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

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CHAPTER 11

GENERAL DISCUSSION

DISCUSSION

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

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

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

Incidence of FNAIT

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

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

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

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

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

Antenatal treatment

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

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

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

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

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

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

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

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

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

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

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

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

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

Burden of disease

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion and Future perspectives

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

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

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

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

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

Other Remaining questions for clinical research to answer are:

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

In Conclusion

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

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