22 fetuses), and in 7/30 (23%) severely hydropic fetuses. Prolonged bleeding (> 300 sec) occurred in 14/215 (7%) cases of transamniotic blood sampling, all with platelets > 50 x 10^9/L. In 5/23 (22%) fetuses with hemorrhagic complications platelet count was < 50 x 10^9/L . Perinatal mortality in fetuses with severe thrombocytopenia was 8/22 (36%).

Conclusion Thrombocytopenia is common in hydropic anemic fetuses. Severe thrombocytopenia is associated with a poor prognosis, irrespective of the presence of hydrops. The option of having platelets available at blood transfusion in severely hydropic anemic fetuses needs further study.

INTRODUCTION

Rhesus D alloimmunization in pregnancy may cause destruction of fetal red cells with progressive fetal anemia, which, untreated, may lead to fetal hydrops and perinatal death. As Rhesus D antigens are exclusively expressed on the surface of the red cells, all sequellae of Rhesus D antibodies are direct consequences of the resulting fetal anemia and hemolysis. In our 20-year experience of treating Rh-alloimmunized pregnancies with intrauterine intravascular blood transfusion for Rhesus D induced fetal anemia, we encountered a number of anemic fetuses in which we additionally, and mostly unexpectedly, found severe thrombocytopenia. A few studies suggested an association between low platelet counts and fetal hydrops[1-3].

Fetal thrombocytopenia may have grave consequences, such as intracranial hemorrhage (ICH) and prolonged, possibly life-threatening bleeding from the puncture site in the umbilical cord. Although thrombocytopenia is generally defined as a platelet count below 150 x 10^9/L, for severe thrombocytopenia with a risk for bleeding problems, a cut-off level of 50 x 10^9/L is commonly used[2,4,5]. Some authors recommended that intrauterine blood transfusions for fetal anemia should be combined with a platelet transfusion when fetal platelets were below 50 x 10^9/L[2].

The aim of our study was to evaluate the incidence and clinical consequences of severe fetal thrombocytopenia in pregnancies complicated by Rhesus D alloimmunization.

MATERIALS AND METHODS

Since 1965, the Leiden University Medical Center is the national referral center for the intrauterine treatment of fetal anemia in The Netherlands. We retrospectively evaluated prospectively collected data from all patients with red cell alloantibodies treated with one or more intrauterine transfusions from January 1988 till December 2005. From this cohort, we selected pregnancies with anti-Rhesus D (including a combination of anti-D and anti-C) alloantibodies in which fetal blood sampling was performed for suspected anemia, with known platelet counts. In a previous study, we found that in our series of Kell alloimmunized pregnancies, severe fetal thrombocytopenia did not occur[6].

All fetal blood samplings were done by inserting a 22 or 20 G needle under continuous ultrasound guidance into the umbilical vein. A sample of 2-3 mL of

pure fetal blood was taken, followed by injection of saline to confirm correct placement of the needle tip. From the sample, 0.2 mL was immediately aspirated into a Sysmex F800 micro cell counter (C.Goffin, IJsselstein, The Netherlands) present in the procedure room, for assessment of hemoglobin concentration, hematocrit, MCV, and platelet count. Another 0.5 mL was collected into an ethylenediaminetetra-acetic acid solution and immediately sent to the hospital's central hematology laboratory for the same measurements and reticulocyte and erythroblast counts. In case of an automated platelet count < 100 x 10 ^{9}/L, a manual count was done. For this study, we used the data obtained from the central laboratory.

Within one minute following the fetal blood sampling, the fetal hemoglobin and hematocrit levels were available from the cell counter, and packed donor red cells were transfused until the desired post transfusion hematocrit of around 45% was reached. Details on our treatment method have been described previously[7]. Transfusion number, gestational age and presence of hydrops at the time of the fetal blood sampling were recorded. Hydrops was further subdivided into mild or severe hydrops, according to previously described criteria[8]. In summary, the presence of a distinct rim of ascites with or without pericardial effusion, hydrops was classified as early or mild hydrops. Hydrops was classified as severe when ascites was abundant (free floating intra-abdominal organs) with or without pericardial effusion, skin edema, and pleural effusion.

Possible associations between severe thrombocytopenia and hydrops, hemorrhagic complications and perinatal outcome were assessed. Hemorrhagic complications were defined as fetal distress during fetal blood sampling with evidence of prolonged bleeding or formation of a hematoma, or evidence of fetal or neonatal ICH. In most cases, successful transfusion causes hydrops to resolve, therefore we assessed the occurrence of thrombocytopenia according to the severity of hydrops using only data from the first fetal blood sampling.

In transfusions performed via transamniotic needling of the cord, the time of bleeding visible on ultrasound from the puncture site was measured after removing of the needle. We investigated a possible association between the bleeding time and the degree of thrombocytopenia.

STATISTICAL ANALYSIS

The association between fetal hemoglobin concentration and fetal platelet count was analyzed using correlation and regression analysis. The platelet counts be-

tween the nonhydropic group, the mild hydrops group and the severe hydrops group were analyzed using analysis of variance followed by pair wise comparisons using the Mann-Whitney test, with *p*-value of less than 0.01 was considered statistically significant, to allow for multiple comparisons. Perinatal mortality rates between the severe thrombocytopenia groups and the other pregnancies were compared using the Fisher exact test.

RESULTS

A total of 982 fetal blood samples were performed in 318 pregnancies with Rhesus D alloimmunization. Platelet count data were unavailable from 68 procedures, leaving 914 blood sampling procedures for analysis. None of the women or fetuses suffered from other diseases known to be associated with fetal thrombocytopenia, such as infection, immune thrombocytopenic purpura, alloimmune thrombocytopenia or chromosomal abnormalities[4]. Fetal hydrops was present in 88/318 (28%) of the Rhesus D alloimmunized pregnancies at the time of the first fetal blood sampling. In 30/318 (9%) the hydrops was classified as severe. Three fetuses were still hydropic at the second transfusion, with severe hydrops in two of them. A median of three transfusions were performed per pregnancy, with a range of 1 to 8. Overall perinatal survival was 296/318 (93%). Demographic characteristics and outcome of pregnancies of the cohort, divided into three groups according to the severity of hydrops, are summarized in Table 1.

Table 1: Demographic characteristics of 318 pregnancies complicated by RhD alloimmunization

	Nonhydropic	Mildly hydropic	Severely hydropic	*P*-value
	n=230	*n*=58	*n*=30	
Gestational age at first IUT (weeks)	28 (16-35)	25 (17-34)	26 (17-34)	0.006
No of IUT	3 (1-8)	3 (1-6)	3 (1-6)	0.003
Gestational age at birth (weeks)	36 (23-38)	35 (26-37)	34 (18-38)	0.001
Vaginal birth, *n* (%)	143 (62)	37 (64)	14 (47)	0.233
Birth weight (gram)	2690 (650-3930)	2665 (1238-3695)	2312 (190-3600)	0.066
Antenatal death, *n* (%)	6 (3)	3 (5)	6 (20)	0.001
Overall survival, *n* (%)	221 (96)	55 (95)	20 (67)	0.001

Values given as median (range) or actual numbers (percentage)
IUT: intrauterine transfusion

Fetal thrombocytopenia (platelet count < 150 x 10^9/L) was found in 241/914 (26%) fetal blood samples. Moderate thrombocytopenia (< 100 x 10^9/L) was present in 85/914 (9%) fetal blood samples, while in 25/914 (3%) platelet counts were below 50 x 10^9/L. These 25 fetal blood samples were taken from 22 fetuses. In 12 cases, severe thrombocytopenia was found at the first fetal blood sampling, in 2 cases at the second, in 6 cases at the third, in 1 case at the fourth and in 1 case at the fifth fetal blood sampling. In Table 2, the fetal platelet counts at the first fetal blood samplings are given and compared according to the severity of hydrops. The median platelet count was significantly lower in the severe hydrops group, compared to the other two groups (p < 0.01).

Table 2: Fetal platelet count and hemoglobin concentration at the first fetal blood sampling in 318 RhD alloimmunized pregnancies according to the severity of hydrops

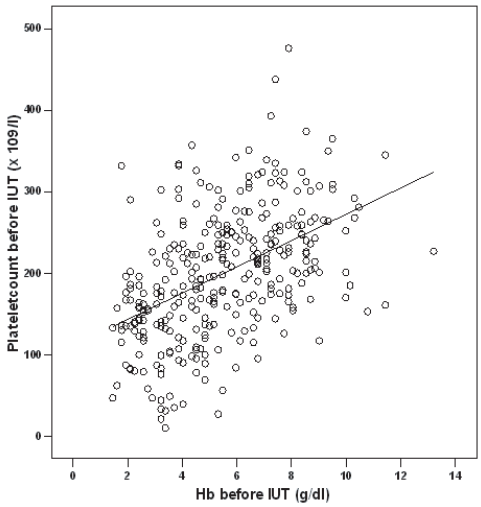
	Nonhydropic	Mildly hydropic	Severely hydropic	*P*-value
	n=230	*n*=58	*n*=30	
Platelet count (x 10^9/L)	219 (3-476)	156 (47-334)	99 (10-332)	0.001
Platelet count < 150 x 10^9/L, *n* (%)	33 (14)	28 (48)	23 (77)	0.001
Platelet count < 50 x 10^9/L, *n* (%)	3 (1)	2 (3)	7 (23)	0.001
Hemoglobin conc. (g/dL)	6.2 (1.9-13.2	3.5 (1.5-8.7)	3.0 (1.5-4.8)	0.001

Values given as median (range) or actual numbers (percentage)

Figure 1 shows the correlation between the fetal platelet count and the hemoglobin concentration at first fetal blood sampling for the complete cohort (R^2 =0.224, p < 0.001).

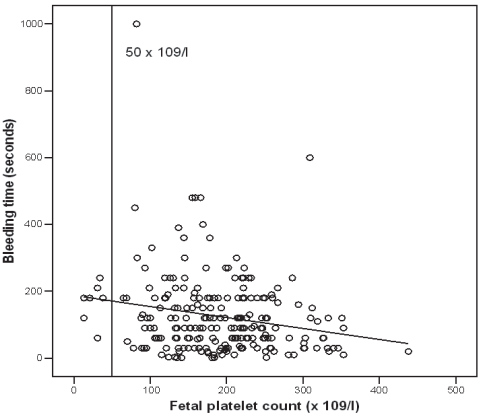
In Figure 2, the duration of bleeding from the puncture site as seen on ultrasound is given in relation to the platelet count before intrauterine transfusion in cases where transamniotic cord needling was done and platelet counts were available (n = 215). The correlation is weak but statistically significant (R^2 = 0.042, p = 0.003). The mean time of visible bleeding was 135 seconds (range 1-600). In 14 fetuses, prolonged bleeding occurred, defined as a duration of > 300 sec[2], all had platelet counts above 50 x 10^9/L. The 7 fetuses with platelet counts below 50 x 10^9/L had a median bleeding time of 180 sec, range 60-240, which was not different from the group of fetuses with platelet counts > 50 x 10^9/L (p = 0.62).

Figure 1: Correlation between fetal platelet count and fetal hemoglobin concentration at the time of the first fetal blood sampling in 318 RhD alloimmunized pregnancies



R^2 Linear = 0.224
$p < 0.001$
Hb fetal hemoglobin concentration
IUT intrauterine transfusion

Figure 2: Correlation between fetal platelet count before intrauterine blood transfusion and the duration of bleeding from the puncture site as seen on ultrasound, in 215 cases of transamniotic fetal blood sampling in RhD alloimmunized pregnancies



R^2 = 0.042
p = 0.003

In a previous study, we analyzed complications of intrauterine transfusions, dividing them in procedure-related or disease-related complications[9]. Using the same definitions, we found 23 procedure-related complications in this cohort of 914 transfusions (3%). In five of these 23 cases (22%), fetal platelet count was found to be below 50 x 10^9/L. In 891 transfusions without procedure-related complications, severe fetal thrombocytopenia was found in 20 blood samples (2%) ($p < 0.001$). Details of the 5 complications associated with low platelet counts were analyzed further, revealing that 3/5 showed excessive hemorrhage or hematoma formation had occurred. One of these survived after an emergency caesarean section at 34 weeks. This neonate had a small grade I bleeding and adverse neurological outcome, possibly related to perinatal asphyxia which caused infantile encephalopathy.

In the group of survivors with fetal thrombocytopenia during at least one fetal blood sampling, 2/14 (14%) had an ICH at cranial ultrasound in the first week of life, versus 4/282 (1%) in the group of fetuses without a severe thrombocytopenia ($p < 0.05$). These six ICH's consisted of four small grade I bleedings, possibly related to prematurity, one large combined subarachnoid and tentorial bleeding (in the severe thrombocytopenic group) and one older hematoma located in the left frontal lobe in a neonate with a congenital cerebellum defect (in the non-severe thrombocytopenic group).

Perinatal mortality in the severely thrombocytopenic group at any fetal blood sampling was 8/22 (36%), significantly higher than the mortality of 14/296 (5%) in fetuses with platelet counts that never dropped below 50 x 10^9/L ($p < 0.001$).

In Table 3, the separate and combined effects of fetal thrombocytopenia and hydrops on perinatal mortality are given.

Table 3: Perinatal mortality and severity of hydrops according to fetal platelet count (plt) in pregnancies with RhD alloimmunization

	Plt > 50x 10^9/L Perinatal mortality	Plt < 50x 10^9/L Perinatal mortality	P-value
No hydrops	8/220 (4%)	1/10 (10%)	0.33
Mild hydrops	2/55 (4%)	1/3 (33%)	0.15
Severe hydrops	4/21 (19%)	6/9 (67%)	0.002
All fetuses	14/296 (5%)	8/22 (36%)	<0.001

COMMENT

Our evaluation of a large cohort of severe Rhesus D alloimmunized pregnancies demonstrates that, in addition to low hemoglobin levels, these fetuses may have decreased platelet counts. Fetal thrombocytopenia occurred more often in hydropic anemic fetuses and was inversely correlated with the severity of hydrops. Perinatal mortality was also highly associated with severe thrombocytopenia, as more than a third of the fetuses in this group died, while more than 95% of non-thrombocytopenic fetuses survived.

Our results confirm findings from two earlier smaller studies. Van den Hof and Nicolaides found platelet deficits > 2SD below the mean for gestational age in one third of fetuses with immune hydrops, and in only 2% of nonhydropic anemic fetuses[1]. Saade *et al.* found severe thrombocytopenia in 10% of the hydropic anemic fetuses versus 2% in the non-hydropic group[3].

In our non-hydropic and mildly hydropic groups however, thrombocytopenia was also associated with poor outcome.

In our study, none of the fetuses with prolonged bleeding had severe thrombocytopenia, and in the severely thrombocytopenic group, bleeding time was within the normal limits. A limitation of this analysis was that this measurement was only possible in transamniotic cordocentesis, while we performed most of our transfusions directly in the cord root or in the intrahepatic portion of the umbilical vein, in which case bleeding either does seem to not occur or is less visible on ultrasound. One previous study reported on post puncture bleeding, and showed an increased mean bleeding time in eight procedures with a fetal platelet count below 50 x 10^9/L[2]. Their mean bleeding time after transamniotic umbilical venous cord puncture with a 22 G needle was 144 seconds, remarkably similar to our findings. They also performed *in vitro* experiments, and estimated an expected blood loss from a venous puncture site to be between 5 and 13 mL/min and probably less in vivo[2]. The impact of such blood loss depends on the fetoplacental volume and thus on gestational age. In extreme cases, prolonged bleeding from the puncture site may cause fetal compromise but other factors than thrombocytopenia are apparently involved.

The main clinically relevant question is whether we can prevent deaths or adverse outcome related to fetal thrombocytopenia using intrauterine platelet transfusions, as was suggested by Saade *et al*[3].

One option would be to have platelets available at any intrauterine blood transfusion in a severely hydropic fetus, including the ability to rapidly perform

a platelet count during the procedure. In our series, this would have meant the preparation and costs for 30 platelet transfusions at a total of 914 (3%) red cell transfusions, actually giving the platelets in nine cases to potentially save three of the six fetuses that died, as the other three deaths were not related to hemorrhagic complications.

A second option would be to include also the mildly hydropic cases. This would mean preparing platelets for 88/914 (10%) transfusions, transfusing platelets in three additional cases to possibly save one additional child.

At least the first option seems a reasonable one. However, platelet transfusion itself may be associated with additional complications due to the extra volume given. This may particularly affect severely hydropic, thus already compromised fetuses. Secondly, there is no guarantee that platelet transfusion prevents hemorrhagic complications in fetuses with thrombocytopenia. Adverse outcome in fetuses suffering from alloimmune thrombocytopenia due to bleeding after cordocentesis despite rapid platelet transfusion has been reported[10,11]. Despite the relatively large series of patients, our study cannot provide strong evidence in favor or against prophylactic platelet transfusion in red cell alloimmunization.

Our analysis was not designed to solve the problem of the etiology of thrombocytopenia in anemic fetuses. Both an increased consumption, increased destruction, a decreased production, or a combination may play a role. In most cases severe thrombocytopenia existed already at the first fetal blood sampling. This rules out the important suppressive role of intra uterine platelet transfusion as a cause for fetal thrombocytopenia.

Given the rarity of this disease, the best way to increase our understanding of the various aspects of thrombocytopenia in red cell alloimmunized pregnancies is a prospective international multicenter collaboration.

In conclusion, our study shows that fetuses with severe anemia due to Rhesus D alloimmunization, particularly those with severe hydrops, may show a decreased platelet count. This group of fetuses constitutes the highest risk group for adverse outcome. Clinicians managing these pregnancies may consider having platelets available when transfusing these fetuses, although at present there is no evidence that this policy does more good than harm.

REFERENCES

1. Van den Hof MC, Nicolaides KH. Platelet count in normal, small, and anemic fetuses. *Am J Obstet Gynecol* 1990; 162: 735-739.

2. Segal M, Manning FA, Harman CR, Menticoglou S. Bleeding after intravascular transfusion: experimental and clinical observations. *Am J Obstet Gynecol* 1991; 165: 1414-1418.

3. Saade GR, Moise KJ Jr, Copel JA, Belfort MA, Carpenter RJ Jr. Fetal platelet counts correlate with the severity of the anemia in red-cell alloimmunization. *Obstet Gynecol* 1993; 82: 987-991.

4. Hohlfeld P, Forestier F, Kaplan C, Tissot JD, Daffos F. Fetal thrombocytopenia: a retrospective survey of 5,194 fetal blood samplings. *Blood* 1994; 84: 1851-1856.

5. Jhawar BS, Ranger A, Steven D, Del Maestro RF. Risk factors for intracranial hemorrhage among full-term infants: a case-control study. *Neurosurgery* 2003; 52: 581-590.

6. Van den Akker ESA, Klumper FJCM, Brand A, Kanhai HHH, Oepkes D. Kell alloantibodies in pregnancy do not cause fetal thrombocytopenia. *Vox Sang in press.*

7. Van Kamp IL, Klumper FJCM, Meerman RH, Oepkes D, Scherjon SA, Kanhai HHH. Treatment of fetal anaemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. *Acta Obstet Gynecol Scand* 2004; 83: 731-737.

8. Van Kamp IL, Klumper FJCM, Bakkum RSLA, Oepkes D, Meerman RH, Scherjon SA, Kanhai HH. The severity of immune fetal hydrops is predictive of fetal outcome after intrauterine treatment. *Am J Obstet Gynecol* 2001; 185: 668-673.

9. Van Kamp IL, Klumper FJCM, Oepkes D et al. Complications of intrauterine intravascular transfusions for fetal anemia due to maternal red cell alloimmunization. *Am J Obstet Gynecol* 2005; 192: 171-177.

10. Overton TG, Duncan KR, Jolly M, Letsky E, Fisk NM. Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. *Am J Obstet Gynecol* 2002; 186: 826-831.

11. Berkowitz RL, Kolb EA, McFarland JG, Wissert M, Primani A, Lesser M, Bussel JB. Parallel Randomized Trials of Risk-Based Therapy for Fetal Alloimmune Thrombocytopenia. *Obstet Gynecol* 2006; 107: 91–96.

Chapter 8

Kell alloimmunisation in pregnancy: associated with fetal thrombocytopenia?

Van den Akker ESA, Klumper FJCM, Brand A, Kanhai HHH, Oepkes D. Kell alloimmunisation in pregnancy: associated with fetal thrombocytopenia? *Vox Sanguinis*, 2008, *in press.*

ABSTRACT

Background and objectives Kell haemolytic disease in pregnancies has been suggested to be associated with decreased fetal platelet counts. The aim of this study was to evaluate the incidence and clinical significance of fetal thrombocytopenia in pregnancies complicated by Kell alloimmunisation.

Materials and methods In this retrospective cohort study, fetal platelet counts were performed in 42 pregnancies with severe Kell alloimmunisation prior to the first intrauterine blood transfusion. Platelet counts from 318 first intrauterine transfusions in Rhesus D alloimmunised pregnancies were used as controls.

Results Fetal thrombocytopenia (platelet count < 150 x 10^9/L) was found in 4/42 (10%) in the Kell group and in 84/318 (26%) in the Rhesus D group. None of the fetuses in the Kell alloimmunised pregnancies, including 15 with severe hydrops had a clinically significant thrombocytopenia defined as a platelet count < 50 x 10^9/L. In the Rhesus D alloimmunised pregnancies 2/230 (1%) of the nonhydropic fetuses and 7/30 (23%) of the severely hydropic fetuses had a clinically significant thrombocytopenia.

Conclusion In contrast to fetuses with severe anaemia and hydrops due to Rhesus D alloimmunisation, fetuses with severe anaemia due to Kell alloimmunisation are generally not at risk for substantial thrombocytopenia.

INTRODUCTION

Kell alloantibodies have been shown to cause fetal anaemia by suppressing fetal non-haemoglobinised erythropoiesis[1-3]. In addition, substantial thrombocytopenia was observed by Wagner *et al.* in three fetuses with anaemia due to Kell alloimmunisation, in contrast to absence of thrombocytopenia in five fetuses with anti-Rhesus D induced anaemia[4]. They suggested that Kell blood group antigens are expressed on erythroid progenitors as well as on megakaryocyte progenitors. However, other investigators could not confirm expression of Kell antigens on myeloid and/or megakaryocyte precursor cells[3,5]. Severe fetal thrombocytopenia is associated with intracranial haemorrhage often resulting in severe handicaps, and may enhance bleeding after intrauterine vascular puncture. As a consequence protocols for intrauterine transfusions require availability of platelets in case of a high probability of fetal thrombocytopenia.

Since the risk of thrombocytopenia in Kell haemolytic disease is unknown, the aim of our study was to evaluate the incidence and clinical significance of fetal thrombocytopenia in pregnancies complicated by Kell alloimmunisation.

METHODS

Since 1965, the Leiden University Medical Centre (LUMC) is the national referral centre for the management and intrauterine treatment of fetal anaemia. We prospectively collected data from all patients with red cell alloantibodies treated with one or more intrauterine transfusions from January 1988 till December 2005. From this cohort, we selected pregnancies with Kell alloantibodies and a confirmed Kell-positive fetus with a known fetal platelet count from fetal blood sampling done for suspected fetal anaemia. As a control group, we selected from the same time period pregnancies with anti-Rhesus D (including a combination of anti-Rhesus D and anti-Rhesus C) alloantibodies in which fetal blood sampling was performed for suspected anaemia and with known platelet counts. Only data from the fetal blood sampling, prior to the first transfusion, were used.

In the LUMC, there is the policy that platelets for transfusion are available in expected cases of (severely) hydropic anaemic fetus and in suspected Parvovirus B19 cases. All fetal blood samplings were done by inserting a 22 or 20 G needle under continuous ultrasound guidance into the umbilical vein. A sample of 2-3 mL of pure fetal blood was taken, followed by injection of saline to confirm correct placement of the needle tip. From the sample, 0.2 mL was imme-

diately aspirated into a Sysmex F800 micro cell counter (C.Goffin, IJsselstein, The Netherlands) present in the procedure room, for assessment of haemoglobin concentration, hematocrit, MCV, and platelet count. Another 0.5 mL was collected into an ethylenediaminetetra-acetic acid solution and immediately sent to the hospital's central haematology laboratory for the same measurements and reticulocyte and erythroblast counts. In case of an automated platelet count < 100 x 10^9/L, a manual count was done. For this study, we used the data obtained from the central laboratory.

Within one minute following the fetal blood sampling, the fetal haemoglobin and hematocrit levels were available from the cell counter, and packed donor red cells were transfused until the desired post transfusion hematocrit of around 45% was reached. Details on our management of red cell alloimmunised pregnancies were described previously[6]. Gestational age, presence or absence of hydrops and estimated fetal weight at the time of the first fetal blood sampling were recorded. Hydrops was further subdivided into mild or severe hydrops, according to previously described criteria[7].

Although thrombocytopenia is defined by a platelet count below 150 x 10^9/L, most studies on fetal thrombocytopenia use a cut-off level of 100 x 10^9/L. To assess the clinical relevance of the problem, severe thrombocytopenia with a risk for bleeding problems, a cut-off level for fetuses with platelet counts below 50 x 10^9/L was used. We analysed the data using all three cut-off levels.

Descriptive statistics are presented as median and range for continuous variables and percentage for categorical data. *P*-values were estimated by Kruskal-Wallis test, and for all categorical data, by chi-square test.

Statistical analysis of the differences in haematological parameters between the Kell group and the Rhesus D group was done using Mann - Whitney U test or Fisher Exact Probability test where appropriate. A *P* value of 0.05 or less was considered statistically significant. All statistical analyses were performed using SPSS 15.0 (SPSS Inc.,Chicago, IL).

RESULTS

A total of 360 fetal blood samples were analysed, from 42 pregnancies with Kell alloimmunisation and 318 pregnancies with Rhesus D alloimmunisation. Demographic characteristics of the two groups are summarised in Table 1. As can be seen in Table 2, the prevalence of fetal thrombocytopenia (platelet count < 150 x 10^9/L) at the first fetal blood sampling was 4/42 (10%) in the Kell group and

Table 1: Characteristics of pregnancies complicated by Kell and Rhesus D alloimmunisation evaluated using fetal blood sampling for suspected fetal anaemia

	Kell (*n*=42)	Rhesus D (*n*=318)	*P*-value
Gravida	3 (1-11)	3 (1-14)	0.148
Gestational age at first IUT(weeks)	24 (18-30)	27 (16-35)	< 0.001
No. of IUT	4 (1-7)	3(1-8)	0.002
Perinatal mortality, *n* (%)	7 (17%)	22 (7%)	0.038
Gestational age at birth (weeks)	34.6 (24-37)	34.9 (18-38)	0.673
Vaginal birth, *n* (%)	31 (74%)	182 (57%)	0.045
Birth weight (gram)	2828 (840-3955)	2628 (190-3930)	0.024

IUT: intrauterine transfusion
Values given as median (range) or actual numbers (percentage).

Table 2: Fetal platelet count and other haematologic parameters at the time of the first fetal blood sampling for suspected fetal anaemia in Kell and Rhesus D alloimmunised pregnancies.

	Kell (*n*=42)	Rhesus D (*n*=318)	*P*-value
Platelet count x 10^9/L	275 (55-450)	199 (10-476)	<0.001
Platelet count <50 x 10^9/L, *n* (%)	0	11 (3%)	0.250
Platelet count <100 x 10^9/L, *n* (%)	1 (2%)	32 (10%)	0.066
Platelet count <150 x 10^9/L, *n* (%)	4 (10%)	84 (26%)	0.007
Haemoglobin conc. (g/dl)	3.5 (1.1-11.9)	5.3 (1.5-13.2)	<0.001
Reticulocyte count	0 (0-109)	0.3 (0-635)	<0.001
per 1000 red blood cells			
(Kell: *n*=37, Rhesus D: n=248)			
Erythroblasts	15 (0-4420)	39 (0-2250)	0.059
per 100 white blood cells			
(Kell: *n*=40, Rhesus D: n=268)			

Values given as median (range) or actual numbers (percentage).

84/318 (26%) in the Rhesus D group. The median platelet count was significantly lower in the Rhesus D group. In contrast, the haemoglobin concentration in the fetus was slightly but significantly lower in the Kell group compared to the Rhesus D group. The reticulocyte counts were significantly lower in the Kell group. The differences in erythroblast count were not statistically significant.

Severe fetal hydrops was present in 15/42 (36%) of the Kell alloimmunised pregnancies, and in 30/318 (9%) of the Rhesus D alloimmunised pregnancies at the time of the first fetal blood sampling.(Table 3) Mild hydrops was present in nine fetuses in the Kell group and in 58 fetuses in the Rhesus D group. In fetuses with severe hydrops, platelet counts were significantly lower both in the Kell and the Rhesus D group as compared to the nonhydropic or mildly hydropic fetuses. For the Kell group, the median platelet count in the severe hydropic group was 186 x 10^9/L (range 55-414) and in the nonhydropic group 326 x 10^9/L (range 148-429) (P=0.005). Only one Kell fetus (2%) showed a definitely too low platelet count (55 x 10^9/L). This severely hydropic fetus was severely anaemic as well at the time of the first fetal blood sampling (2.6 g/dL), but the neonate survived without any complications after four intrauterine transfusion and one exchange transfusion after birth. For Rhesus D group, the median platelet count in the severe hydropic group was 99 x 10^9/L (range 10-332) and in the nonhydropic group 219 x 10^9/L (range 27-476) ($P < 0.001$). From the 15 Kell alloimmunised pregnancies with severe hydrops, 12 neonates survived, two died antenatal and one died two months after birth. This baby was born vaginally after a gestational age of 29 weeks after spontaneous rupture of membranes and died after 68 days of severe pulmonary hypertension and cardiac failure. From the 30 Rhesus D alloimmunised pregnancies with severe hydrops, 20 neonates survived, six died antenatally and four neonates died after birth. Two of them after an emergency caesarean section after complications caused by the intra uterine transfusion. The other two because of pulmonary complications and cardiac failure often seen in neonates with severe hydrops.

Clinically significant thrombocytopenia, defined as a platelet count < 50 x 10^9/L, occurred only in the Rhesus D alloimmunised pregnancies in 2/230 (1%) of the nonhydropic fetuses, and in 7/30 (23%) of the severely hydropic fetuses ($P < 0.001$).

Table 3: Fetal platelet counts and outcome in Kell and Rhesus D alloimmunised pregnancies, in fetuses with severe hydrops at the time of the first fetal blood sampling

	Kell (*n*=15)	Rhesus D (*n*=30)	*P*-value
Platelet count (x 10^9/L)	186 (55-414)	99 (10-332)	<0.005
Platelet count <50 x 10^9/L, *n* (%)	0	7 (23%)	<0.05
Platelet count <100 x 10^9/L, *n* (%)	1 (7%)	15 (50%)	<0.005
Platelet count <150 x 10^9/L, *n* (%)	4 (27%)	23 (77%)	<0.005
Gestational age at first IUT (weeks)	23 (18-31)	26 (17-34)	0.081
No. of IUT	4 (2-7)	3 (1-6)	<0.05
Gestational age at birth (weeks)	36 (28-38)	34 (18-38)	0.109
Vaginal birth, n (%)	10 (67%)	14 (47%)	0.171
Birth weight (gram)	2860 (2000-3270)	2312 (190-3600)	<0.05
Antenatal death, *n* (%)	2 (13%)	6 (20%)	0.458
Overall survival, *n* (%)	12 (80%)	20 (67%)	0.285
Postnatal death, *n* (%)	1 (7%)	4 (13%)	0.511

Values given as median (range) or actual numbers (percentage)
IUT: intrauterine transfusion

DISCUSSION

Our study confirmed that fetal anaemia due to red cell alloimmunisation can be associated with low fetal platelet counts. Fetal thrombocytopenia below 150 x 10^9/L, occurred in more than 25% of anaemic fetuses with Rhesus D alloimmunisation and in 10% of cases with Kell haemolytic disease. Clinically significant thrombocytopenia however, with a risk for intracranial or other bleeding, defined as a platelet count < 50 x 10^9/L was only found in fetuses with Rhesus D related anaemia.

These results differ from observations by Wagner *et al.* who observed platelets counts ≤ 70 x 10^9/L in three fetuses with Kell haemolytic disease and a mean platelet count of 252 ± 15 x 10^9/L in five fetuses with an anaemia due to Rhesus D alloimmunisation[4]. Our much larger series of consecutive patients represents an unselected population. The main difference between this study and the observations by Wagner *et al.* was that the majority of the fetuses in this study affected by Kell haemolytic disease did not have thrombocytopenia of any degree. Our

observation that Kell alloantibodies are not associated with severe fetal thrombocytopenia is in agreement with *in-vitro* studies showing that although Kell antigens are expressed very early in the erythroid lineage, Kell antibodies exclusively inhibit the growth of the erythroid progenitor cells and spares nonerythroid haematopoietic lineages[3,5]. The observed deeply depressed erythropoiesis, reflected by low or absent reticulocytes and erythroblast in the majority of fetuses in the Kell group is in accordance with the in-vitro observed wide range of erythroid inhibition with different anti-K sera[3].

Our most relevant finding for clinical practice is the absence of platelet counts below 50 x 10^9/L in any fetus with Kell induced anaemia. This is the generally accepted cut-off, above which the risk of bleeding is negligible[10,11]. Even severe hydrops due to Kell alloimmunisation in our series was not associated with severe low platelet counts. This is in contrast to findings in Rhesus D alloimmunisation, both in our current control group as well as in previously published series, in which between 10% to 33% of hydropic fetuses were thrombocytopenic[9,10,12]. This intriguing difference deserves further study.

Some authors recommend that in severe Rhesus D alloimmunisation, clinicians should consider having platelets ready at each intrauterine blood transfusion in hydropic anaemic fetuses[9,10,12]. For safe fetal blood transfusion in Kell induced anaemia, our data suggest that there seems to be no need to be prepared for additional platelet transfusion. These findings should obviously be interpreted with caution because of the limited sample size.

Perinatal survival is significantly worse in case of severe fetal immune hydrops, and the underlying mechanisms are still poorly understood[7,13]. Since in our relatively large series, both Kell and Rhesus D had similar poor survival rates in case of severe hydrops, it seems unlikely that a low platelet count is a major contributor to this poor outcome. More research is needed to provide insight in the aetiology of fetal thrombocytopenia in Rhesus D alloimmunisation and especially in fetal hydrops.

In conclusion, our study shows that fetuses with severe anaemia due to Kell alloimmunisation are generally not at risk for substantial thrombocytopenia.

REFERENCES

1. Vaughan JI, Warwick R, Letsky E, Nicolini U, Rodeck CH, Fisk NM. Erythropoietic suppression in fetal anaemia because of Kell alloimmunization. *Am J Obstet Gynecol* 1994; 171: 247-252.

2. Weiner CP, Widness JA. Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anaemia. *Am J Obstet Gynecol* 1996; 174: 547-551.

3. Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anaemia. *N Engl J Med* 1998; 338: 798-803.

4. Wagner T, Bernaschek G, Geissler K. Inhibition of megakaryopoiesis by Kell-related antibodies. *N Engl J Med* 2000; 343: 72.

5. Pu JJ, Redman CM, Visser JW, Lee S. Onset of expression of the components of the Kell blood group complex. *Transfusion* 2005; 45: 969-974.

6. Van Kamp IL, Klumper FJCM, Meerman RH, Oepkes D, Scherjon SA, Kanhai HHH. Treatment of fetal anaemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. *Acta Obstet Gynecol Scan* 2004; 83: 731-737.

7. Van Kamp IL, Klumper FJCM, Bakkum RSLA, Oepkes D, Meerman RH, Scherjon SA, Kanhai HH. The severity of immune fetal hydrops is predictive of fetal outcome after intrauterine treatment. *Am J Obstet Gynecol* 2001; 185: 668-673.

8. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell isoimmunisation. *Lancet* 1988; 1:1073-1075.

9. Van den Hof MC, Nicolaides KH. Platelet count in normal, small, and anaemic fetuses. *Am J Obstet Gynecol* 1990; 162: 735-739.

10. Segal M, Manning FA, Harman CR, Menticoglou S. Bleeding after intravascular transfusion: experimental and clinical observations. *Am J Obstet Gynecol* 1991; 165: 1414-1418.

11. Jhawar BS, Ranger A, Steven D, Del Maestro RF. Risk factors for intracranial hemorrhage among full-term infants: a case-control study. *Neurosurgery* 2003; 52: 581-590.

12. Saade GR, Moise KJ Jr, Copel JA, Belfort MA, Carpenter RJ Jr. Fetal platelet counts correlate with the severity of the anaemia in red-cell alloimmunization. *Obstet Gynecol* 1993; 82 987-991.

13. De Groot CJM, Oepkes D, Egberts J, Kanhai HHH. Evidence of endothelium involvement in the pathophysiology of hydrops fetalis? *Early Hum Dev* 2000; 57: 205–209.

Chapter 9

Thrombocytopenia in hydropic fetuses with parvovirus B19 infection

De Haan TR, Van den Akker ESA, Porcelijn L, Oepkes D, Kroes ACM, Walther FJ. Thrombocytopenia in hydropic fetuses with parvovirus B19 infection: Incidence, treatment and correlation with fetal B19 viral load. *British Journal of Obstetrics and Gynaecology* 2008, 115: 76-81.

ABSTRACT

Objective To examine (1) the incidence of fetal thrombocytopenia in hydropic fetuses with congenital B19 virus infection, (2) the effect of intrauterine platelet transfusions and (3) the correlation between fetal B19 viral load and severity of thrombocytopenia.

Design Retrospective analysis of data from prospectively collected fetal blood samples.

Setting Leiden University Medical Centre, the national centre for management of intrauterine fetal disease in the Netherlands.

Population Thirty hydropic fetuses treated with intrauterine red blood cell and platelet transfusions for human B19 virus-induced severe fetal anaemia and thrombocytopenia over a 10-year period.

Methods Fetal blood samples (n = 30) taken before and after intrauterine transfusion were investigated. No cases were excluded, and there was no loss to follow up.

Main outcome measures Parameters recorded were gestational age, experienced fetal movements, gravidity and parity, severity of fetal hydrops, severity of fetal anaemia and thrombocytopenia and megakaryocyte and reticulocyte counts. Survival and procedure-associated complications were documented. Quantitative B19 viral load measurements were performed on all fetal samples.

Results Forty-six percent of all hydropic fetuses showed severe thrombocytopenia. No antenatal intracerebral haemorrhage or procedure-associated bleeding occurred. Overall, survival was 77%. Platelet counts increased following platelet transfusion and decreased significantly following red blood cell transfusion alone. No correlation was found between fetal viral loads and platelet counts.

Conclusion Thrombocytopenia was frequently encountered in fetal B19V infection, but fetal bleeding complications were not noted. Absence of a direct relationship between fetal B19 viral load and platelet counts suggests a temporal dissociation between these findings. Dilutional thrombocytopenia is frequently seen

in the fetus following red blood cell transfusion alone. The clinical significance of this phenomenon is unclear. The risk of fluid overload by fetal platelet transfusion in a severely hydropic fetus should be weighed against the low incidence of fetal bleeding complications.

INTRODUCTION

Congenital human parvovirus B19 (B19V) infection may lead to fetal hydrops, anaemia or fetal demise. In a series of 485 cases of fetal hydrops, 7% of all cases were due to congenital B19V infection.[1] Therapeutic intrauterine erythrocyte and platelet transfusion for severe fetal anaemia or thrombocytopenia is feasible using percutaneous umbilical cord blood sampling techniques.[2] The fetal loss rate due to this procedure is approximately 1.6% in experienced hands.[3,4] An increased rate of fetal loss due to exsanguination from the umbilical cord puncture site has been noted in thrombocytopenic fetuses.[5,6] Three studies describe the incidence of fetal thrombocytopenia in B19V infection during gestation.[1,5,7] Segata *et al.*[7] reported demise of two severely thrombocytopenic fetuses due to exsanguination from the umbilical cord puncture site. None of these studies evaluated platelet counts before and after transfusion for each individual case.

The cause of thrombocytopenia in human B19V infection is unclear. B19V shows primary erythroid tropism in bone marrow through the cellular globoside receptor (blood group P antigen).[8–11] Viral genome has been shown in megakaryocytes *in vitro*, but viral replication of B19V in megakaryocytes has not been shown as yet. A significant inhibitory effect of B19V on megakaryocyte colony formation has been observed *in vitro*. The NS1 gene of B19V may be responsible for this direct cytotoxicity to megakaryocytes.[12] An inhibitory effect of this NS1 protein on the colony-forming ability of megakaryocytic progenitor cells *in vivo* was confirmed in an animal model of parvovirus infection. The degree of inhibitory effect was dependent on the parvovirus infective load, and the depletion of megakaryocyte progenitor cells in the bone marrow appeared to coincide with erythroid progenitor depletion.[13] Lesions in cell chromatin and possible induction of apoptotic events may be responsible for this cytotoxic effect.[14]

No studies on correlates of the B19V viral load and B19Vinduced fetal thrombocytopenia has been published yet. This study was conducted to evaluate: (1) the incidence of thrombocytopenia in hydropic fetuses with congenital B19V infection, (2) the correlation between B19 viral load and pre-intrauterine transfusion (IUT) fetal platelet counts, (3) the effect of intrauterine platelet transfusions in

congenital parvovirus B19 infection and (4) the incidence of procedure-associated complications due to thrombocytopenia.

METHODS

Clinical data

The Leiden University Medical Centre, Leiden, is the single national referral centre for the management and intrauterine treatment of fetal anaemia in the Netherlands. We retrospectively studied the prospectively collected clinical and laboratory data from severely hydropic and anaemic fetuses, with a proven B19V infection during gestation from January 1997 until August 2006. As this study was performed on materials collected during intention-to-treat sessions and did not involve any new subject contact, the study was approved by the institutional ethics committee.

From this cohort, all cases requiring an IUT were included. Maternal parameters noted were: maternal age in years, gravidity and parity, fetal movements (or any reduction in fetal movements) as experienced by the mother, gestational age at the time of the IUT session and outcome. The indication to perform fetal blood sampling in all cases was a combination of signs of fetal hydrops and an increased middle cerebral artery peak systolic flow velocity (MCA-PSV) (>1.5 MoM).[15,16]

All fetal blood sampling procedures were performed by inserting a 22- or 20-G needle under continuous ultrasound guidance into the umbilical vein. From the sample, 0.2 ml was immediately aspirated into a Sysmex F800 micro cell counter (Goffin, IJsselstein, The Netherlands), present in the procedure room, for immediate assessment of haemoglobin concentration and platelet values and for immediate decisions on transfusion. Another 0.5 ml was immediately sent to the hospital's central haematology laboratory for confirmation of this measurement and manual differential counts. Fetal haematological parameters, that is haemoglobin (in g/dl), total leucocyte count (x 10^9/l), erythroblast count (/100 white blood cells), reticulocyte count (%), platelet count (x 10^9/l) and the number of circulating megakaryocytes (/100 white blood cells), were noted before and after IUT.

In all cases of this series, red blood cell transfusion was given first. Our protocol for IUT in anaemic and hydropic fetuses known or likely to be due to parvovirus B19 infection includes having platelets ready for transfusion in all cases.

Platelet transfusion is performed when the pretransfusion platelet count is below 50 x 10^9/l using the following equation to determine the volume required:

$$\text{Transfused volume (in ml)} = \frac{\text{Fetal Placental Blood Volume (FPBV) x (desired- current platelet count) x 2}}{\text{Platelet count in transfused concentrate}}$$

The FPBV was calculated by multiplying the estimated fetal weight (in g) by 0.14. The factor 2 is used in the numerator of the equation to allow for possible platelet sequestration in the spleen or liver.[6] Since both fetal haemoglobin concentrations and platelet counts vary with gestational age, reference ranges published by Forestier *et al.*[17] were used. These reference ranges were used to be able to compare our results with those of Schild's group. Mild thrombocytopenia was defined as platelet counts < 150 x 10^9/l, moderate thrombocytopenia as counts < 100 x 10^9/l and severe thrombocytopenia was defined as fetal platelet counts < 50 x 10^9/l.

Virological investigations

All fetal blood samples were evaluated for quantitative B19 viral load. The quantitative real-time polymerase chain reaction (PCR) used in this study has been described before.[18,19] Briefly, DNA was isolated from 200 µl serum using a QIAamp DNA Mini Blood Kit (Roche Applied Science 51106; Roche Diagnostics, Almere, the Netherlands) or a MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche Molecular Diagnostics 3038505). For quantitative detectionof B19V DNA in blood, a real-time PCR assay using Taqman polymerase was developed. Primers were selected on the NS part of the B19V genome. Sensitivity of the real-time B19V DNA PCR was 100 iu/ml by duplo measurements of ten-fold dilutions of WHO B19V DNA international standard[16].

Statistical analysis

Statistical analysis was performed using the SPSS version 11.5.1 data manager program (SPSS 11.5.1; SPSS Inc., Chicago, IL, USA). A *P*-value of < 0.05 was considered statistically significant. As parameters were not distributed normally, correlations were computed using Spearman's ranking coefficient, and differences between pretransfusion and posttransfusion values were calculated using the Wilcoxon signed ranks test. All values are reported as median with minimum and maximum values.

RESULTS

Clinical parameters

During the study period, a total of 30 consecutive cases were included. All cases were diagnosed as congenital parvovirus B19 infection by serology and PCR on maternal and fetal blood samples. Population characteristics are depicted in Table 1. The original data series are shown in Table 2. Twenty-five out of 30 (83%) women reported decreased fetal movements before the IUT session and clearly increased fetal movements after IUT. Median gestational age at IUT was 22.5 weeks (minimum: 18 weeks; maximum: 28 weeks). At ultrasound investigation before IUT, all cases showed a MCA-PSV >1.5 MoM, indicative of severe fetal anaemia. Eight fetuses were diagnosed as mildly hydropic and 22 fetuses were diagnosed as severely hydropic. No fetuses were noted with a raised MCA-PSV without signs of hydrops, indicating the clinical severity of cases in this cohort. Two fetuses died of heart failure and irreversible bradycardia during the IUT session directly following the red blood cell transfusion. There were no cases of haemorrhage from the cordocenteses puncture site in our series. Three fetuses died *in utero* following IUT due to progressive cardiac decompensation (range: one day to four weeks following IUT). Two fetuses died after a failed neonatal resuscitation at 29 and 33 weeks of gestational age following spontaneous premature delivery and initial stabilisation on the neonatal unit. The total mortality in this cohort was 7/30 (23%).

Haematological parameters

Platelets

Table 1 shows the haematological parameters. At blood sampling before IUT, 14/30 (46%) fetuses had severe thrombocytopenia (median platelet value 29 x 10^9/l), 12/30 (40%) had moderate thrombocytopenia (median platelet value 77 x 10^9/l), and 3/30 (10%) had mild thrombocytopenia (median platelet value 131 x 10^9/l). In only one case, normal values were noted. All severely thrombocytopenic fetuses received a platelet transfusion to prevent procedure-associated bleeding. In two cases with moderate thrombocytopenia, the operator chose to transfuse platelets (with platelet counts of 54 and 59 x 10^9/l). Mild thrombocytopenic cases received no transfusions. Although the median platelet value following platelet transfusion exceeded the pretransfusion value, this difference was not statistically significant (P = 0.143). This is probably due to the small sample size. In three cases, platelet values after IUT were unavailable due to needle displacement.

All fetuses receiving red blood cells only (n = 14) showed a significant decrease

Table 1: Maternal characteristics and median fetal blood values at IUT.

	Median	**Minimum and maximum values (range)**
Maternal age (years)	28.0	19.0 – 39.0
Gravidity	3	1 – 4
Parity	1	0 – 3
Gestational age (weeks)	22.5	18.0 –28.0
Hb pre-IUT (g/dl)	3.6	0.9 – 11.0
Hb post-IUT (g/dl)	12.3	1.1 - 18.7
Platelet value pre-IUT (x 10^9/l)	57	4 – 238
Platelet value post-IUT (x 10^9/l)	392	14 – 406
Platelets <50 (x 10^9/l) (*n*=14)	29	4 – 49
Platelets 50-100 (x 10^9/l) (*n*=12)	77	54 – 96
Platelets > 100 (x 10^9/l) (*n*=4)	134	128 – 238
Leukocytes pre-IUT(x 10^9/l)	2.1	0.8 – 20
Megakaryocytes (/100 WBC)	2	0 – 32
Erythroblasts (/100 WBC)	314	24 – 4208
Reticulocytes (in %)	10.1	1.8 – 79.7
Viral load (Log value)	8.1	3.7 - 11.3

Hb: haemoglobin value; Plt: platelet value; IUT: intra-uterine transfusion; Platelet group: values depicted for all cases pre IUT; WBC: white blood cells.
Median values and range.

Table 2: Individual haematological data

GA (weeks)	Pretransfusion values					
	Hb (g/dl)	**Plt (10^9/l)**	**Leuc (10^9/l)**	**Ery (/100 WBC)**	**Mkc (/100 WBC)**	**B19 viral load (log value)**
20	6.5	96	1.2	314	21	NK
22	8.3	76	3.1	842	6	8.80
23	2.4	88	2.8	76	9	9.80
27	11.0	37	3.8	24	1	6.20
20	4.9	87	1.1	824	NK	7.40
23	3.2	70	3.1	1180	32	11.30
25	9.4	238	2.0	303	3	7.00
20	2.4	80	2.0	66	1	NK
22	5.4	54	1.8	479	4	7.20
18	4.0	128	1.4	28	0	11.00
21	2.7	37	2.3	0	1	8.10
22	4.4	48	2.7	83	2	9.60
20	7.5	38	4.3	180	1	8.00
23	4.0	7	2.1	73	1	9.80
28	4.9	137	2.3	213	5	NK
23	4.4	79	4.3	0	0	6.40
26	2.2	32	1.9	481	2	10.00
25	2.5	45	1.6	864	2	9.20
23	4.6	70	20.0	411	10	10.70
24	.96	36	1.2	27	3	7.70
26	1.4	4	1.1	2115	0	7.80
25	2.5	12	0.8	4208	0	3.74
19	1.4	39	1.3	24	0	6.40
25	7.3	96	1.3	64	14	8.00
22	2.8	60	2.6	753	2	8.00
21	5.1	73	2.0	1900	7	7.50
21	3.0	49	3.6	2311	5	10.40
21	1.9	6	NK	NK	NK	8.60
27	2.0	24	2.9	3196	2	8.10
20	3.2	131	1.7	205	0	9.60

D, died; Ery, erythroblast count; GA, gestational age at time of IUT; Hb, haemoglobin value; Leuc, leucocyte cou
S, survived; WBC, white blood cells.

Volume transfused (ml)		Posttransfusion values		
RBC	**Plt**	**Hb post (g/dl)**	**Plt post (10^9/l)**	**S/D**
16	-	13.9	62	S
6	-	13.9	39	S
27	-	9.7	68	S
	14	12.8	214	S
13	-	18.7	19	S
28	NK	11.3	406	S
21	-	12.9	208	S
20	2	11.8	40	S
27	-	NK	NK	S
19	-	17.6	40	S
25	5	NK	NK	S
28	NK	13.4	207	S
42	20	12.3	87	S
40	3	9.7	23	S
87	-	10.8	107	S
24	-	9.7	70	S
52	5	10.5	86	D
69	7	9.7	158	D
38	-	13.4	33	D
NK	-	1.1	14	D
52	7	8.9	106	S
50	5	7.2	50	D
16	2	12.3	122	S
42	10	13.9	194	S
28	5	12.3	182	S
20	4	14.2	72	S
29	4	11.5	217	S
10	5	NK	NK	S
62	-	10.7	17	D
24	-	15.0	35	D

cyte count; Mkc, megakaryocyte count; NK, missing (not known) value; Plt, platelet count; RBC, red blood cells;

in platelet values following transfusion (P = 0.001). In 8/14 (57%) cases, platelet values dropped below 50 x 10^9/L, but in no cases was a platelet transfusion given after red blood cell transfusion for posttransfusion thrombocytopenia. In 22 of all 30 (73%) cases, megakaryocytes were detected in the differential counts. We did not observe a statistically significant correlation between pre-IUT platelet counts and megakaryocyte counts/100WBC (P= 0.123).

Haemoglobin and leucocyte counts

All cases were anaemic with a median pre-IUT haemoglobin value of 3.6 g/dl (minimum: 0.96 g/dl; maximum: 11.0 g/dl). In one case, a pretransfusion haemoglobin level of 11 g/dl was noted in the presence of a raised MCA-PSV and signs of fetal hydrops. We speculated that in this case, the contribution of fetal myocarditis may have outweighed possible earlier effects of fetal anaemia. All remaining 29 fetuses received a red blood cell transfusion with a median post-IUT haemoglobin value of 12.3 g/dl (minimum: 1.12 g/dl; maximum: 18.7 g/dl). The rise in haemoglobin value following transfusion was statistically significant (P < 0.001). A statistically significant correlation existed between the severity of fetal anaemia and the MCA-PSV values (P = 0.007). In 18/30 (60%) cases, erythroblastosis was present according to gestational age corrected reference ranges.[17] Leucocyte and neutrophil counts were all normal when compared with gestational age adjusted reference values.[17]

Quantitative viral load measurements

To evaluate any correlation between the severity of thrombocytopenia and fetal B19 viral load values, all fetal blood samples were tested for B19 viral load (Table 2). Twenty-seven samples were tested; in three samples, insufficient material had been preserved for reliable testing. We found no correlation between fetal quantitative B19 viral load values and fetal pre-IUT platelet counts (P = 0.580) nor did we detect any significant correlation between haemoglobin values and fetal B19 viral load (P = 0.459).

DISCUSSION

In this cohort of pregnancies complicated by parvovirus B19 infection and hydrops, we found a 46% incidence of severe concomitant thrombocytopenia. Combined intrauterine treatment with both red blood cell and platelet transfusion was associated with a survival rate of 77%. These results are in accordance

with the few other series reporting survival rates ranging from 54 to 85% after IUT for parvovirus B19 infection in pregnancy.[1,5,20] Although the mechanisms of disease cannot be fully compared, intrauterine treatment for severe fetal anaemia with hydrops due to red blood cell alloimmunisation is associated with a survival rate of 55%.[16]

Our findings confirm the limited existing data on the association between B19V infection and fetal thrombocytopenia, with incidences ranging from 29 to 64%.[5,7] We realise that our data only apply to the selected group of hydropic fetuses treated with intrauterine blood transfusion. Whether thrombocytopenia occurs in all fetuses affected by parvovirus B19 infection is unknown and difficult if not impossible to determine.

In our report, all 30 cases were evaluated on one occasion only. There were no follow-up transfusions performed. The presented data show that platelet transfusions resulted in an adequate direct increment of the platelet count. However, whether this increase is sustained remains uncertain, as this would require serial sampling procedures with inherent risks.

A clinically important observation was the decrease in platelet count after transfusion of red blood cells alone. In 8/14 cases, posttransfusion platelet counts dropped to values below 50 x 10^9/l. Red blood cell transfusion-related thrombocytopenia has been reported previously, before the routine use of leucocyte depletion of blood components. Platelet counts dropped after transfusion due to adherence of platelets to microaggregates (composed of leucocytes and platelets) in transfused blood.[21] Most centres, including ours, nowadays use leucocyte-depleted and irradiated donor blood to eliminate any leucocyte activity. In this cohort, therefore, another explanation must be considered. In adult patients, dilutional thrombocytopenia during massive erythrocyte transfusion is a common finding.[22–24] This mechanism is counteracted by endogenous platelet release, and standard prophylactic platelet transfusion is considered not warranted.[23,24] In B19V infection with bone marrow depression and a possible reduction in platelet production, this endogenous platelet release may be insufficient. We do think that dilutional thrombocytopenia in B19V-infected fetuses with an already lower platelet count is responsible for the low platelet counts in some of the posttransfusion samples. The clinical significance of this 'dilutional thrombocytopenia' remains to be elucidated. In the cases with severe dilutional thrombocytopenia, 2/8 (25%) fetuses died following intrauterine red blood cell transfusion compared with 5/14 (35%) fetuses with severe thrombocytopenia before IUT. Fetal demise was not due to bleeding complications in any case.

When performing any invasive intervention, benefits should always be weighed against possible complications. Evidence for the need of fetal platelet transfusion for human B19 virus-induced thrombocytopenia is limited. Reviewing the literature on complications of severe thrombocytopenia in fetal B19 virus infection, we found only one report by Segata *et al.*[7] reporting exsanguination of two thrombocytopenic fetuses following cordocentesis for B19V-induced severe fetal anaemia. In a study by Kiefel *et al.*,[25] bleeding complications of cord vessel puncture in fetuses with normal platelet counts were seen in 5% of cases. Paidas *et al.*[26] reported an increased risk of fetal loss related to cordocenteses in alloimmune thrombocytopenic fetuses. However, this is an entirely different condition with a high risk of fetal haemorrhage. Therefore, the need for fetal platelet transfusion in congenital B19V infection remains a matter of debate. In our series of 30 cases, we did not observe any procedure-associated haemorrhage. We also did not note any cases of antenatal or postnatal bleeding complications. As it is our current practice to perform platelet transfusions in all fetuses with pre-IUT platelet counts < 50 x 10^9/l, we are not able to provide evidence on the natural course of severely thrombocytopenic cases in this disease. As the fetus is in a state of severe cardiovascular compromise possibly due to a combination of fetal myocarditis and severe fetal anaemia, platelet transfusion may prove hazardous due to the extra fluid overload. We conclude that the risk of exacerbating cardiovascular compromise should be carefully weighed against the risk of procedure-associated bleeding as described by Segata *et al.*[7] Larger studies are needed to evaluate the incidence of bleeding from the sampling site.

In contrast with reports that speculate about an association between the B19V viral load and thrombocytopenia [12–14], we found no such correlation in our series. This may be due to a temporal dissociation between the peak value of viral DNA and the resulting decline of platelet counts. Another possible result of platelet and megakaryocyte destruction may be the release of internalised thrombopoietin (Tpo) and the decrease of Tpo receptors, resulting in an increased level of free circulatory thrombopoietin. This could compensate for fetal platelet destruction by B19 virus at the time of treatment.[26–30]

We were unable to show any correlation between the pretransfusion platelet count and megakaryocyte count. We speculate that this might be explained to a reduced fetal response to thrombopoietin, since in preterm infants, the response to thrombopoietin was shown to be reduced.[31] This hypothesis warrants further studies. In our series, we did not observe leucopenia or neutropenia, and fetal white blood cell progenitors do not seem to be influenced by congenital B19V

infection.

A limitation of our study is the fact that all values were obtained at one single time point of the total infectious course. Serial fetal sampling would be extremely interesting. However, given the risks, this is not acceptable in continuing pregnancies, and future investigations should be directed towards development of an animal model of congenital B19V infection, with the possibility of serial fetal sampling sessions to answer questions on the course of haematological and virological parameters.

In conclusion, we found that anaemic and hydropic fetuses with a fetal B19V infection have a high risk of concomitant severe thrombocytopenia. There was no correlation between the fetal B19V viral load and the severity of thrombocytopenia. Fetal haemorrhage was not seen as a complication of thrombocytopenia. Intrauterine platelet transfusion can be performed relatively safely, although the risk of fluid overload in the hydropic fetusmay outweigh possible benefits. If fetal treatment consists of transfusion of red blood cells only, posttransfusion dilutional thrombocytopenia occurs in a majority of cases. The clinical significance of this dilutional thrombocytopenia needs to be elucidated.

REFERENCES

1 Schild RL, Bald R, Plath H, Eis-Hubinger AM, Enders G, Hansmann M. Intrauterine management of fetal parvovirus B19 infection. *Ultrasound Obstet Gynecol* 1999;13:161–6.

2 Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling via the umbilical cord using a needle guided by ultrasound. Report of 66 cases. *Prenat Diagn* 1983;3:271–7.

3 Duchatel F, Oury JF, Mennesson B, Muray JM. Complications of diagnostic ultrasound-guided percutaneous umbilical blood sampling: analysis of a series of 341 cases and review of the literature. *Eur J Obstet Gynecol Reprod Biol* 1993;52:95–104.

4 Van Kamp IL, Klumper FJ, Oepkes D, Meerman RH, Scherjon SA, Vandenbussche FP, *et al*. Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red-cell alloimmunization. *Am J Obstet Gynecol* 2005;192:171–7.

5 Forestier F, Tissot JD, Vial Y, Daffos F, Hohlfeld P. Haematological parameters of parvovirus B19 infection in 13 fetuses with hydrops foetalis. *Br J Haematol* 1999;104:925–7.

6 Murphy MF, Waters AH, Doughty HA, Hambley H, Mibashan RS, Nicolaides K, *et al*. Antenatal management of fetomaternal alloimmune thrombocytopenia—report of 15 affected pregnancies. *Transfus Med* 1994;4:281–92.

7 Segata M, Chaoui R, Khalek N, Bahado-Singh R, Paidas MJ, Mari G. Fetal thrombocytopenia secondary to parvovirus infection. *Am J Obstet Gynecol* 2007;196:61–4.

8 Chisaka H, Morita E, Yaegashi N, Sugamura K. Parvovirus B19 and the pathogenesis of anaemia. *Rev Med Virol* 2003;13:347–59.

9 Corcoran A, Doyle S. Advances in the biology, diagnosis and host-pathogen interactions of parvovirus B19. *J Med Microbiol* 2004;53:459–75.

10 Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* 1993;262:114–17.

11 Brown KE, Young NS. Parvoviruses and bone marrow failure. *Stem Cells* 1996;14:151–63.

12 Srivastava A, Bruno E, Briddell R, Cooper R, Srivastava C, van BK, *et al*. Parvovirus B19-induced perturbation of human megakaryocytopoiesis in vitro. *Blood* 1990;76:1997–2004.

13 Lamana ML, Albella B, Bueren JA, Segovia JC. In vitro and in vivo susceptibility of mouse megakaryocytic progenitors to strain i of parvovirus minute virus of mice. *Exp Hematol* 2001;29:1303–9.

14 Op De Beeck A, Caillet-Fauquet P. The NS1 protein of the autonomous parvovirus minute virus of mice blocks cellular DNA replication: a consequence of lesions to the chromatin? *J Virol* 1997;71:5323–9.

15 Mari G, Detti L, Oz U, Zimmerman R, Duerig P, Stefos T. Accurate prediction of fetal hemoglobin by Doppler ultrasonography. *Obstet Gynecol* 2002;99:589–93.

16 van Kamp IL, Klumper FJ, Bakkum RS, Oepkes D, Meerman RH, Scherjon SA, *et al*. The severity of immune fetal hydrops is predictive of fetal outcome after intrauterine treatment. *Am J Obstet Gynecol* 2001;185:668–73.

17 Forestier F, Daffos F, Catherine N, Renard M, Andreux JP. Developmental hematopoiesis in normal human fetal blood. *Blood* 1991; 77:2360–3.

18 Saldanha J, Lelie N, Yu MW, Heath A; B19 Collaborative Study Group. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. *Vox Sang* 2002;82:24–31.

19 Beersma MF, Claas EC, Sopaheluakan T, Kroes AC. Parvovirus B19 viral loads in relation to VP1 and VP2 antibody responses in diagnostic blood samples. *J Clin Virol* 2005;34:71–5.

20 Enders M, Weidner A, Zoellner I, Searle K, Enders G. Fetal morbidity and mortality after human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. *Prenat Diagn* 2004;24:513–8.

21 Schuh A, Atoyebi W, Littlewood T, Hatton C, Bradburn M, Murphy MF. Prevention of worsening of severe thrombocytopenia after red cell transfusions by the use of leucocyte-depleted blood. *Br J Haematol* 2000;108:455–7.

22 Levy JH. Massive transfusion coagulopathy. *Semin Hematol* 2006;43 (1 Suppl 1):S59–63.

23 Reed RL, Ciavarella D, Heimbach DM, Baron L, Pavlin E, Counts RB, *et al.* Prophylactic platelet administration during massive transfusion. A prospective, randomized, double-blind clinical study. *Ann Surg* 1986; 203:40–8.

24 Ishida A, Handa M. Examination and treatment for dilutional thrombocytopenia. *Rinsho Byori* 2005;53:654–7.

25 Kroll H, Kiefel V, Giers G, Kaplan C, Murphy M,Waters A, *et al.* Prenatal management of fetuses with alloimmune thrombocytopenia. *Beitr Infusionsther Transfusionsmed* 1996;33:150–5.

26 Paidas MJ, Berkowitz RL, Lynch LL, Lockwood CJ, Lapinski R, Mc Farland JG, *et al.* Alloimmune thrombocytopenia: fetal and neonatal losses related to cordocenteses. *Am J Obstet Gynecol* 1995;172:475–9.

27 Dame C, Cremer M, Ballmaier M, Bartmann P, Bald R, Schild RL, *et al.* Concentrations of thrombopoietin and interleukin-11 in the umbilical cord blood of patients with fetal alloimmune thrombocytopenia. *Am J Perinatol* 2001;18:335–44.

28 Porcelijn L, Folman CC, Bossers B, Huiskes E, Overbeeke MA, van der Schoot CE, *et al.* The diagnostic value of thrombopoietin level measurements in thrombocytopenia. *Thromb Haemost* 1998;79:1101–5.

29 Folman CC, Linthorst GE, van Mourik J, van Willigen G, de Jonge E, Levi M, *et al.* Platelets release thrombopoietin (Tpo) upon activation: another regulatory loop in thrombocytopoiesis? *Thromb Haemost* 2000;83:923–30.

30 Porcelijn L, Folman CC, de Haas M, Kanhai HH, Murphy MF, von dem Borne AE, *et al.* Fetal and neonatal thrombopoietin levels in alloimmune thrombocytopenia. *Pediatr Res* 2002;52:105–8.

31 Watts TL, Murray NA, Roberts IA. Thrombopoietin has a primary role in the regulation of platelet production in preterm babies.*Pediatr Res* 1999;46:28–32.

Chapter 10

General discussion and future perspectives

GENERAL DISCUSSION

Soon after the introduction of imaging techniques to visualise the fetus, both diagnosis and in some conditions treatment of fetal diseases became feasible. Especially the widespread availability of high-resolution ultrasound has contributed to the commonly accepted concept of the fetus as a patient. Access to the fetal circulation has enabled clinicians to study fetal haematological parameters and abnormalities. The same technique of ultrasound guided fetal blood sampling or cordocentesis can be used to deliver packed red cells, platelets or medication to the fetus.

Since the early 1960s, one of the main interests of the Department of Obstetrics of the Leiden University Medical Centre has been the field of fetal therapy. A few but increasing number of fetal diseases that otherwise would lead to death or severe handicap are amenable to in utero intervention. This interest originated in the 1960s after Prof. Bennebroek Gravenhorst visited Liley in New Zealand, who just introduced intrauterine blood transfusion for Rhesus alloimmunisation.

This thesis focuses on a group of fetal patients which have one dangerous and life-threatening symptom in common: severe thrombocytopenia. In itself, a low platelet count is not harmful, but since the primary function of platelets is the prevention of bleeding, severe thrombocytopenia is a major risk factor for haemorrhagic complications. The most devastating of these complications is intracranial haemorrhage. From our perspective as a centre for fetal therapy, we are especially interested in those pregnancies which we can identify as having a risk for the development of fetal thrombocytopenia, and for which we have some potentially useful prenatal strategy available to prevent either the decrease of the platelet count or its consequences, haemorrhage.

The studies described in this thesis were clinical studies in human pregnancies, designed to further improve the outcome of pregnancies complicated by fetal thrombocytopenia due to potentially treatable causes.

The primary focus was on pregnancies known to be complicated by fetal and neonatal alloimmune thrombocytopenia (FNAIT). In addition, in two other potentially lethal fetal diseases amenable to in utero therapy, red cell alloimmunisation and Parvovirus B19 infection, a subset of the affected fetuses were found not only to have anaemia but also dangerously low platelet counts. In this discussion section, the findings of the respective studies are summarised. Furthermore, based

on our finding we provide speculations and ideas with regard to future developments and research in this still relatively new and exciting area of perinatal medicine.

FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

As screening programs are not routinely performed, the diagnosis FNAIT is almost exclusively made after birth when signs of bleeding or asymptomatic thrombocytopenia in the neonate are recognised. In the most severe cases, the first symptoms of the disease are fetal intracranial haemorrhage (ICH) or even intrauterine death. Extensive work-up may be required to identify the underlying cause of the bleeding or low platelet counts. Preventive strategies can only be employed in a next pregnancy. Some authors suggest that like in Rhesus disease, untreated FNAIT is likely to show a more severe course in subsequent pregnancies[1-3]. However, unlike Rhesus disease, very little is known about the natural history of FNAIT. The assumption mentioned above seems to be based on a few case-reports only. Given the apparent success of treatment however, clinicians understandingly are unwilling to study the natural history by allowing placebo-arms in their treatment trials.

Antenatal management is ultimately aimed at preventing ICH. To achieve this goal, various preventive strategies have been designed to increase or maintain the fetal platelet count above the level that is regarded 'safe'. Commonly used cut-off levels are 50 x 10^9/L and 30 x 10^9/L, although in adult medicine 20 x 10^9/L and 10 x 10^9/L are also used[4,5]. Controversy still exists on the optimal antenatal management of FNAIT. A range of treatment modalities is available, varying from the most invasive option, using serial fetal blood samplings and platelet transfusions, to a completely noninvasive protocol using 'blind' administration of intravenous immunoglobulin (IVIG).

The last decade, there is an increasing tendency to avoid fetal blood sampling (FBS) as much as possible, because of its inherent risks, especially in cases of fetal thrombocytopenia. Based on a review of the literature, the complication rate of FBS and intrauterine platelet transfusion in FNAIT pregnancies was calculated as 1.6% fetal loss and 2.4% other complications 6. Combined data from three recent studies showed a 6% fetal loss rate directly related to FBS[7-9].

The results from the evaluation of patients treated in our centre (*Chapter 3*) show that in a relatively large series of consecutive pregnancies complicated by FNAIT, the gradual change over time from an invasive management protocol to a completely noninvasive approach resulted in excellent outcome for all noninvasively treated patients.

We compared our results with two recently published studies describing results from more invasive management protocols[8,10]. The studies by Birchall *et al.* and Berkowitz *et al.* describe a considerable number of complications and adverse outcomes associated with FBS. However, such risks could be acceptable if the invasive management would result in a better overall outcome when compared with a completely noninvasive approach.

We conclude that based on our data and the currently available literature, there seems to be no advantage to the use of FBS in the management of pregnancies complicated by FNAIT. For clinically relevant endpoints, the noninvasive management strategy using IVIG without pretreatment or confirmatory FBS seems both effective and safe[6]. Adherence to the principle of *primum non nocere* means, in our view, that potentially hazardous diagnostic procedures should only be employed when proven to do more good than harm. Our view was recently supported by Ghevaert *et al*[11]. They studied prospectively 200 cases of FNAIT, including 47 pregnancies with a known history of FNAIT. Of the total of six intrauterine deaths that occurred, at least four were directly caused by complicated fetal blood samplings. In the IVIG treated group (n=7), no ICH occurred, while in the group treated with serial platelet transfusions 4/40 cases of ICH occurred of which two died in utero.

In the antenatal management, most clinicians make a distinction in the severity of the disease based on the clinical course of the previous affected pregnancy. Unfortunately thus far, other than presence or absence of antenatal ICH in a previous affected sibling, there are no reliable predictors of the severity of the thrombocytopenia in subsequent pregnancies. Bussel *et al.* (1997) suggest that if an older sibling had severe FNAIT, subsequent infants might have disease that is at least as severe[3]. Bussel & Primiani proposed in a recent protocol a grading system with the arbitrary names: Very High Risk, High Risk and Standard Risk[12].

They state that patients are at Very High Risk if they are antigen positive and have an older affected sibling with an antenatal ICH that occurred before 28 weeks gestation. Patients are at High Risk if they are antigen positive and have a previous sibling who suffered an antenatal ICH between 28 and 36 weeks gesta-

tion or a perinatal ICH. Patients affected by FNAIT who have no history of a sibling with an ICH and have an initial fetal platelet count greater than 20 x 10^9/L are called to be at Standard Risk.

In *Chapter 4* we report our less invasive treatment strategy in FNAIT in cases at high risk for ICH. In this study, we included patients that, when using Bussel's new grading system, would fall into the Very High Risk and High Risk categories. In contrast to the proposed management strategies by Bussel, our data again suggest that the effectiveness of maternally administered IVIG allows a further avoidance of FBS even in these most severe cases of FNAIT. We do acknowledge however that our series is small, and in the literature a few cases have been described of ICH in pregnancies treated with IVIG therapy alone[13-16]. The obvious conclusion is that more research needs to be done to find the most effective and least harmful preventive strategies for all risk categories. In view of the rarity of this condition, international collaboration is required in order to be able to arrange large prospective studies, RCT's, and a registry of all cases.

In FNAIT pregnancies without an ICH in a previous child, Caesarean section is often routinely employed for delivery. Practice guidelines advise vaginal delivery as an option in case of a platelet count > 50 x 10^9/L established by FBS, with or without an intrauterine platelet transfusion[17-19].

In our experience with vaginal delivery in FNAIT pregnancies without ICH in a previous child, none of the thirty-two neonates developed an ICH, although in four cases the platelet count was below 50 x 10^9/L (*Chapter 5*). Three of these four children were born vaginally.

We think these findings are important because for the mother the benefits of a vaginal delivery against a Caesarean section are obvious and although a Caesarean section is safer then ever before, risks are not negligible. Maternal morbidity - thrombosis, hysterectomy, infections, extended hospital stay and chance of rehospitalisation - is higher[20,21]. Most importantly however, uterine scarring is associated with increased risks for both mother and child in future pregnancies [22-24]. Another principle question also needs to be asked: is there any evidence or logical background for the assumption that a normal vaginal delivery poses the thrombocytopenic fetus at greater risk for intrapartum ICH than an elective Caesarean section? Almost all FNAIT patients are multiparous in which a relatively smooth delivery might be expected. Furthermore, the idea that a Caesarean section is always a completely nontraumatic birth without any pressure on the fetal

head only lives in the minds of those who never witnessed a Caesarean section. Although this is difficult to prove, we cannot exclude the possibility that fear for medical-legal consequences play a role in the decision of clinicians to recommend elective Caesarean sections for all FNAIT patients. We acknowledge that in some cultures, these concerns are more serious than in others. Based on strictly scientific criteria however, we believe that for FNAIT pregnancies, in analogy to the avoidance of FBS unless proven benificial, the more harmful Caesarean section should only be recommended as a routine when proven or highly likely to provide better outcome for the fetus. The currently available literature does not seem to support this.

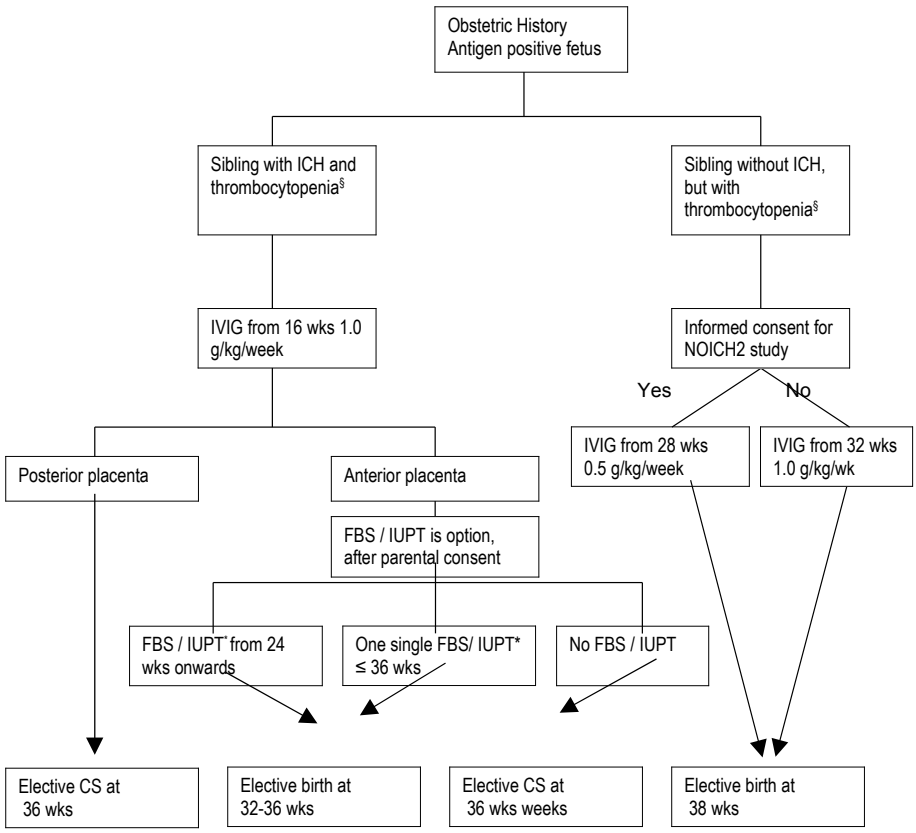
In collaboration with our colleagues from the Karolinska Institute in Sweden and the University of Northern Norway, we started the NOICH (No Intra Cranial Haemorrhage) randomised controlled trial (RCT), in combination with an international registry both via the website www.noich.org.

Both initiatives, the RCT and the registry, gained a lot of attention during the presentation at the annual meeting of the International Fetal Medicine and Surgery Society meeting in Denmark in 2005. Since then, many colleagues in all parts of the world have contributed and entered patient data into the registry. However, only a few managed to actually get the NOICH RCT going in their own centre, either due to difficulties with IRB (Institutional Review Board) approval, lack of patients or being too busy with patient care and research for more frequently occurring diseases. Our efforts to help others to overcome these issues mostly did not have the desired effect, and finally almost three years after starting we had to decide to end the RCT prematurely. The useful lessons we learned, and an analysis of the still interesting data we gathered from the small group of randomised patients are summarised in *Chapter 6*.

Not surprisingly, we could only conclude that we had insufficient data to show equivalence between the low dose (0.5 gram) versus the standard dose (1 gram) IVIG. The fact that almost all eligible patients in the participating centres agreed to be randomised and completed the assigned treatment, in our view, proved the feasibility of such a study. Furthermore, the data also did not show any benefit of the 1 gram dose, the possibility of equivalence is still there.

The information on the FNAIT patients entered in the NOICH study registry hold a great potential to help solve other important issues in the management of this rare disease. We plan to continue the use of the NOICH website hosted by

Figure: The new Leiden management protocol of FNAIT



§ defined as a neonatal platelet count of < 100 x 10^9/L
* in case of a fetal platelet count < 100 x 10^9/L

ICH intracranial haemorrhage
IVIG intravenous immunoglobulins
FBS fetal blood sampling
IUPT intrauterine platelet transfusion
CS Caesarean section

the MedSciNet group for further international multicentre studies.

After ending the NOICH trial, there was an obvious need for reconsidering our management protocol for pregnant women at risk for FNAIT. The two presumptions that were the basis to start this trial, still hold: 1. There is no scientific evidence supporting the dose of 1 gram, this was entirely empiric, and 2. Our previous work on the levels of IgG in the mother and the foetus were confirmed by the trial results, suggesting that there is no benefit to increase the dose to more than 0.5 gram since this does not result in any increase of IgG in the fetus. We therefore propose to use a dose of 0.5 gram IVIG per kg per week, starting at 28 weeks gestation, in women at risk for FNAIT who's previous affected child did not have an ICH. We consider it mandatory to propose this experimental treatment only as part of a prospective study, for which we have asked our colleagues already participating in the NOICH registry project to join us. A flow chart of our newly proposed preventive protocol is given in the Figure.

RHESUS D ALLOIMMUNISED PREGNANCIES

We found that severe thrombocytopenia is common in hydropic anaemic fetuses in Rhesus D alloimmunised pregnancies. These cases had a strikingly high mortality rate. Surprisingly, also in absence of severe hydrops, severe thrombocytopenia in addition to Rhesus D related anaemia was associated with a poor prognosis.

The logical clinically relevant question is then whether we can prevent death or adverse outcome related to fetal thrombocytopenia using intrauterine platelet transfusions, as was suggested by Saade *et al*[25]. One option would be to have platelets available at any intrauterine blood transfusion in a severely hydropic foetus, together with the ability to rapidly perform a platelet count during the procedure.

However, platelet transfusion itself may be associated with additional complications due to the extra volume given. This may particularly affect severely hydropic, thus already compromised fetuses. Secondly, there is no guarantee that platelet transfusion prevents haemorrhagic complications in fetuses with thrombocytopenia. Adverse outcome in fetuses suffering from alloimmune thrombocytopenia due to bleeding after cordocentesis despite rapid platelet transfusion has been reported[7,10]. Severe hydrops due to Rhesus D alloimmunisation is an increasingly rare condition, We continue to prospectively collect as much data as possible, and we plan to further increase our collaboration with fetal medicine centres around the world to both to gain more insight in the cause of the low

platelet counts in some of the Rhesus D affected fetuses and to evaluate the effects of platelet transfusions.

KELL ALLOIMMUNISATION

In contrast to hydropic fetuses with Rhesus D haemolytic disease, we found that fetuses with severe anaemia due to Kell alloantibodies are generally not at risk for substantial thrombocytopenia. Surprisingly even severe hydrops due to Kell alloimmunisation in our series was not associated with low platelet counts. For safe fetal blood transfusion in Kell induced anaemia, our data suggest that there seems to be no need to be prepared for additional platelet transfusion. These are preliminary conclusions based on relatively few cases. Since the cause for the low platelet counts in the Rhesus D cases is yet unknown, it is also difficult to speculate on the reasons for the difference observed in the hydropic Kell cases. We are currently collecting as many haematologic parameters as we can from all hydropic fetuses with anaemia, with the aim to answer some of these questions. As a result of the routine screening, since 1998, for Kell alloantibodies in our country the number of severely hydropic fetuses with Kell disease is fortunately decreasing, which makes it unlikely that we will find all answers on short notice. Again, multicentre international collaboration may be very valuable here as well.

PARVOVIRUS B19 INFECTION

Although thrombocytopenia was frequently encountered in hydropic anaemic fetuses with Parvovirus B19 infection, fetal bleeding complications were not noted in our series. As in hydropic fetuses caused by Rhesus D alloimmunisation, the risk of fluid overload by fetal platelet transfusion in these often extremely hydropic compromised fetuses should be weighed against the apparently low incidence of fetal bleeding complications. In our experience, referrals for fetal anaemia due to Parvovirus B19 infection still tend to occur in late stages of the disease, due to a combination of patients delay and doctors delay. We are putting a lot of effort in educating both the public and our colleagues about the hazards of Parvovirus B19 in pregnancy, to reduce the number of extremely hydropic and deeply anaemic fetuses by earlier detection and referral. Our current policy is still to try and have platelets available at every blood transfusion for Parvovirus B19 anaemia, but we are reluctant to transfuse platelets if the fetus shows signs of serious cardiac compromise. Fetal Parvovirus B19 infection is another rare and potentially lethal

disease that deserves further study. Not only the issue of more timely detection policies and the role of thrombocytopenia, but especially the long term follow up of survivors is an important aspect for further research, again best done by international multicentre collaboration.

FUTURE PERSPECTIVES

Almost all publications on FNAIT describe observational studies. Until recently, only the group of Bussel at Cornell University had performed randomised controlled trials (RCT). Although our first attempt, with the international NOICH study group, to carry out a large RCT had to be stopped prematurely for not reaching the required sample size, we do consider this trial a successful pilot study. In the participating centres, practically all patients consented to be randomised, almost all received and completed the assigned treatment and none were lost to follow-up. The single reason for not completing the study within a reasonable time frame was the limited number of centres participating. We do therefore believe that in the future, adequately powered RCT's in this field are certainly possible, provided that a large number of centres in many countries together agree to commit to collaboration.

Based on our experience with the NOICH trial, we regard the following prerequisites to be fulfilled in order to expect such RCT's to become a success:

- Clinical relevance of the study question clear to all potential participants
- Willingness to go through the invariably time-consuming IRB approval process for a usually very limited number of patients that each centre can contribute
- Adequate funding for all additional work, including laboratory, mailing and data-entering expenses
- One dedicated research person in each participating centre with enough time, motivation and alertness to ensure that the few eligible patients per year are not missed, are informed in time, are randomised and are followed according to protocol.
- Web-based randomisation and easy to use study database.

These items may all seem straightforward. One has to realise however that apart from a few centres around the world in countries with a high level of centrali-

sation, most centres see less than five FNAIT pregnancies per year. For most, this particular disease, however devastating it can be, is not one of their main interests in terms of fetal medicine research. To still collaborate in studies with FNAIT patients, participation must be made very easy and as attractive as possible. This remains a challenge, however, we feel that, based on our many contact with colleagues around the world, it should be possible to carry out relatively large prospective studies and RCT's with FNAIT patients. There may be a role for existing Fetal Medicine networks, such as the International Society for Fetal Medicine and Surgery (IFMSS), the Eurofoetus group and the North-American Fetal Therapy Network (NAFTNET), to facilitate communication and possibly funding.

What are the most pressing remaining questions for clinical practice to answer in this particular disease?

- Is routine screening of all pregnant women useful, cost-effective and feasible, and what to offer the screen-positive group?
- Can we better define the group of pregnant women with alloantibodies against fetal platelets at true risk for fetal intracranial haemorrhage?
- Is safe and effective treatment truly possible without the use of fetal blood sampling?
- Is IVIG treatment the best option, what is the optimal dose, what is the best time to initiate the treatment? What is the underlying difference between responders and nonresponders, and can we use this to guide our management?
- Is there a role for additional medication such as steroids? If so, for a specific group, and do the benefits outweigh the maternal and fetal side-effects?
- What is the best mode and time of delivery?

Several other important 'basic science' issues that deserve to be studied:

- Is there a way to measure fetal platelet levels noninvasively?
- Would it be possible to develop anti-HPA prophylaxis similar to the anti-D prophylaxis in Rhesus D alloimmunisation?
- Why do some women easily produce alloantibodies and others do not? More insight in these immune responses to pregnancy is needed for this and many other diseases of pregnancy.
- Why do some fetuses or neonates with low platelet counts have an ICH

and others with similar platelet counts do not? Are there genetic differences, does the endothelium play a role?
- How does IVIG actually work in FNAIT, and is there really a protective effect on the endothelium?

Some of these issues may be studied in the laboratory, using e.g. endothelial cells, in vitro placenta models or animals. An promising example is the recently developed mouse model for FNAIT, by Dr Ni and colleagues in Toronto, Canada[25].

In the other conditions studied in this thesis, red cell alloimmunisation and Parvovirus B19 infection, many questions also remain. The main threat to the fetus is anaemia, this is relatively easily diagnosed using noninvasive Doppler measurements, and effectively treated with intrauterine blood transfusion. The minority of these fetuses that suffer from accompanying thrombocytopenia is more difficult to identify, and whether or not this condition requires adding platelets to the transfusion therapy is unclear. Our limited data suggest that in these diseases, low fetal platelet count are associated with a worse prognosis. Future studies therefore should answer both clinical questions such as the benefit versus harm of platelet transfusion, as well as more basic questions on the cause of the thrombocytopenia and its direct or indirect relationship with adverse outcome.

In conclusion, after the almost five years of research reflected in the chapters of this thesis, some advances have been made. However, we still have more questions than answers on many aspects of fetal thrombocytopenia. The research group of the department of Obstetrics of the LUMC, together with our local colleagues in the departments of Haematology and Neonatology and the foreign research partners, will continue to initiate and collaborate in both clinical and basic science research in this field, with the ultimate goal to help eradicate all adverse effects this disease can have on the health of fetuses and newborns.

REFERENCES

1. Friedman JM, Aster RH. Neonatal alloimmune thrombocytopenic purpura and congenital porencephaly in two siblings associated with a "new" maternal antiplatelet antibody. *Blood* 1985; 65: 1412–1415.

2. Herman JH, Jumbelic MI, Ancona RJ. In utero cerebral hemorrhage in alloimmune thrombocytopenia. *Am J Pedaitr Hemotol Oncol* 1986; 8: 312-317.

3. Bussel JB, Zabusky MR, Berkowitz RL & McFarland JG Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997; 337: 22–24.

4. Jhawar BS, Ranger A, Steven D, et al. Risk factors for intracranial hemorrhage among full-term infants: a case-control study. *Neurosurgery* 2003; 52: 581-590.

5. Bussel JB, Zacharoulis S, Kramer K, McFarland JG, Pauliny J, Kaplan C. Clinical and diagnostic comparison of neonatal alloimmune thrombocytopenia to non-immune cases of thrombocytopenia. *Pediatr Blood Cancer* 2005; 45: 176-183.

6. Radder CM, Brand A, Kanhai HHH. Will it ever be possible to balance the risk of intracranial haemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? *Vox Sang* 2003; 84: 318-325.

7. Overton TG, Duncan KR, Jolly M, Letsky E, Fisk NM. Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. *Am J Obstet Gynecol* 2002; 186: 826-831.

8. Birchall JE, Murphy MF, Kaplan C, Kroll H. European collaborative study of the antenatal management of feto-maternal alloimmune thrombocytopenia. *Br J Haematol* 2003; 122: 275-288.

9. Berkowitz RL, Bussel JB, McFarland JG. Alloimmune Thrombocytopenia: State of the art 2006. *Am J Obstet Gynecol* 2006; 195: 907-913.

10. Berkowitz RL, Kolb EA, McFarland JG, Wissert M, Primani A, Lesser M, Bussel JB. Parallel Randomized Trials of Risk-Based Therapy for Fetal Alloimmune Thrombocytopenia. *Obstet Gynecol* 2006; 107: 91–96.

11. Ghevaert C, Campbell K, Walton J, Smith GA, Allen D, Williamson LM, Ouwehand WH, Ranasinghe E. Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. *Transfusion*. 2007 ;47 :901-10.

12. Bussel JB, Primiani A, Fetal and neonatal alloimmune thrombocytopenia: progress and ongoing debates, *Blood Reviews* 2008; 22: 33-52.

13. Kroll H, Kiefel V, Giers O, Bald R, Hoch J, Hanfland P, Hansmann M, Mueller/Eckhardt C. Maternal intravenous immunoglobulin treatment does not prevent intracranial haemorrhage in fetal alloimmune thrombocytopenia. *Transfusion Medicine* 1994; 4: 293-296.

14. Murphy MF, Metcalfe P, Waters AH, et al. Antenatal management of severe feto-maternal alloimmune thrombocytopenia: HLA incompatibility may affect responses to fetal platelet transfusions. *Blood* 1993; 81: 2174–2179.

15. Murphy MF, Waters AH, Doughty A, Hambley H, Mibashan S, Nicolaides K, Rodeck CH. Antenatal management of fetomaternal alloimmune thrombocytopenia - report of 15 affected pregnancies. *Transfusion Medicine* 1994; 4: 281-292.

16. Sainio S, Jarvenpaa AL, Renlund M, et al. Thrombocytopenia in term infants: a population-based study. *Obstet Gynecol* 2000; 95: 441-446.

17. Reznikoff-Etievant MF. Management of alloimmune neonatal and antenatal thrombocytopenia. *Vox Sang* 1988; 55: 193–201.

18. Bussel JB, Berkowitz RL, Lynch L et al. Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: A randomized trial of the addition of low-dose steroid to intravenous gamma-globulin. *Am J Obstet Gynecol* 1996; 174: 1414–1423.

19. Kaplan C, Murphy MF, Kroll H, Waters AH. Feto-maternal alloimmune thrombocytopenia: antenatal therapy with IVIGG and steroids--more questions than answers. European Working Group on FMAIT. *Br J Haematol* 1998; 100: 62-65.

20. Ham van MAPC, Dongen van PWJ, Mulder J. Maternal consequences of Caesarean section: a retrospective study of intraoperative and postoperative maternal complications of Caesarean section during a 10-year period. *Eur J Obstet Gynecol Reprod Biol* 1997; 74: 1–6.

21. Lydon-Rochelle M, Holt VL, Martin DP, Easterling TR. Association between method of delivery and maternal rehospitalization. *JAMA* 2000; 283: 2411–2416.

22. Miller DA, Chollet JA, Goodwin TM. Clinical risk factors for placenta previa-placenta accreta. *Am J Obstet Gynecol* 1997; 177 210–214.

23. Greene MF. Vaginal delivery after cesarean section—is the risk acceptable? *N Engl J Med* 2001; 345: 54–55.

24. Kwee A, Bots ML, Visser GH, Bruinse HW. Obstetric management and outcome of pregnancy in women with a history of caesarean section in the Netherlands. *Eur J Obstet Gynecol Reprod Biol.* 2000; 132; 171-176.

25. Saade GR, Moise KJ Jr, Copel JA, Belfort MA, Carpenter RJ Jr. Fetal platelet counts correlate with the severity of the anemia in red-cell alloimmunization. *Obstet Gynecol* 1993; 82: 987-991.

26. Ni H, Chen P, Spring CM, Sayeh E, Semple JW, Lazarus AH, Hynes RO, Freedman J. A novel murine model of fetal and neonatal alloimmunethrombocytopenia: response to intravenous IgG therapy. *Blood* 2006; 107: 2976-2983.

Chapter 11

Summary / samenvatting

SUMMARY

Intracranial haemorrhage (ICH) among term neonates is associated with neonatal death or lifelong disability. Between all the proposed aetiological mechanisms, including impairments in coagulation, hypoxic-ischemic injury and birth related trauma, thrombocytopenia seems to be the most important predictor of ICH among term neonates and is also associated with the most severe forms of haemorrhage.

Neonatal thrombocytopenia

Neonatal thrombocytopenia is defined as a platelet count < 150 x 10^9/L. The incidence of thrombocytopenia (< 150 x 10^9/L) in all newborns is 1-4%. However, due to absence of clinical signs, mild or moderate thrombocytopenia is often not noted.

Symptoms that might occur are petechiae, haematomas, gastro-intestinal bleedings and intracranial haemorrhages. Especially neonates with severe thrombocytopenia (< 50 x 10^9/L) are at risk for bleeding problems.

In general, the aetiology of thrombocytopenia can be classified into disorders associated with increased destruction, including consumption, or decreased production of platelets.

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of thrombocytopenia, especially in otherwise healthy term newborns.

Most patients at risk for a fetal platelet disorder are identified only after a baby is born with a low platelet count. It is vital to identify the cause of the thrombocytopenia as quickly as possible, primarily to be able to start the correct treatment without delay. In addition establishing the cause of any neonatal thrombocytopenia is essential to institute proper management in the next pregnancy.

Aim of the studies

The aim of the studies described in this thesis was to contribute to improve the outcome of pregnancies complicated by fetal thrombocytopenia, caused by alloimmune thrombocytopenia, red cell alloimmunisation (Rhesus D and Kell) and Parvovirus B19 infection.

In *Chapter 2*, an extensive review of the literature is given on fetal and neonatal alloimmune thrombocytopenia. FNAIT is caused by maternal Immunoglobu-

lin G (IgG) antibodies against paternal human platelet antigens (HPA) on fetal platelets. The mechanism is the platelet equivalent on Rhesus disease. FNAIT is the most common cause of severe thrombocytopenia in the term neonate. Most cases are identified after birth of an affected child, either through observation of clinical signs such as purpura, petechiae, bruising or by coincidental detection of thrombocytopenia and absence of clinical signs. The most feared complication of a low platelet count in the fetus or the neonate is intracranial haemorrhage (ICH), subsequent neurological handicaps and even death. The incidence of FNAIT is estimated to be one in 1500 births. As routine screening for HPA antibodies is not done, it invariably occurs unexpectedly. Unlike Rhesus disease, it can occur in a severe form in the first pregnancy.

The immunodominant antigen in Caucasian in individuals is the HPA-1a, which is responsible for approximately 85% of FNAIT cases.

The recurrence rate in a next pregnancy is 85% and like Rhesus disease, FNAIT seems to worsen in subsequent pregnancies.

Untreated newborns with FNAIT are reported to be affected by ICH in 7% of cases, if the previous sibling did not have an ICH. If the previous sibling did have an ICH, the recurrence rate without treatment is estimated to be 79% (CI 61-97%).

Antenatal management is ultimately aimed at preventing ICH. Over the last 15 years, there has been a gradual change in antenatal treatment, from an invasive management protocol via a less invasive management protocol to a completely noninvasive approach. Preventive noninvasive maternal treatment with weekly IVIG is in most cases preferred above invasive fetal blood samples combined with intrauterine platelet transfusions. The risk of developing an ICH has to be balanced against the risk of serious complications from invasive procedures. However, internationally, there is still controversy over the optimal medical treatment regimen and the role of diagnostic invasive procedures in the management of FNAIT.

In *Chapter 3*, we describe the outcome of pregnancies with fetal and neonatal alloimmune thrombocytopenia (FNAIT) treated in our centre, in relation to the invasiveness of the management protocol.

We performed a retrospective analysis of prospectively collected data. The population existed of 98 pregnancies in 85 women with FNAIT having a previous child with thrombocytopenia with (n=16) or without (n=82) an ICH. Our management protocol evolved over time from serial fetal blood samplings (FBS)

and platelet transfusions (*n*=13) via combined FBS with maternal IVIG (*n*=33) to completely noninvasive treatment with IVIG only (*n*=52 pregnancies, resulting in 53 neonates). Ninety-seven pregnancies ended in a live birth; none of the neonates had an ICH. In the first two groups, three emergency caesarean sections were performed after complicated FBS, resulting in two healthy babies and one neonatal death. We concluded that noninvasive antenatal management of pregnancies complicated by FNAIT appears to be both effective and safe.

In *Chapter 4*, we analysed more thoroughly the patients that had a previous child with a thrombocytopenia and an ICH. In seven pregnancies with a history of ICH in the older sibling, weekly intravenous IVIG therapy, without initial cordocentesis was started at a median gestational age of 16 weeks. In four pregnancies, cordocentesis was avoided. This group delivered by elective caesarean section. One predelivery cordocentesis with platelet transfusion was performed in three further cases, after which delivery was induced. Although none of the cases had a platelet count of > 50 x 10^9/L at cordocentesis predelivery or at birth, no ICH's were observed. Only the neonates without a predelivery cordocentesis needed one platelet transfusion shortly after birth. However we acknowledge that our series is small, we suggest that the effectiveness of maternally administered IVIG allows a further avoidance of cordocentesis even in the most severe cases of FNAIT.

After balancing the risk for serious complications from cordocentesis for fetal blood sampling on one hand and ICH on the other, we designed a protocol to further reduce invasive procedures in these patients.

In FNAIT pregnancies without an ICH in a previous child, Caesarean section is often routinely employed. Practice guidelines advise vaginal delivery as an option in case of a platelet count > 50 x 10^9/L established by FBS, with or without an intrauterine platelet transfusion. In *Chapter* 5, we report our experience with the safety of a vaginal delivery in FNAIT pregnancies without ICH in a previous child. None of the thirty-two neonates developed an ICH, although in four cases the platelet count was below 50 x 10^9/L. Three of these four children were born vaginally. We think these findings are important because for the mother the benefits of a vaginal delivery against a Caesarean section are obvious. Especially the risks on uterine scarring or a placenta accreta are associated with increased risks for both mother and child in future pregnancies. Besides, another question remains: is there any evidence or logical background for the assumption that a normal vaginal delivery poses the thrombocytopenic fetus at greater risk for intra-

partum ICH than an elective Caesarean section? Almost all FNAIT patients are multiparous in which a relatively smooth delivery might be expected.

In collaboration with our colleagues from the Karolinska Institute in Sweden and the University of Northern Norway, we started the NOICH study (No Intra Cranial Haemorrhage).

The results from the NOICH study are reported in *Chapter 6*. In this randomised trial the hypothesis was tested that IVIG in a low dose of 0.5 g/kg/wk was at least as effective as the standard dose of 1.0 g/kg/wk in preventing ICH and severe fetal thrombocytopenia in pregnancies at risk for FNAIT.

The calculated sample size was two arms of 106 patients. After almost three years of recruitment, a total of only 23 pregnancies had been randomised, which led to the decision by the steering committee to prematurely end the recruitment. We concluded that whether the effectiveness of a low dose of IVIG is equivalent to the standard dose in the treatment of FNAIT remains uncertain. The trial can be regarded as a successful pilot, showing feasibility and acceptability of the study design both for patients and clinicians.

In *Chapter 7* we evaluated the clinical significance of fetal thrombocytopenia in Rhesus D alloimmunised pregnancies. We found that severe thrombocytopenia is common in hydropic anaemic fetuses in Rhesus D alloimmunised pregnancies. These cases had a high mortality rate. Surprisingly, also in absence of severe hydrops, severe thrombocytopenia in addition to Rhesus D related anaemia was associated with a poor prognosis. The logical clinically relevant question is then whether we can prevent death or adverse outcome related to fetal thrombocytopenia using intrauterine platelet transfusions. However, platelet transfusion itself may be associated with additional complications due to the extra volume given. This may particularly affect severely hydropic, thus already compromised fetuses. Secondly, there is no guarantee that platelet transfusion prevents haemorrhagic complications in fetuses with thrombocytopenia.

In contrast to hydropic fetuses with Rhesus D haemolytic disease, we describe in *Chapter 8* that fetuses with severe anaemia due to Kell alloimmunisation are generally not at risk for substantial thrombocytopenia. Surprisingly, even severe hydrops due to Kell alloimmunisation in our series was not associated with low platelet counts. Since the cause for the low platelet counts in the Rhesus D cases is yet unknown, it is also difficult to speculate on the reasons for the difference

observed in the hydropic Kell cases. New prospective research might give answers to these questions.

Finally, in *Chapter 9*, we evaluated the significance of thrombocytopenia in hydropic anaemic fetuses with congenital Parvovirus B19 infection. Although thrombocytopenia was frequently encountered in hydropic anaemic fetuses with parvovirus B19 infection, fetal bleeding complications were not noted in our series. As in hydropic fetuses caused by Rhesus D alloimmunization, the risk of fluid overload by fetal platelet transfusion in these often extremely hydropic compromised fetuses should be weighed against the apparently low incidence of fetal bleeding complications.

In the general discussion in *Chapter 10*, suggestions for future research are given. We emphasise the importance of international collaboration for these uncommon diseases.

The most important clinical remaining questions are:

- Is routine screening of all pregnant women useful, cost-effective and feasible, and what to offer the screen-positive group?
- Can we better define the group of pregnant women with alloantibodies against fetal platelets at true risk for fetal intracranial haemorrhage?
- Is safe and effective treatment truly possible without the use of fetal blood sampling?
- Is IVIG treatment the best option, what is the optimal dose, what is the best time to initiate the treatment? What is the underlying difference between responders and nonresponders, and can we use this to guide our management?
- Is there a role for additional medication such as steroids? If so, for a specific group, and do the benefits outweigh the maternal and fetal side-effects?
- What is the best mode and time of delivery?

Other, more basic science issues that should be studied are:

- Is there a way to measure fetal platelet levels noninvasively?
- Would it be possible to develop anti-HPA prophylaxis similar to the anti-D prophylaxis in Rhesus D alloimmunisation?
- Why do some women easily produce alloantibodies and others do not? More insight in these immune responses to pregnancy is needed for this and many other diseases of pregnancy.
- Why do some fetuses or neonates with low platelet counts have an ICH and others with similar platelet counts do not? Are there genetic differences, does the endothelium play a role?
- How does IVIG actually work in FNAIT, and is there really a protective effect on the endothelium?

In the other conditions studied in this thesis, red cell alloimmunisation and Parvovirus B19 infection, many questions also remain. The main threat to the fetus is anaemia, this is relatively easily diagnosed using noninvasive Doppler measurements, and effectively treated with intrauterine blood transfusion. The minority of these fetuses that suffer from accompanying thrombocytopenia is more difficult to identify, and whether or not this condition requires adding platelets to the transfusion therapy is unclear. Our limited data suggest that in these diseases, low fetal platelet counts are associated with a worse prognosis. Future studies therefore should answer both clinical questions such as the benefit versus harm of platelet transfusion, as well as more basic questions on the cause of the thrombocytopenia and its direct or indirect relationship with adverse outcome.

Conclusion

In conclusion, after the almost five years of research reflected in the chapters of this thesis, some advances have been made. However, we still have more questions than answers on many aspects of fetal thrombocytopenia. The research group of the department of Obstetrics of the LUMC, together with our local colleagues in the departments of Haematology and Neonatology and the foreign research partners, will continue to initiate and collaborate in both clinical and basic science research in this field, with the ultimate goal: a healthy child and a happy mother.

SAMENVATTING

Bij een pasgeborene is een hersenbloeding een ernstige aandoening met een handicap of zelfs sterfte als mogelijk gevolg. Hersenbloedingen bij pasgeborenen kunnen verschillende oorzaken hebben. Naast stollingsafwijkingen, hypoxisch-ischemische schade en schade ten gevolge van de bevalling, is trombocytopenie de meest voorkomende oorzaak. Daarnaast is trombocytopenie geassocieerd met de meest ernstige vormen van hersenbloedingen.

Neonatale trombocytopenie is gedefinieerd als een trombocytengetal lager dan 150 x 10^9/L. De incidentie van trombocytopenie onder alle neonaten is 1-4%. Dit wordt vaak niet onderkend, aangezien een (milde) trombocytopenie in veel gevallen symptoomloos verloopt. Symptomen die kunnen optreden zijn petechiën, hematomen, darmbloedingen en hersenbloedingen. Vooral neonaten met een ernstige trombocytopenie (< 50 x 10^9/L) lopen een groot risico op bloedingsproblemen.

Neonatale trombocytopenie

Neonatale trombocytopenie wordt veroorzaakt door afwijkingen die geassocieerd zijn met een verhoogde afbraak van trombocyten (inclusief verhoogde consumptie) of door een verlaagde aanmaak van trombocyten.

Foetale en neonatale alloimmuun trombocytopenie (FNAIT) is de meest voorkomende oorzaak van trombocytopenie in verder gezonde à terme neonaten.

Meestal wordt FNAIT pas postpartum vastgesteld. Het is bij neonatale trombocytopenie van groot belang de oorzaak op korte termijn vast te stellen, ten einde zo snel mogelijk met adequate therapie te kunnen starten. De diagnose FNAIT is in verband met het herhalingsrisico ook belangrijk om het beleid bij een eventuele volgende zwangerschap vast te stellen.

Doel van de studies

Het doel van de studies die in dit proefschrift worden beschreven, was het verbeteren van de uitkomsten van zwangerschappen die gecompliceerd zijn door foetale trombocytopenie.

In *Hoofdstuk 2* wordt een literatuuroverzicht gegeven over FNAIT.

Tijdens de zwangerschap kan de aanstaande moeder antistoffen aanmaken tegen een of meer paternale trombocytenkenmerken (Human Platelet Antigens (HPA)) als zij zelf negatief is voor deze kenmerken. Deze antigenen bevinden

zich op de glycoproteïnen in de celmembraan van de trombocyten. De alloimmuunreactie is vergelijkbaar met immunisatie tegen het meer bekende Rhesus D antigeen op rode bloedcellen. De door het immuunsysteem gevormde IgG-antistoffen in de moederlijke circulatie passeren de placenta, en kunnen afbraak van de foetale trombocyten veroorzaken. In tegenstelling tot Rhesus D immunisatie treedt FNAIT in 50% van de gevallen op in de eerste graviditeit.

FNAIT is de meest voorkomende oorzaak van ernstige trombocytopenie bij de à terme neonaat. Dit kan symptoomloos verlopen, maar kan ook met petechiën, hematomen en intracraniële bloedingen (ICH) gepaard gaan. In het ernstigste geval treedt intra-uteriene sterfte op. De incidentie wordt geschat op 1 op 1000 tot 2000 geboorten. Omdat geen standaardonderzoek naar HPA-antistoffen in de zwangerschap wordt verricht, wordt de aandoening vaak pas ontdekt als bij een neonaat alloimmuun trombocytopenie wordt vastgesteld. Het komt ook voor dat onderzoek naar HPA- type en HPA-antistoffen wordt ingezet nadat een zus van de zwangere een kind heeft gekregen met FNAIT. In de Kaukasische populatie is HPA1a het immunodominante antigeen dat verantwoordelijk is voor 85% van alle FNAIT-gevallen.

In een volgende zwangerschap is de kans op herhaling 85%, waarbij de symptomen vaak in ernst toenemen. Als geen behandeling wordt gegeven, wordt de incidentie van ICH bij FNAIT geschat op 7%, als het vorige kind geen ICH had. Als een vorig kind wel een ICH had is de herhalingskans zonder behandeling 79% (betrouwbaarheidsinterval: 61-97%). De afgelopen 15 jaar heeft een geleidelijke verandering plaatsgevonden in de behandeling van FNAIT in de volgende zwangerschap. Van een aanvankelijk invasief behandelprotocol is, via een tussenperiode met een minder invasief behandelprotocol, de behandeling momenteel in Nederland volledig niet-invasief. Preventieve niet-invasieve behandeling met wekelijks immunoglobulines (IVIG) heeft in het merendeel van de casus de voorkeur boven invasieve diagnostische navelstrengpuncties met therapeutische trombocytentransfusies. De kans op het optreden van een ICH moet worden afgewogen tegen de kans op het optreden van een complicatie die wordt veroorzaakt door de diagnostiek of de behandeling zelf. Internationaal echter bestaat nog steeds een verschil van mening omtrent de juiste behandeling.

In *Hoofdstuk 3* wordt nader ingegaan op de uitkomst van door FNAIT gecompliceerde zwangerschappen in relatie tot de invasiviteit van het behandelprotocol. Prospectief verzamelde patiëntengegevens uit het LUMC werden geanalyseerd.

De populatie bestond uit 98 zwangerschappen van 85 vrouwen met FNAIT die een eerder kind hadden met een trombocytopenie èn een ICH (*n*=16) of een eerder kind hadden met een trombocytopenie zonder ICH (*n*=82). Het behandelprotocol veranderde tijdens de studieperiode van seriële navelstrengpuncties met intra-uteriene trombocytentransfusies (*n*=13) via een combinatie van deze navelstrengpuncties en intraveneuze immunoglobulines (IVIG) (*n*=33) tot volledig niet-invasieve behandeling met uitsluitend IVIG (*n*=52 zwangerschappen en 53 neonaten).

Zevenennegentig van de 98 zwangerschappen eindigden in een levend geboren kind; geen van de neonaten had een ICH. In de eerste twee groepen vonden drie spoedkeizersnedes plaats ten gevolge van een gecompliceerde navelstrengpunctie, resulterend in twee gezonde baby's en één neonatale sterfte. Geconcludeerd wordt dat niet-invasieve behandeling van FNAIT-zwangerschappen veilig en effectief lijkt te zijn.

In *Hoofdstuk 4* wordt ingegaan op de groep vrouwen die een eerder kind hadden met een trombocytopenie èn een hersenbloeding. (De hoog-risico groep, volgens indeling van Bussel) Bij in totaal zeven zwangerschappen met een voorgeschiedenis van een hersenbloeding bij een eerder kind, werd gestart met IVIG vanaf een mediane zwangerschapsduur van 16 weken. Bij vier zwangerschappen werd geen navelstrengpunctie verricht. Deze groep beviel per electieve keizersnede. Bij drie zwangerschappen werd een navelstrengpunctie met intra-uteriene trombocytentransfusie verricht vlak voor de bevalling, waarna de bevalling werd ingeleid. Geen enkele neonaat had trombocyten > 50 x 10^9/L ondanks meerdere weken IVIG. Toch traden in deze groep geen neonatale bloedingsproblemen op. Alleen de neonaten die geen intra-uteriene trombocytentransfusie ondergingen, hadden een eenmalige trombocytentransfusie postpartum nodig.

Na het afwegen van de risico's die kunnen optreden ten gevolge van de navelstrengpuncties en de intra-uteriene bloedtransfusies, ontwikkelden we een protocol om ook in deze hoog-risico groep patiënten minder invasief te kunnen behandelen.

Bij FNAIT-zwangerschappen met een eerder kind zonder hersenbloeding in de voorgeschiedenis wordt vaak routinematig een electieve keizersnede verricht. Richtlijnen adviseren dat een vaginale partus mogelijk is als de trombocyten > 50 x 10^9/L zijn, verkregen bij een navelstrengpunctie met of zonder intra-uteriene transfusie. In *Hoofdstuk 5* rapporteren we over onze ervaringen met betrekking tot

de veiligheid van een vaginale bevalling in FNAIT-zwangerschappen bij vrouwen met een eerder kind met een trombocytopenie, maar zonder hersenbloeding. Van de bestudeerde 32 neonaten ontwikkelde geen enkele neonaat een hersenbloeding, terwijl in vier gevallen de trombocyten < 50 x 10^9/L waren. Drie van deze vier neonaten werden vaginaal geboren.

Deze uitkomsten zijn van belang, met name gezien de maternale voordelen van een vaginale bevalling in vergelijking tot een keizersnede, en de nadelen van een keizersnede zoals vooral het risico op een uterusruptuur of een placenta accreta in een volgende zwangerschap. Daarnaast is het maar de vraag of een neonaat met een trombocytopenie een groter risico loopt op een hersenbloeding ten tijde van een vaginale bevalling in vergelijking met een electieve keizersnede. Hiervoor is geen wetenschappelijk bewijs. Bijna alle FNAIT-patiënten zijn al eens eerder bevallen, zodat bij hen een relatief vlotte vaginale partus mag worden verwacht.

In samenwerking met onze collega's van het Karolinska Instituut in Zweden en van de Universiteit van Noord Noorwegen is de NOICH-studie (No IntraCranial Haemorrhage) opgestart. In *Hoofdstuk 6* worden de resultaten van de NOICH studie beschreven. In deze prospectieve, gerandomiseerde, multicentrische studie werd de hypothese getoetst dat IVIG in een lage dosis van 0,5 gram per kilogram maternaal lichaamsgewicht per week minstens even effectief is als de standaarddosis van 1,0 gram/kg/week. Als uitkomstmaten werd het voorkomen van een hersenbloeding en een ernstige trombocytopenie genomen. De gecalculeerde sample size was twee armen van elk 106 patiënten. Na bijna drie jaar van rekruteren waren pas 23 zwangerschappen gerandomiseerd. Hierop werd door de stuurgroep besloten de studie voortijdig af te breken.

Geconcludeerd werd dat het nog steeds onzeker is of de lage dosis IVIG equivalent is aan de standaarddosis. Toch kan de trial als een succesvolle pilot worden gezien, omdat de uitvoerbaarheid en acceptatie van een eventuele vervolgstudie zowel voor patiënten als voor behandelend artsen kon worden aangetoond.

In *Hoofdstuk 7* hebben we het klinische belang van trombocytopenie bij Rhesus D geïmmuniseerde zwangerschappen geëvalueerd. We vonden dat ernstige trombocytopenie vaak voorkomt bij hydropische, anemische foetussen, veroorzaakt door Rhesus D immunisatie. In deze groep vonden we een hoge mortaliteit. Maar ook in niet-hydropische anemische foetussen bleek een ernstige trombocytopenie gerelateerd te zijn aan een slechte prognose. Mogelijk zou deze slechte

prognose verbeterd kunnen worden met intra-uteriene trombocytentransfusies. Echter, intra-uteriene trombocytentransfusies kunnen ook geassocieerd zijn met extra complicaties ten gevolge van het extra volume dat in deze reeds hydropische foetussen wordt gegeven. Daarnaast is het onzeker of een trombocytentransfusie een eventuele bloeding zal voorkomen.

In *Hoofdstuk 8* wordt vastgesteld dat, in tegenstelling tot de hydropische foetussen ten gevolge van Rhesus D immunisatie, foetussen met ernstige anemie ten gevolge van Kell immunisatie geen risico lopen op ernstige trombocytopenie. Zelfs ernstige hydrops, gecombineerd met ernstige anemie, was niet geassocieerd met ernstige trombocytopenie. Aangezien de oorzaak van de trombocytopenie in de Rhesus D gevallen nog niet bekend is, is het zoeken naar de reden waarom deze trombocytopenie in de Kell gevallen niet optreedt, moeilijk. Prospectief onderzoek zal mogelijk antwoorden gaan geven.

Tenslotte wordt in *Hoofdstuk 9* het optreden van trombocytopenie in combinatie met Parvovirus B19 infectie in de zwangerschap besproken. Hoewel trombocytopenie frequent wordt vastgesteld in hydropisch-anemische foetussen met een Parvovirus B19 infectie, werden in onze serie geen foetale bloedingscomplicaties gevonden. Zoals bij hydropische foetussen veroorzaakt door Rhesus D immunisatie, moet het risico van een intra-uteriene trombocytentransfusie met extra volumebelasting voor de reeds overvulde hydropische foetus worden afgewogen tegen de blijkbaar lage incidentie van het optreden van foetale bloedingscomplicaties.

In de algemene discussie in *Hoofdstuk 10* worden suggesties gedaan voor toekomstig onderzoek. Hierbij wordt het belang van internationale samenwerking in multicentrische studies onderstreept.

Klinische vragen met betrekking tot FNAIT die nog openstaan, zijn de volgende:

- Is routinematig screenen van alle zwangeren zinvol, kosteneffectief en haalbaar? Welk behandeltraject kan de positief gescreende groep worden aangeboden?
- Kunnen we een betere selectie maken van die zwangeren met HPA-antistoffen die echt een hoog risico op foetale hersenbloedingen hebben?
- Is veilige behandeling inderdaad mogelijk zonder navelstrengpuncties?
- Is IVIG de beste behandeling? En wat is dan de optimale dosis?
- Is er een rol weggelegd voor aanvullende medicamenten, zoals bijvoorbeeld steroïden?
- Wat is de beste manier van bevallen en wat is het beste tijdstip?

Meer basaal wetenschappelijke onderzoeksvragen waarnaar nader onderzoek kan worden gedaan, zijn:

- Is er een manier om de foetale trombocytenaantallen langs niet-invasieve weg te voorspellen?
- Is het mogelijk om anti-HPA profylaxe te ontwikkelen, vergelijkbaar met anti-D profylaxe bij Rhesus D immunisatie?
- Waarom onderscheiden vrouwen zich van elkaar met betrekking tot het aanmaken van antistoffen?
- Waarom krijgen sommige foetussen met lage trombocytenaantallen een hersenbloeding en andere, met vergelijkbare trombocytenaantallen, niet? Bestaan er genetische verschillen? Speelt het endotheel een rol?
- Hoe werkt IVIG eigenlijk? Is er inderdaad een effect op het endotheel te meten?

In het geval van de andere oorzaken van foetale trombocytopenie (erythrocytenimmunisatie en ParvovirusB19 infectie) zijn er eveneens nog vele vragen onbeantwoord. De belangrijkste dreiging voor deze foetussen is anemie, die relatief gemakkelijk gediagnosticeerd kan worden met behulp van niet-invasieve Dopplermetingen en die effectief behandeld kan worden met intra-uteriene bloedtransfusies. De minderheid van foetussen die daarnaast ook een trombocytopenie hebben, is moeilijker te identificeren; en of behandeling met intra-uteriene trombocytentransfusie nuttig is, is nog niet duidelijk. Het verdient aanbeveling

toekomstige studies onder andere te richten op deze vraag, en op meer basale vragen zoals die naar de oorzaak van de trombocytopenie en de relatie van de trombocytopenie met de slechte uitkomst van de zwangerschap.

Conclusie

Concluderend mogen we na vijf jaar onderzoek stellen, dat er enige vooruitgang is geboekt. Er zijn echter nog steeds meer vragen dan antwoorden met betrekking tot de vele aspecten van foetale trombocytopenie. De onderzoeksgroep van het LUMC gaat samen met de lokale collega's van de afdelingen neonatologie en hematologie, en met de buitenlandse onderzoekspartners door met het uitvoeren van klinisch en basaal wetenschappelijk onderzoek, met als ultiem doel: een gezond kind en een gelukkige moeder.

LIST OF ABBREVIATIONS

B19V	congenital human parvovirus B19
CMV	cytomegalovirus
CS	Caesarean section
DIC	diffuse intravascular coagulation
FBS	fetal blood sampling
FcR	Fc-receptor
FNAIT	fetal and neonatal alloimmune thrombocytopenia
GA	gestational age
GBS	Group B Streptococcus
GP	glycoprotein
Hb	haemoglobin
HIV	human immunodeficiency virus
HPA	human platelet antigen
IRB	institutional review board
ICH	intracranial haemorrhage
IgG	Immunoglobulin G
IVIG	intravenous immunoglobulin
ITP	idiopathic thrombocytopenic purpura
IUGR	intrauterine growth restriction
IUPT	intrauterine platelet transfusion
IUT	intrauterine transfusion
LUMC	Leiden University Medical Centre
MCA-PSV	middle cerebral artery peak systolic velocity
NOICH	No Intra Cranial Haemorrhage
PCR	polymerase chain reaction
PLC	platelet count
RCT	randomised controlled trial
RhD	Rhesus D
SLE	systemic lupus erythematosus
TAR	thrombocytopenia absent radius

AUTHORS AND AFFILIATIONS

From the Division of Fetal Medicine, Department of Obstetrics, Leiden University Medical Centre, Leiden:
Dick Oepkes, Frans Klumper, Humphrey Kanhai

From the Division of Neonatology, Department of Paediatrics, Leiden University Medical Centre, Leiden:
Enrico Lopriore, Frans Walther, Timo de Haan (presently AMC)

From Sanquin SW & the Department of Immunohematology, Leiden University Medical Centre, Leiden:
Anneke Brand

From the Center for Fetal Medicine, Karolinska Institute, Huddinge, Stockholm, Sweden:
Magnus Westgren

From the Department of Immunology and Transfusion Medicine, University Hospital of Northern Norway, Tromsø, Norway:
Anne Husebekk

From the Platelet/Leucocyte Serology Department, Sanquin Diagnostics Service, Amsterdam:
Leendert Porcelijn

From the Department of Medical Microbiology, Leiden University Medical Centre, Leiden:
Louis Kroes

PUBLICATIONS

Van den Akker ESA, Oepkes D. Fetal and neonatal alloimmune thrombocytopenia. Best Practice & Research Clinical Obstetrics and Gynaecology 2008; 22: 3-14.

Van den Akker ESA, Klumper FJCM, Brand A, Kanhai HHH, Oepkes D. Kell alloimmunisation in pregnancy: associated with fetal thrombocytopenia? Vox Sanguinis, 2008, in press.

De Haan TR, *Van den Akker ESA*, Porcelijn L, Oepkes D, Kroes ACM, Walther FJ. Thrombocytopenia in hydropic fetuses with parvovirus B19 infection: Incidence, treatment and correlation with fetal B19 viral load. British Journal of Obstetrics and Gynaecology 2008, 115: 76-81.

Porcelijn L, *Van den Akker ESA*, Oepkes D. Fetal thrombocytopenia. Semin Fetal Neonatal Med. 2008 in press.

Porcelijn L, *Van den Akker ESA*, Kanhai HHH. Diagnosis and management of fetal platelet disorders. In: Rodeck CH, Whittle MJ eds. Fetal Medicine. Churchill Livingstone, London 2008 in press.

Te Pas AB, Lopriore E, *Van den Akker ESA*, Oepkes D, Kanhai HHH, Brand A, Walther FJ. Postnatal management of fetal and neonatal alloimmune thrombocytopenia: the role of matched platelet transfusion and IVIG. Eur J Pediatr 2007; 166: 1057-1063.

Van den Akker ESA, Oepkes D, Lopriore E, Brand A, Kanhai HHH. Noninvasive antenatal management of fetal and neonatal alloimmune thrombocytopenia: safe and effective British Journal of Obstetrics and Gynaecology 2007; 114: 469-473.

Kanhai HHH, *Van den Akker ESA*, Walther FJ, Brand A. Intravenous immunoglobulins without initial and follow-up cordocentesis in alloimmune fetal and neonatal thrombocytopenia at high risk for intracranial hemorrhage Fetal Diagnosis and Therapy 2006; 21: 55-60.

Van den Akker ESA, Oepkes D, Brand A, Kanhai HHH. Vaginal delivery for fe-

tuses at risk of alloimmune thrombocytopenia? British Journal of Obstetrics and Gynaecology 2006; 113: 781-783.

Van den Akker ESA, Oepkes D, Kanhai HHH. Beleid bij zwangerschappen met een verhoogd risico van foetale of neonatale allo-immuun trombocytopenie (FNAIT). Tijdschrift voor Geneeskunde 2005; 61; 1531-1536.

Vrouenraets FPJM, Roumen FJME, Dehing CJG, *Van den Akker ESA*, Aarts M.B, Scheve EJT. Bishop score and risk of cesarean delivery after induction of labor in nulliparous women. Obstet Gynecol 2005; 105: 690-697.

Van den Akker ESA, Oepkes D, Kanhai HHH. Foetale en neonatale alloimmuun trombocytopenie (FNAIT). In: Vandenbussche FPHA, Kanhai HHH, Walther FJ eds. Vorderingen in de foetale geneeskunde. Boerhaave commissie PAOG. Leiden 2005; 61-69.

Van den Akker ESA, Rijken J, Theunissen PHMH, Roumen FJME. Colitis Ulcerosa en zwangerschap: Een casus met een fulminant verloop. Ned Tijdschr Obstet Gyn 2003; 116: 188-191.

Van den Akker ESA, Stikkelbroeck MML, Menheere PPCA, Roumen FJME, Otten BJ. Succesvolle behandeling en zwangerschap bij een vrouw met de niet-klassieke vorm van het adrenogenitaal syndroom. Ned Tijdschr Geneesk 2002; 146: 268-270.

.

DANKWOORD

Dit proefschrift kon alleen tot stand komen door de welwillende medewerking van velen. Ik ben hen allen veel dank verschuldigd. Dat geldt in de eerste plaats voor de zwangere vrouwen en hun partners die aan de verschillende studies hebben deelgenomen, vaak onder voor hen zorgelijke omstandigheden.

Ik dank Jennie Verdoes, Clara Kolster en Yvonne Beuger, de research nurses die verantwoordelijk waren voor de logistiek rond de bloedafnames bij de zwangeren, het verzamelen van navelstrengbloed en het organiseren van de wekelijkse IVIG-toedieningen.

Ik dank Godelieve de Groot-Swings van het laboratorium verloskunde voor het bewerken en opslaan van de bloedmonsters.

Ik dank Robertjan Meerman voor alle praktische hulp rond de FNAIT-database en Ivanka Bekker voor de secretariële ondersteuning.

Ik dank Christine Kramer van Sanquin divisie Plasmaproducten voor het verstrekken van de IVIG en voor de organisatie en het overleg met de apothekers van de bij de NOICH-studie betrokken ziekenhuizen.

Ik dank Leendert Porcelijn van Sanquin Diagnostiek voor het uitvoeren van de verschillende extra laboratoriumbepalingen.

A special thanks to Anne Husebekk from the University of Northern Norway in Tromsø and Magnus Westgren from the Karolinska Institute in Stockholm for the collaboration and their inspiring role in the NOICH-study. I recall with pleasure our meetings in Paris, Leiden, Stockholm en Tromsø. I am looking forward to a continuation of our teamwork in the future. Takk! & Tack så mycket!

Degenen die ik noemde, waren allen op heel directe wijze bij de studies betrokken. Ik kan zeggen dat de samenwerking met ieder van hen uiterst plezierig is verlopen. Ik ben hen daar dankbaar voor.

Er zijn veel meer mensen die mijn promotie hebben mogelijk gemaakt. Tot hen behoren degenen die mij gevormd hebben tot de gynaecoloog die ik nu ben. Ik

heb veel geleerd van de gynaecologen van het Atrium Medisch Centrum te Heerlen, waar ik gedurende vier jaar als AGIO werkzaam was. De daarop volgende twee jaar waren de gynaecologen van het Leids Universitair Medisch Centrum in Leiden mijn leermeesters en lichtende voorbeelden; dat gold voor één van hen in het bijzonder…

Hans Duvekot dank ik voor de uiterst leerzame periode die ik onder zijn supervisie mocht doorbrengen als fellow perinatologie aan het Erasmus Medisch Centrum te Rotterdam.

Mijn beide paranimfen Ingrid en Anneke ken ik vanuit mijn opleidingstijd. Daarnaast is het fellowship perinatologie onze verbindende factor. Ik ben er trots op dat zij vandaag naast mij staan.

Intussen maak ik anderhalf jaar deel uit van de vakgroep gynaecologie van het Onze Lieve Vrouwe Gasthuis in Amsterdam. Ik krijg er de kans om de kennis en kunde die ik heb opgedaan in praktijk te brengen en -meer nog- verder te ontwikkelen. Ik zie uit naar een langdurige collegiale samenwerking.

Een meelevend thuisfront is ter ondersteuning van het moreel van de promovendus onontbeerlijk. Mijn lieve zus Carolien en haar vriend Jeroen dank ik voor hun betrokkenheid. Ik ben heel blij met de vormgeving van dit proefschrift die door Jeroen is verzorgd. Mijn lieve ouders dank ik voor hun meeleven bij alles wat belangrijk is in mijn leven. Ik kan altijd een beroep op hen doen. Het is veelzeggend dat de titelpagina van mijn proefschrift geïllustreerd is met een houtskooltekening van Käthe Kollwitz, dezelfde die in 1977 het omslag van het proefschrift van mijn vader over ongehuwd moederschap sierde.

Dick en Teun, dit proefschrift is aan jullie opgedragen. Dat zegt alles!

De vreugde om een promotie is groot, maar zij valt in het niet bij het wonder van de geboorte van een kind. Beroepsmatig maak ik dit wonder dagelijks mee, maar de waarachtigheid daarvan is voor mij pas echt gaan leven bij de geboorte van mijn eigen zoon Teun. Ik ben er van overtuigd dat de ervaringen die ik als moeder opdoe, mij helpen bij het uitoefenen van mijn prachtige beroep.

CURRICULUM VITAE

Eline van den Akker, auteur van dit proefschrift, werd op 14 maart 1972 geboren in Tilburg. Met haar jongere zus Carolien groeide zij op in het ouderlijk gezin in Riel. Van 1984 tot 1990 bezocht zij het Theresia Lyceum in Tilburg, een periode die zij afsloot met het behalen van het diploma Gymnasium-ß. In 1990 begon zij met de studie geneeskunde aan de Rijksuniversiteit Leiden. Nadat zij in 1997 was geslaagd voor het artsexamen, was zij tijdelijk werkzaam als AGNIO gynaecologie in het AMC in Amsterdam. Hierna begon zij aan haar opleiding tot gynaecoloog. Van 1999 tot 2003 was zij daartoe verbonden als AGIO aan het Atrium Medisch Centrum in Heerlen, met als opleiders Dr. J.E.G.M. Stoot en Dr. F.J.M.E. Roumen. In de twee jaar daarna vervolgde zij haar opleiding aan het Leids Universitair Medisch Centrum in Leiden, waar Prof. Dr. H.H.H. Kanhai haar opleider was. In deze periode is zij aan dit proefschrift begonnen.

Onder leiding van Prof. Dr. E.A.P. Steegers en Dr. J.J. Duvekot was zij gedurende de jaren 2005 en 2006 fellow perinatologie in het Erasmus Medisch Centrum te Rotterdam. Sinds 1 januari 2007 maakt zij deel uit van de vakgroep gynaecologie in het Onze Lieve Vrouwe Gasthuis te Amsterdam, waar de verloskunde haar speciale aandachtsgebied is.

Eline woont thans in een voormalig klooster te Lisse. Zij is getrouwd met Dick en samen hebben zij een prachtige zoon Teun.