Faculty of Health Science

Antenatal treatment and neonatal outcome of fetal and neonatal alloimmune thrombocytopenia – a 20-years´experience from Norway

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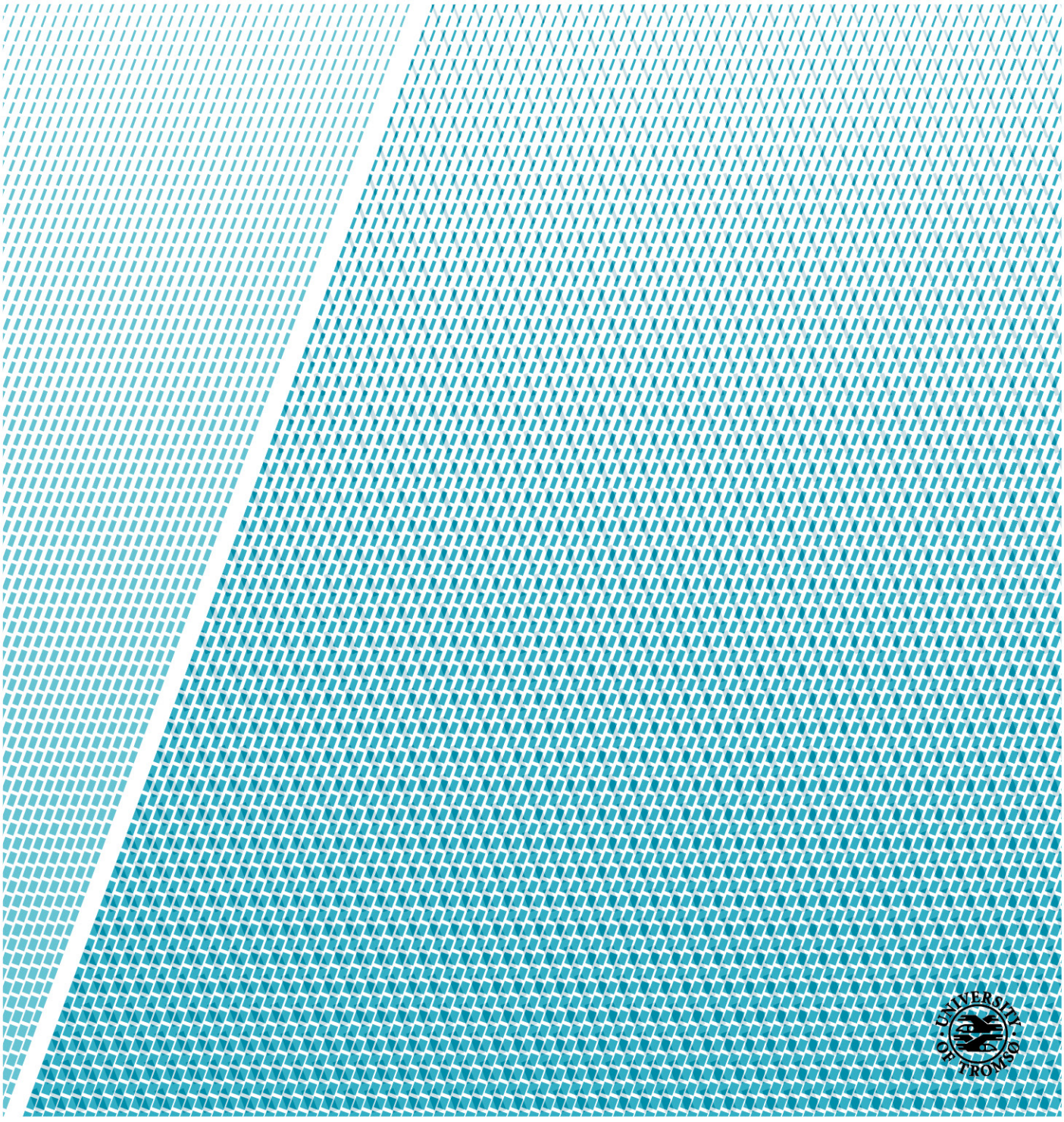

Preface

Since I started my medical studies I have had an interest in immunology, and when I by chance met Heidi Tiller, who later became my main supervisor, her engagement for FNAIT captured my interest. Furthermore, I find the paradox that Norway has a different antenatal treatment strategy for fetal and neonatal alloimmune thrombocytopenia (FNAIT) than other countries, to be an interesting field. I am grateful for receiving the opportunity to participate in evaluating the antenatal treatment and neonatal outcome in FNAIT in Norway during the last 20 years.

I want to express my gratitude to my skilful main supervisor, Heidi Tiller, for helping me out, for her engagement, and for being so optimistic and supportive during the whole process of working with the thesis.

I also want to thank my co-supervisor, Siw Ernstsen, for the good collaboration working closely together during the project period, and Eirin Listau Bertelsen for contributing with important work collecting data material.

Last, but not least, I want to thank my family and friends for being supportive and helpful, for participating in both my worries and achievements, and for giving good advice along the way.

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Summary

Introduction

Internationally, recognized anti-HPA-1a-immunized pregnant women are typically treated with high-dose intravenous immunoglobulins (IVIg), although conclusive evidence on IVIg's efficacy in preventing severe FNAIT is lacking. In Norway, however, IVIg is rarely used. It is therefore relevant to compare FNAIT pregnancy outcome in Norway with other countries where IVIg is more frequently used. The objectives of this study were to assess neonatal outcome (ICH) of FNAIT due to anti-HPA-1a in Norway, and to assess the risk of ICH in subsequent, untreated pregnancies following a history of FNAIT.

Materials and method

Data were collected 20 years back in time from time of initiation of the study (1997-2017). Potential patients were identified from the register at the Norwegian National Unit for Platelet Immunology (NNUPI) in Tromsø and from the previous HPA-1 screening study. Antenatal management and neonatal outcome was retrieved from medical records with informed consent.

Results

In total, 269 pregnancies from 175 women were included. Antenatal IVIg was given in eight (3 %) of these pregnancies, where none resulted in FNAIT with ICH. Seventeen of the 261 untreated pregnancies were complicated by FNAIT with ICH (6.9 %). Fifteen of ICH cases had no known risk of FNAIT before birth. We identified 7 subsequent, untreated pregnancies where an older sibling had FNAIT with ICH. 2 out of these 7 (29 %) subsequent pregnancies were complicated by ICH in the fetus/neonate. We identified 75 subsequent, untreated pregnancies where a previous child had FNAIT but without ICH. We identified one ICH in this group. The risk of ICH in subsequent, untreated pregnancies was therefore 1.3 %.

Conclusion

In the majority of ICH cases the risk of FNAIT was not known before delivery. The risk of ICH in subsequent, untreated pregnancies significantly lower than previously reported by others. By implementing antenatal weekly high dose IVIg treatment to all subsequent pregnancies at

risk we would not have prevented any ICH cases identified through 1997-2017. Therefore, we consider it safe to continue with the Norwegian guidelines.

Abbreviations

FNAIT: Fetal and neonatal alloimmune thrombocytopenia

IVIg: Intravenous immunoglobulins

ICH: Intracranial haemorrhage

HPA: Human platelet antigen

HLA: Human leucocyte antigen

MHC: Major histocompatibility complex

NNUPI: Norwegian National Unit for Platelet Immunology

ESPGI: European Symposium on Platelet and Granulocyte Immunology

1 Introduction

1.1 Definition of fetal and neonatal alloimmune thrombocytopenia

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is defined as fetal or neonatal thrombocytopenia caused by maternal alloantibodies directed against fetal platelets due to incompatibility between fetal and maternal platelet antigens. Thrombocytopenia is defined as a platelet count < $150x10^9$/L. Severe thrombocytopenia is defined as a platelet count < $50x10^9$/L.

1.2 Pathophysiology

Platelets, also named thrombocytes, are blood cells that play an important role in blood clotting. Thrombocytopenia is a blood disease characterized by an abnormally low number of platelets in the bloodstream. Platelet antigens are proteins found on the surface of platelets. Antigens that varies between different individuals within the same species are called alloantigenes. Difference between the platelet antigens of an unborn baby and their mother, because of a fetal platelet alloantigen that is inherited from the father and not shared by the mother, can lead to production of alloantibodies in the mother. The connection between platelet specific alloantibodies in the mother and low platelet count in the fetus/newborn is known and can cause the disease fetal and neonatal alloimmune thrombocytopenia (FNAIT).

In the platelets membrane there are glycoprotein receptors that play fundamental roles in platelet function, such as adhesion and aggregation. The human platelet antigens (HPAs) are located on these glycoproteins. If there is incompatibility in the human platelet antigen (HPA) between the mother and the fetus, the mother may develop an immune response with alloantibodies targeting fetal platelets for destruction. The most common cause is alloantibodies against the HPA-1a (1). The HPA-1a antigen is located on the β3 integrin (GPIIIa) (2), and is expressed on fetal platelet surface from gestational week 16 (3). HPA-1a

antibodies of IgG class from the mother can traverse the placenta and get attached to the surface of fetal platelets, which makes them an easy target for destruction by the fetus. The opsonized platelets are phagocytosed by macrophages in the fetal spleen. As a result, the fetus may become thrombocytopenic.

1.3 Clinical characteristics

Low platelet count in fetal life or just after birth can lead to bleeding complications, either before birth or peripartum. The clinical consequences span a continuum, from no symptoms, to petechiae, mucosal bleedings, hematomas, retinal bleedings, and internal haemorrhages in the fetus or the newborn – where intracranial haemorrhage (ICH), in particular, may lead to intrauterine death or lifelong disability (4, 5).

1.4 Epidemiology

Thrombocytopenia is present in 1 – 3 % of an unselected population of newborns and is associated with a variety of conditions, such as prematurity, infections, thrombosis, and certain congenital disorders (6). FNAIT is the single most common cause of severe neonatal thrombocytopenia (platelet counts less than 50×10^9/L) in otherwise healthy term-born neonates (7, 8). Thrombocytopenia due to FNAIT occurs in around 1 in 1000 newborns (9).

Among Caucasians, around 85 % of the FNAIT cases are caused by an alloantibody to the human platelet antigen-1a (HPA-1a). The ability to develop an immune response against HPA-1a is strongly associated with the human histocompatibility complex (MHC) class II allele HLA-DRB3*01:01 (10). 90 % of HPA-1a immunized women in the large Norwegian screening study were positive for this HLA allele (11), while the frequency of HLA-DRB3*01:01 in a random population is 28 % (10). Around 2 % of the Caucasian population have the platelet type HPA-1bb, and if a pregnant woman with this platelet type produces alloantibodies against the HPA-1a, she can deliver a child with FNAIT (11). Around 10 % of the women with HPA-1bb platelet type will be alloimmunized (make anti-HPA-1a antibodies)

in pregnancy, and 30 % of them will have a child with FNAIT. Furthermore, around 10 % of children with FNAIT is reported to have ICH (12), which can result in serious morbidity and mortality of the child. The incidence of ICH due to anti-HPA-1a-associated FNAIT has been estimated to be around 1 in 10,000 newborns (9) – and the recurrence risk of ICH in subsequent pregnancies of women with a history of FNAIT with ICH is previously reported to be 79 % (13).

1.5 Management

Most cases of fetal and neonatal alloimmune thrombocytopenia (FNAIT) are discovered when a child is born at term with petechiae or other signs of bleeding in the absence of any other condition known to be associated with neonatal thrombocytopenia. Occasionally, these bleeding are suspected during fetal life in case of ultrasound abnormalities, especially in the brain. The majority of FNAIT-affected newborns will not have ICH before delivery, so preventing ICH by increasing platelet counts immediately after birth is considered a neonatal emergency. Therefore, whenever FNAIT is suspected, the newborn should immediately receive a transfusion of compatible platelets without awaiting laboratory confirmation of the diagnosis. The platelets should preferably be from a donor who is HPA-1a negative, but random donor platelets can also be used (14).

As there is currently no screening program for HPA-1a negative pregnant women (pregnancies at risk), primary prevention is not an option. Secondary prevention/treatment is only possible in those cases where the risk of FNAIT is known before birth, typically because the pregnant woman previously has given birth to a child with FNAIT.
There is currently no international consensus regarding the optimal antenatal management for these high-risk pregnancies. None of the different therapeutic approaches that are currently used, have been tested in randomized controlled trials (RCTs) with an adequate control group where IVIg is not given (15, 16). It is therefore challenging to determine the optimal antenatal management of FNAIT. Recently a systematic review compared different antenatal treatment strategies complicated by FNAIT (16). This review has proposed that a first line antenatal management in FNAIT is weekly IVIg administration, with or without the

addition of corticosteroids. This review included amongst other 4 RCTs. Importantly, none of these RCTs included had an adequate control group, that is; a group of HPA-1a-alloimmunized women with subsequent pregnancies that received placebo or no treatment.

However, most Western countries treat recognized HPA-1a-immunized pregnant women with high-dose intravenous immunoglobulin (IVIg) with or without additional glucocorticoids, with varying degree of clinical follow-up of the fetal development during pregnancy (17, 18). Despite the widespread use, solid evidence on IVIg's efficacy in preventing severe FNAIT is lacking (15, 16). Some centres stratify the treatment according to the presence or absence of ICH in the previous child and the timing of when ICH occurred, i.e., early in pregnancy, late in pregnancy or perinatally (19). Based on risk assessment, the dosage of IVIg used for treatment of HPA-1a-immunized women varies from 0.5 – 2.0 g/kg/week. Gestational age at onset also varies from 12-16 weeks in high-risk pregnancies, to 24 weeks in standard-risk pregnancies (18, 19). The costs of IVIg treatment should also be considered in the debate of IVIg as an antenatal treatment for FNAIT.

The use of intrauterine transfusion of compatible allogeneic (or washed maternal) platelets has also been applied but has now been abandoned at most maternal-fetal centres because of the high risk of procedure-related fetal demise (13, 16).

In Norway, the FNAIT management strategy has for more than two decades differed from most other countries. The Norwegian strategy has most likely been influenced by the results obtained in the Norwegian screening and intervention study that was conducted in Norway from 1996 until 2004 (11). Thus, IVIg is only rarely used for treating HPA-1a-immunized women. Since 2014 the National clinical guidelines for obstetricians changed from not recommending IVIg at all, to recommending antenatal IVIg to women who previously have had a child with FNAIT-induced ICH. This change was based on some evidence of reduced risk of fetal/neonatal ICH in subsequent pregnancies in cases where an older sibling suffered with ICH (12). For HPA-1a-immunized pregnant women where a previous child had FNAIT, but not ICH, IVIg is usually not given. Instead, immunized women are carefully monitored through the pregnancy with frequent clinical examinations including ultrasonographic examination of the fetal brain, fetal growth and maternal anti-HPA-1a quantifications (figure

1). Women with high anti-HPA-1a antibody levels during pregnancy (> 3 IU/mL) are delivered by elective caesarean section at around gestational week 38-39 in hospitals with a neonatal intensive care unit as well as facilities to offer HPA-1a negative platelet transfusion to the newborn, if necessary (figure 1).

The question has been raised as to whether the pregnancy outcome of women who previously have had a fetus/child with FNAIT, is worse in Norway, where very few women are treated with IVIg, as compared to other countries where such women are typically treated with IVIg.

2 Materials and method

2.1 Objective

The main objective of this study was to assess neonatal outcome (i.e. ICH) of FNAIT due to anti-HPA-1a alloimmunization in Norway, stratified on antenatal treatment (IVIg or no treatment). Secondly, to assess the risk of ICH in subsequent, untreated pregnancies following a history of FNAIT (both if FNAIT in index child was complicated by ICH and not). Since IVIg is rarely given in Norway, the large population of untreated FNAIT cases in Norway represents a control group to those FNAIT populations where IVIg is typically given during pregnancy.

2.2 Type of study

A retrospective observational study was conducted where antenatal treatment and outcome-related data for the newborn was retrieved from women in Norway who previously have had a fetus/newborn with FNAIT, either identified in the clinic or through a previous HPA-1a screening study.

2.3 Selection of the study population

Data was collected 20 years back in time from time of initiation of the study (1997-2017). Potential patients were identified from the register at the Norwegian National Unit for Platelet Immunology (NNUPI) in Tromsø (referred to as "routine population/-cases") and from a previous HPA-1 screening study in Norway (referred to as "screening population/-cases") (11).

Norwegian National Unit for Platelet Immunology (NNUPI)

All cases of suspected FNAIT cases in Norway should be referred to the Norwegian National Unit for Platelet Immunology (NNUPI) at UNN Tromsø. At NNUPI, detection, identification and quantification of both auto- and alloantibodies directed against thrombocyte is performed, together with analyses for identification of thrombocyte specific antigens. The service contributes with clinical counselling in connection with diagnostics and treatment of thrombocytopenia caused by immune disorders, and can provide compatible blood products to patients with special and complicated transfusion requirements. Detection and identification of antibodies against thrombocytes, in addition to identification of thrombocyte antigens, is necessary for medical review and making diagnosis of thrombocytopenia caused by immune disorders, and choice of treatment for these.

The Immunologic Research Group (IRG)

The Immunologic Research Group at IMB, UiT The Artic University of Norway is internationally leading in the research field for FNAIT. The group consist of around 10 members who all are active in research at UNN and UiT and have access to research infrastructure in both institutions. The group have external support from NFR, the research foundation from the regional health authorities (Helse Nord), UiT The Arctic University of Norway, EU and EØS. They are doing research that include basal research linked to the immune response in FNAIT together with clinical and epidemiological research.

2.4 Definitions and categories of clinical parameters

Our definition of index cases is the first pregnancy with recognised FNAIT after year 1997. Subsequent pregnancies are all recognises pregnancies, both FNAIT and no FNAIT, after the index pregnancy during the study period. This means that there were a few women who had their first FNAIT-child born before 1997, but where we have defined a younger sibling as the index case.

Since we wanted to assess neonatal outcome of FNAIT stratified on antenatal treatment, we have divided included pregnancies in two categories; antenatal treatment with IVIg, and untreated pregnancies. Untreated refers to no IVIg-treatment antenatally, it does not imply that there was no other intervention for the mother and/or child.

Furthermore, we have stratified the outcomes on whether the risk for FNAIT was known before birth or not; screening (both index and subsequent pregnancies) and subsequent pregnancies identified through routine had known risk, while index cases in routine had no known risk.

At last, we wanted to assess the risk of ICH in subsequent, untreated pregnancies following a history of FNAIT – both if FNAIT in index child was complicated by ICH and not. Therefore, we have divided this population into two groups; index child with ICH and index child without ICH.

2.5 Ethics

The National reference laboratory for clinical platelet immunology at UNN Tromsø routinely asks HPA-1a alloimmunized women identified through clinical investigation at the lab for written consent. Some of the patients in the register at the NRLCPI had therefor already consented to be contacted by NRLCPI for research projects before we started this study. For all women previously participating in the Norwegian HPA-1a screening study, the consent

obtained as part of that study was found to cover the study at hand. All potential participants who did not already give consent was asked for a written consent.

In cases where the mothers journal wasn't sufficient, we also asked the hospitals for copies of relevant parts of the children's journal. Since all children born in- and before 2002 is over the age of 16, we needed these children them self for consent to extract data from their journals. We have asked REK permission for this but are still waiting for such approval. Some of medical data for children > 16 years are therefore still missing.

The project is approved by the REK (2009/1585).

The study was conducted according to the principles laid down in the 7th version of the Declaration of Helsinki (20) and in compliance with the Norwegian data protection laws and regulations.

2.6 Hospital Charts

After obtaining written consent, copies of relevant parts of the hospital charts was retrieved from the hospitals. For women who belongs to our own health region (Health North), we were able to use DIPS to access the relevant parts of the medical journals. During the project period we were three persons with relevant competence who evaluated the journals and extracted relevant data before plotting it into a database made for the purpose of this study. The database is stored electronically with limited access. All participating women was given an ID-number, so we have the opportunity to extract the non-identifiable data from the database.

2.7 Statistics

As the present study is a retrospective descriptive study, only descriptive statistics is used.

2.8 Project organization

The project is a part of my Master thesis, which was written during Spring 2018. I participated in the data collection, extraction of medical data from the patient records, plotting of data into the project database and analyses of the data. I contributed in planning a presentation and poster for presentation at the ESPGI (European Symposium on Platelet and Granulocyte Immunology) in Netherlands May 2018. I prepared the tables and figures to be used in this preparation. My report, including tables and figures presenting the results, will also be part of a planned scientific publication. I will be co-author of this publication.

Resident at Department of Laboratory Medicine at UNN Tromsø, Siw Ernstsen, was my co-supervisor for this master thesis. During the project period we worked closely together doing data collection, database plotting and analysis. She will also be first-author of the planned publication.

Heidi Tiller, senior consultant at UNN and associate professor at IMB, UiT The Arctic University of Norway, was my main supervisor. She contributed in planning of the project, organizing and supervising retrieval and plotting of medical data, analysing the data and writing of the thesis and the manuscript for ESPGI. She was also project leader and will be anchor author of the publication.

Eirin Listau Bertelsen is a research engineer/ technical staff at the NRLCPI and was a collaborator and will be co-author in the project. She contributed by obtaining informed consents, retrieval of medical records, plotting of medical data and analysing the data.

Jens Kjeldsen-Kragh was a collaborator on the project and will be co-author of the publication. He was contributing in planning the project, analysis of the data and writing of the manuscript.

Bjørn Skogen was collaborator on the project and will be co-author of the publication. He was contributing in planning the project, analysis of the data and writing of a manuscript.

3 Results

3.1 Overview study population

In total we identified 171 pregnancies trough the routine and 120 from screening, including both index and subsequent pregnancies (figure 2). We excluded 15 pregnancies from the routine and 7 pregnancies from the screening due to missing consent or missing data. This gave us in total 269 included pregnancies, from 175 different women. The number of index pregnancies were 180, including 5 twin pregnancies, while 89 were subsequent pregnancies (figure 2).

3.2 Antenatal treatment – IVIg

Of the 269 pregnancies included, antenatal treatment with IVIg was given in 8 pregnancies during the study period (figure 3). Five of these treatments were given to women who previously had given birth to a child with FNAIT not complicated with ICH. Two women received IVIg because they previously had given birth to a child with FNAIT with ICH. One of these women got IVIg in two subsequent pregnancies. One of the mothers received IVIg because of rheumatic disease; during the pregnancy she received a total of 3 treatments with Octagam 0,4g/kg/day for 5 subsequent days.

None of the pregnancies treated with antenatal IVIg resulted in FNAIT complicated by ICH (figure 3). Two of the pregnancies resulted in children born without FNAIT, both children had incompatible genotype (HPA1-ab) compared to their mothers (HPA1-bb).

3.3 Neonatal outcome – platelet counts

To study the FNAIT severity, we looked at neonatal platelet counts at birth, and compared platelet counts from index pregnancies with subsequent pregnancies. We also compared the platelet counts between index pregnancies originating from the screening with those from routine. We excluded pregnancies where the mother had received antenatal IVIg, the

neonate was HPA-1bb, or pregnancies where the neonate had normal platelet count where data on neonatal HPA-1 genotype was missing. Of 261 untreated pregnancies, there were 5 children with confirmed compatible platelet type (HPA1-bb), and 6 children without FNAIT where we are missing the HPA-type. In total, data for 250 pregnancies were included for platelet count analyses (table 1). Among these 250 cases, we have compared mean platelet counts between different categories (figure 4).

Mean platelet count was 22 in index routine (SD 22), 44 in index screening (SD 38), 47 in subsequent routine (SD 57) and 45 in subsequent screening (SD 52).

Index routine had a significant lower mean platelet count than subsequent routine (independent sample t-test, p = 0.004, figure 4). There was also significantly lower mean platelet count in index routine compared to index screening (independent sample t-test, p = 0.000, figure 4). Comparing mean platelet count between index screening and subsequent screening gave no significant difference (independent sample t-test, p = 0.958, figure 4).

3.4 Neonatal outcome of untreated pregnancies - intracranial haemorrhage

In the total number of 261 untreated pregnancies, there were 14 subsequent pregnancies without FNAIT. This gives us 247 untreated pregnancies with FNAIT. Among these, ICH due to FNAIT occurred in 17 pregnancies from 15 women. This gives an overall occurrence risk of 6.9 % for ICH due to FNAIT.

The risk of FNAIT was known before delivery in only 2 pregnancies (figure 5). Both were women who previously delivered a child with ICH, none of these received antenatal IVIg in the subsequent pregnancy – and both resulted in ICH in the next child.

In the remaining 15 of these pregnancies, the risk of FNAIT was not known before delivery (figure 5). Twelve of these women were nulliparous, while 3 were multiparous. Although 3 were multiparous, the risk of FNAIT was not known before delivery because two of them had their first FNAIT child in this current pregnancy. The remaining woman who were

multiparous probably had an FNAIT child earlier when reviewing her medical records, however this was not recognised as FNAIT at the time and therefore not followed up in the subsequent pregnancy.

We wanted to assess the recurrence risk of ICH for untreated pregnancies in women who previously delivered a child with ICH due to FNAIT. We identified nine subsequent pregnancies from eight mothers after having a previous child with ICH. 2 of these received antenatal IVIg, while 7 was untreated (6 from routine). Of these 7 untreated pregnancies, there were 2 cases with ICH (figure 6). This gives a Norwegian ICH recurrence risk on 28.6 %.

Furthermore, we wanted to estimate the risk of ICH in subsequent untreated pregnancies where a previous child had FNAIT not complicated by ICH. From 261 untreated pregnancies there was only one pregnancy with FNAIT not complicated by ICH in index pregnancy who had FNAIT with ICH in the subsequent pregnancy. We identified 75 untreated subsequent pregnancies, of which 44 came from the routine (table 2). The overall risk of ICH in subsequent untreated pregnancies where a previous child had FNAIT without ICH was calculated to be 1.3 % (figure 7). If we only included pregnancies from the routine, the risk was 2.3 %.

We are missing ICH data in < 10 % of the untreated FNAIT cases.

4 Discussion

First of all, I want to summarize main findings. The occurrence rate of ICH in our study was 6.9 %. When a previous child had FNAIT complicated by ICH, the recurrence risk in subsequent untreated pregnancies was 28.6 %. In cases where a previous child had FNAIT without ICH, the risk of ICH in subsequent untreated pregnancies was calculated to be 1.3 %.

Second, I want to mention some methodological considerations. We have merged retrospective and prospective data, something we think is appropriate in this study because we have looked at the outcome of patients who have been given equal treatment, rather than frequency.

It is possible to criticise inclusion of the screening because there were probably discovered FNAIT cases with a less severe outcome which would not have been discovered by routine, and furthermore the subsequent pregnancies had a better outcome because they were followed up (without screening these subsequent pregnancies may have been index pregnancies). Therefore, it is a point to take out the retrospective cases from the routine. When we do that, the recurrence risk was 33.4 % rather than 28.6 %.

When it comes to subsequent untreated pregnancies, the population of 75 women may include some HPA-1 compatible children because we lack data on genotype in 47 of the children – and therefore some compatible children may be included in our data. Among these 47 children, 40 had thrombocytopenia and are therefore most likely incompatible with the mother. Of the 7 cases without thrombocytopenia, there are 1 case where the father is typed to be HPA1-aa, meaning that the child has to be HPA-1ab. Then we are left with 6 cases without thrombocytopenia where the child possibly is compatible (1 screening, 5 routine). Among the 28 subsequent untreated pregnancies of which we know the genotype, there are 4 confirmed compatible (HPA1-bb) pregnancies (3 from screening, 1 routine). So, if we exclude these 4 confirmed compatible pregnancies and the 6 possible compatible pregnancies; the risk of ICH in subsequent untreated pregnancies where a previous child had FNAIT without ICH was then 1 / (75-10) = 1.5 % among all, and 1 / (44-6) = 2.6 % only including pregnancies from the routine.

The occurrence rate of ICH in our study population of 6.9 % suggests that the Norwegian FNAIT population is comparable with what has previously been reported for other populations (21, 22). There is therefore no indication that Norway has any lower or higher prevalence of FNAIT complicated by ICH than other countries.

In the NOICH study from 2013 (5), they found that IVIg treatment during pregnancy seemed to be protective with regards to ICH, reducing the ICH recurrence risk from 79 % as previously reported (13), to 11 %. In our material we found recurrence risk of 28-34 % - without antenatal treatment, which is significantly lower than previously reported. The difference may be explained by publication bias in the earlier reported risk on 79 % (13).

The risk of ICH in subsequent untreated pregnancies where a previous child had FNAIT without ICH was found to be 1-3 % in our material, and this number is also lower than what has previously been reported by others (13).

ICH due to FNAIT occurred in 17 pregnancies. Only in 2 of these the risk of FNAIT was known before delivery. Both were women who previously delivered a child with ICH, none of these received antenatal IVIg in the subsequent pregnancy – and both resulted in ICH in the next child. In the remaining 15 of these pregnancies, the risk of FNAIT was not known before delivery. So, the potential avoidable ICH-cases in a routine setting were 2. These two cases where the woman previously gave birth to a child with FNAIT with ICH occurred before 2014, and would therefore nowadays have been offered antenatal IVIg according to the Norwegian treatment strategy – and by giving antenatal IVIg to these two women it is likely that ICH would have been avoided.

A recent systematic review in Blood is recommending that weekly administration of IVIg is the best treatment for women who has delivered a child with FNAIT earlier (16). Of all the 17 ICH-cases in our material there were unknown risk for FNAIT before birth in 15 of the cases, and these women would normally not be offered antenatal IVIg – because the risk of FNAIT was not identified before birth. So, these cases of FNAIT with ICH would not have been avoided by changing the Norwegian treatment strategy. None of the other ICH-cases would have been avoided if the Norwegian FNAIT-strategy had included IVIg as standard choice of treatment of all subsequent pregnancies. This is something to consider in the further process making an international consensus on the optimal antenatal management of FNAIT in subsequent pregnancies.

Regarding neonatal platelet counts, our data showed that there were no worse outcomes in platelet counts among untreated, subsequent pregnancies than among index pregnancies. Mean platelet count in index routine was significant lower than in subsequent routine, while there was no significant difference between index screening and subsequent screening. Thus, increased neonatal platelet count in a subsequent FNAIT pregnancy may not always reflect antenatal treatment effect. This was also presented in a recent Norwegian prospective study (2004-2012), where the natural course of FNAIT in several subsequent pregnancies after an index pregnancy in the screening study (1995-2004) (11), was reported

for the first time (23). The data showed that younger siblings of FNAIT-affected children had unchanged or higher platelet counts without antenatal treatment in the majority of subsequent pregnancies. The data material we have used in our study includes the same cases, but we have in addition included routine cases from our whole study period (1997-2017), and an extended study period of subsequent pregnancies from screening cases (2012-2017).

Strengths with our study is that we have a relative high number of included cases, which we can use as an adequate control group to earlier reported studies (13, 16), and our study population is considered to be representative of a larger study population. There is no publication bias in our data. Competent personal have evaluated the cases and outcomes. We also have minimal undetermined or missing data. Missing ICH data is < 10 % of the untreated FNAIT cases. Before our planned article publication, we will hopefully have these data. There are still some missing cases (cases not registered in the population and excluded cases because of no consent or missing data) and therefore it is possible that there are indeed some more ICH cases among the included pregnancies.

Clinical implications

By implementing antenatal weekly high dose IVIg treatment to all subsequent pregnancies at risk we would not have prevented any of the ICH cases identified during 20 years in Norway. Both of the 2 ICHs in the 7 subsequent untreated pregnancies after an index pregnancy with FNAIT complicated by ICH, would have been treated with the guidelines we use in Norway today. Therefore, we consider it safe to continue with the Norwegian guidelines, and thereby continuing to have different clinical practice from our colleagues throughout the world.

However, we don't know anything about the efficacy of IVIg as antenatal treatment in a prospective setting. So, if a screening programme is implemented, the antenatal treatment still is questionable. Our data supports that not necessarily all HPA-1 immunized women should be treated with IVIg during pregnancy, but up to date we don't have the knowledge to identify those who wold need this treatment in a screening setting – better methods to

identify pregnancies at risk for ICH among women without a previous history of FNAIT is required.

5 Conclusions

The occurrence rate of ICH in our study was 6.9 %. The risk of FNAIT was not known before delivery in the majority of ICH cases.

In our material, the Norwegian recurrence risk for ICH in subsequent untreated pregnancies was 28-34 %. The risk of ICH in subsequent, untreated pregnancies following a history of FNAIT without ICH was 1-3 %. Both these numbers are considerably lower than previously reported (13).

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7 Tables

Table 1. Neonatal outcome – platelet counts.

Category	Number
Untreated pregnancies	261
Confirmed HPA1-bb	5
Untreated pregnancies without confirmed HPA1-bb	256
No FNAIT	9
No FNAIT and in addition missing HPA-type	6
Untreated pregnancies without confirmed HPA1-bb, and no FNAIT + missing HPA-type	250

Of 261 untreated pregnancies, there were 5 children with confirmed compatible platelet type (HPA1-bb), and 6 children without FNAIT where we are missing the HPA-type. So, untreated pregnancies, without confirmed compatible pregnancies and possible compatible pregnancies, is 250.

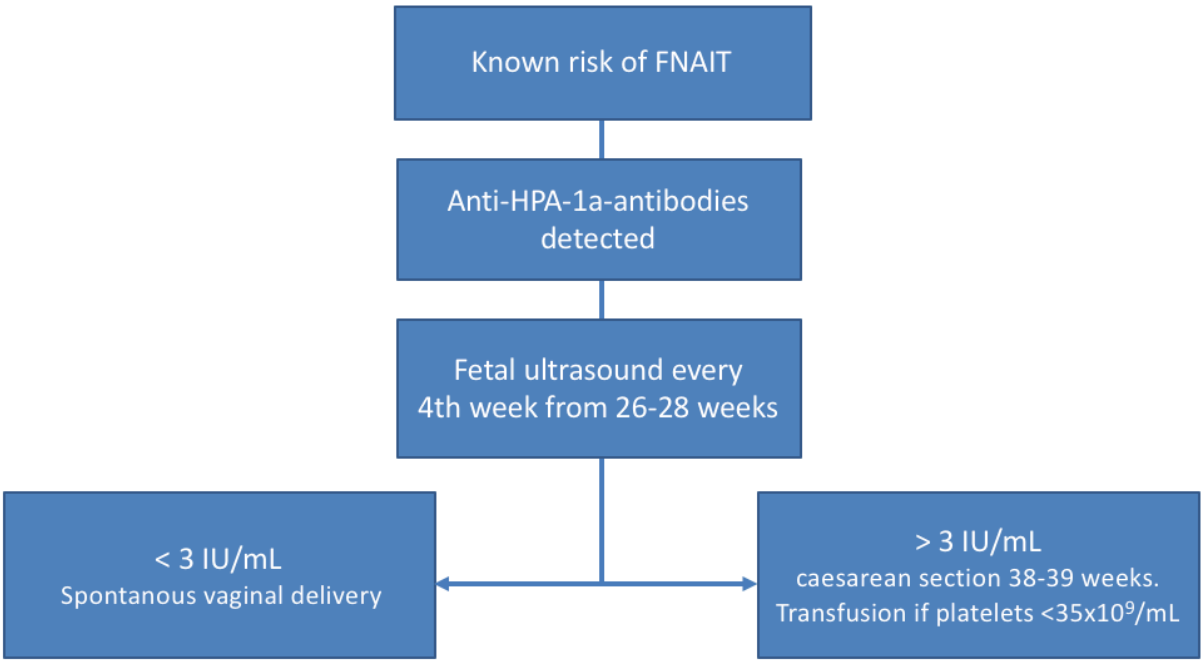
Table 2. Subsequent untreated pregnancies with FNAIT not complicated by ICH in first pregnancy.

Category	Number		
Subsequent pregnancies	89		
Subsequent pregnancies with ICH in index pregnancy	9		
Subsequent pregnancies without ICH in index pregnancy	89 - 9 = 80		
Subsequent pregnancies treated with IVIG	7		
Number of IVIG pregnancies after ICH in index pregnancy	2		
Subsequent untreated pregnancies without ICH in index pregnancy	80 - (7-2) = 75	44 rutine, 31 screening	

We identified 75 untreated subsequent pregnancies, of which 44 came from the routine.

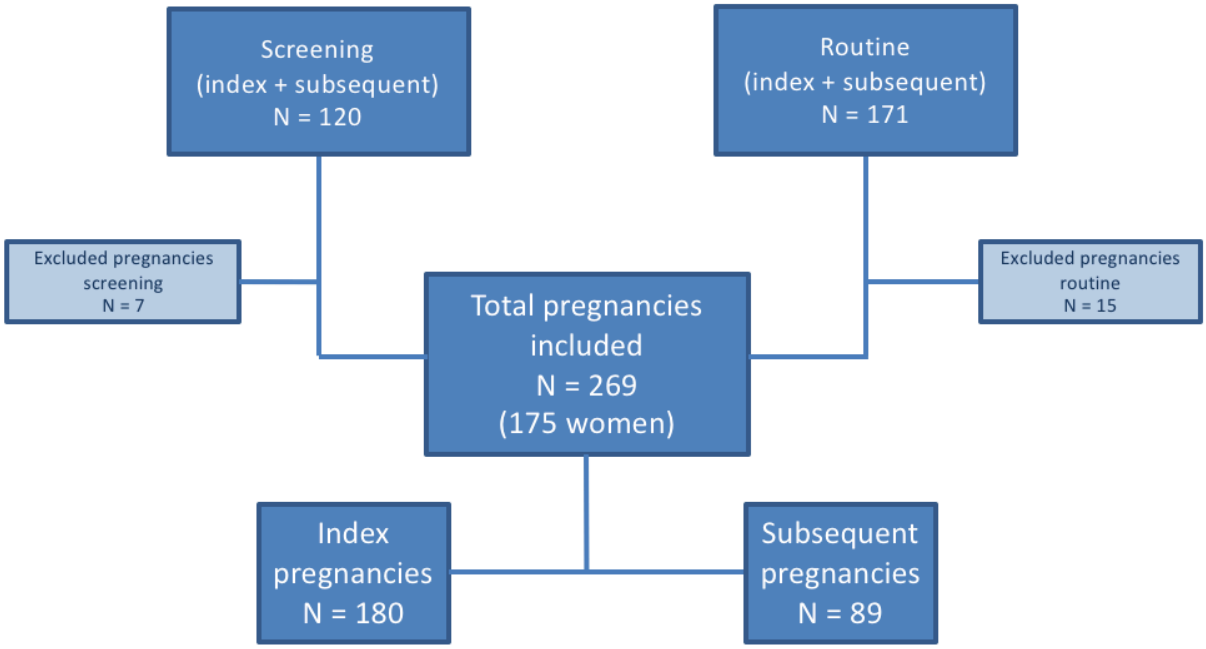
8 Figures

Figure 1. The FNAIT management strategy in Norway.



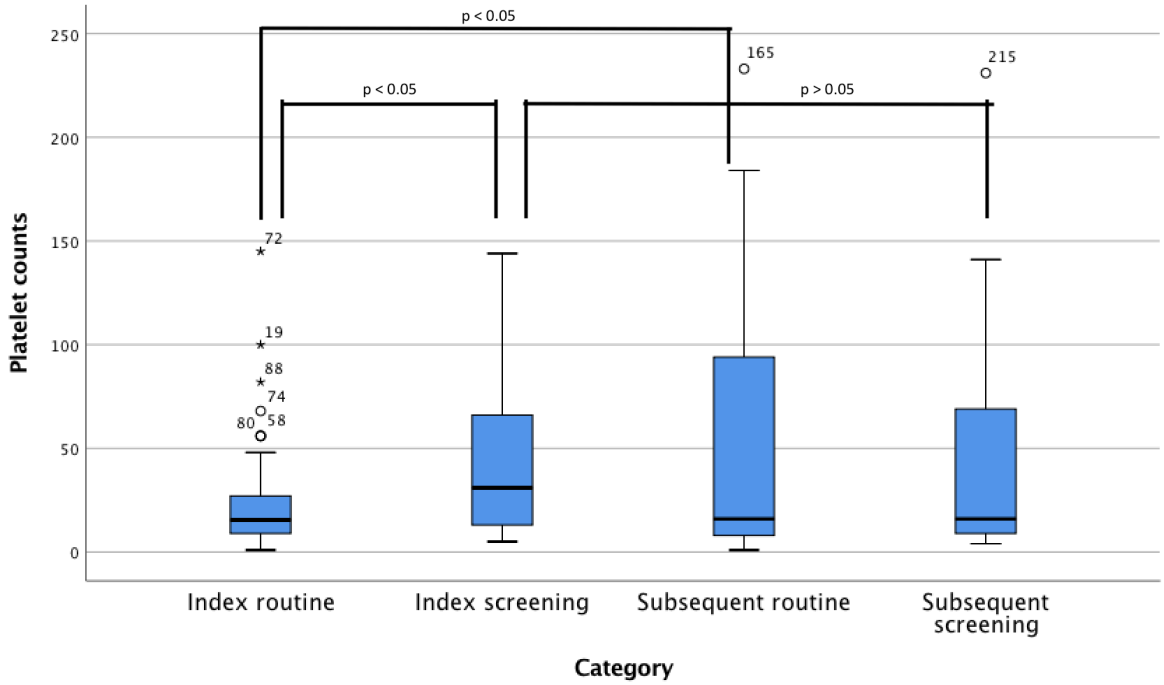
HPA-1a-immunized pregnant women are carefully monitored through the pregnancy with frequent clinical examinations including ultrasonographic examination of the fetal brain, fetal growth and maternal anti-HPA-1a quantifications.

Figure 2. Overview of the study population.



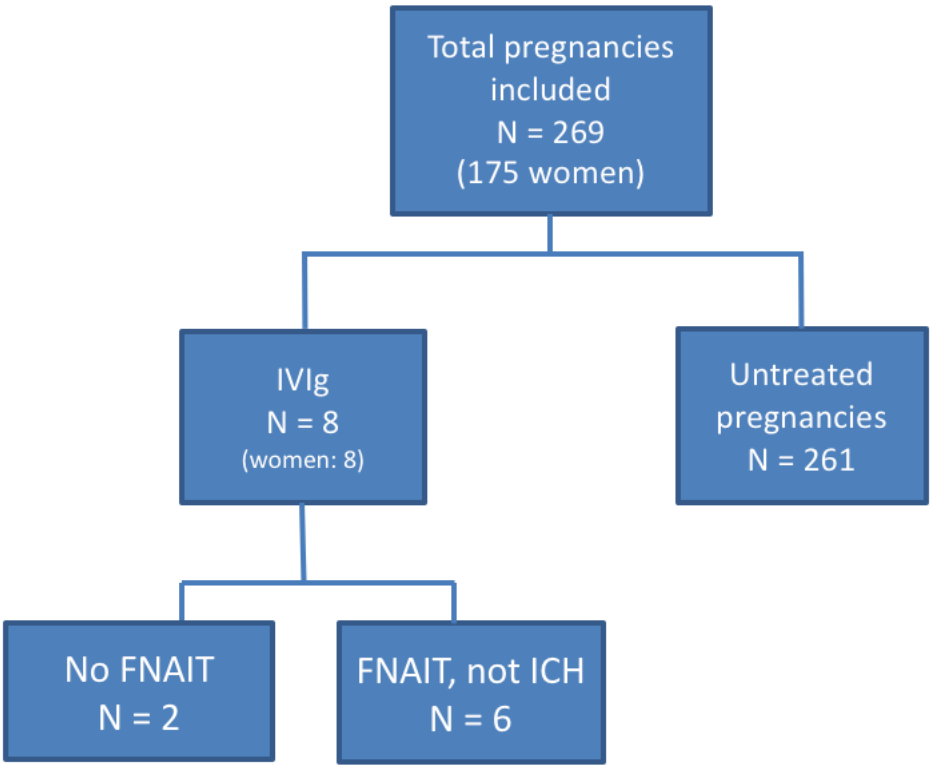
We identified 171 pregnancies trough the routine and 120 from screening. We excluded 15 pregnancies from the routine and 7 pregnancies from the screening. In total, 269 pregnancies were included. The number of index pregnancies were 180, while 89 were subsequent pregnancies.

Figure 3. Neonatal outcome – platelet counts.



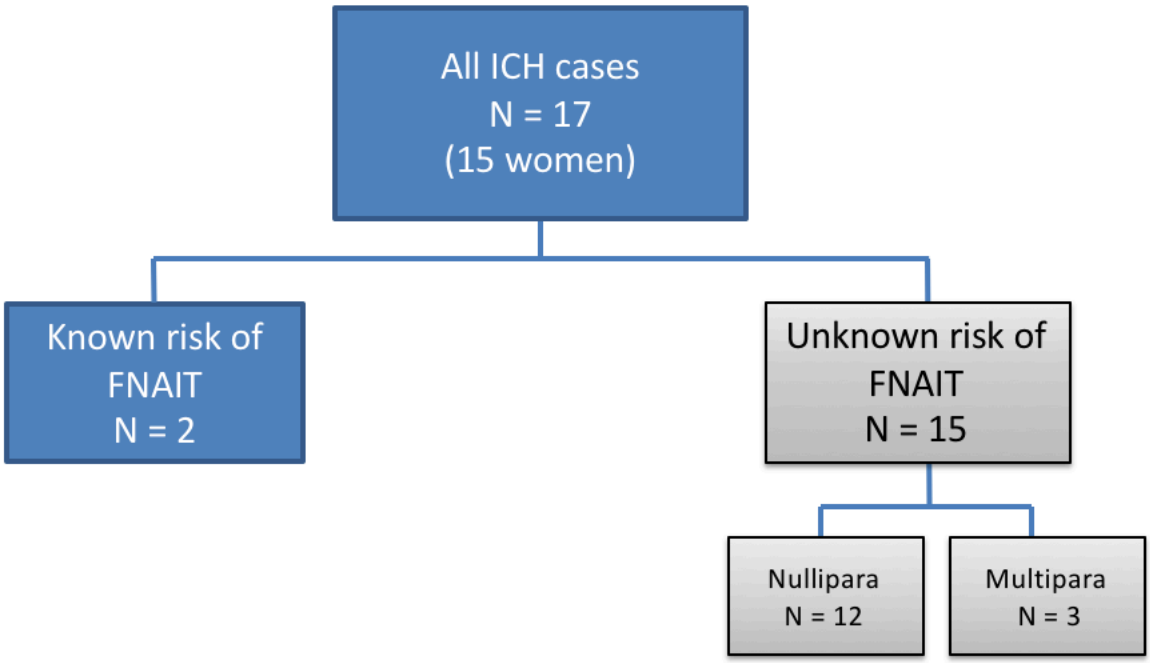
Mean platelet count in index routine was significant lower than in subsequent routine. There was also significant lower mean platelet count in index routine compared to index screening. Comparing mean platelet count between index screening and subsequent screening gave no significant difference.

Figure 4. Antenatal treatment – IVIg.



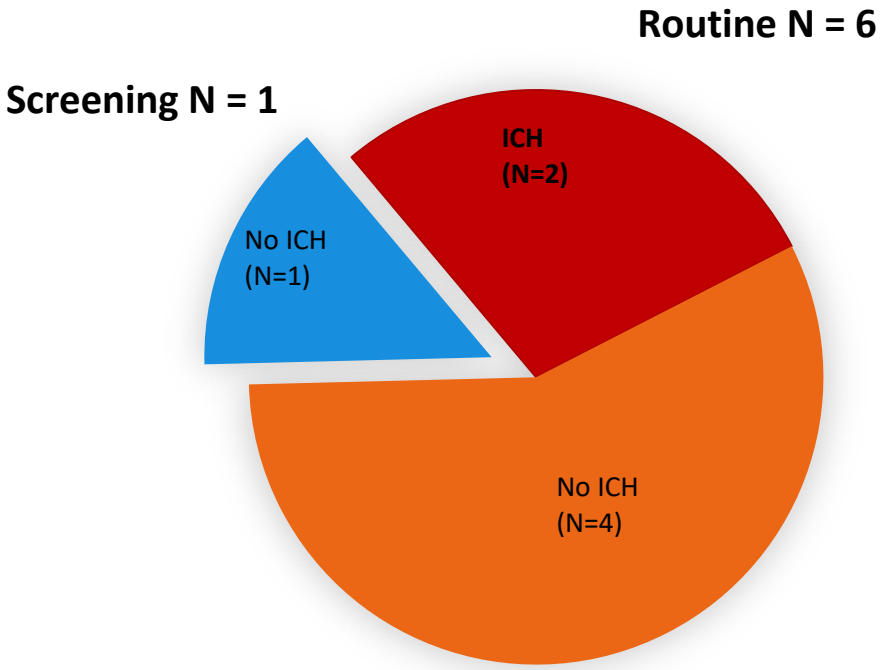
Of the 269 pregnancies included, antenatal treatment with IVIg was given in 8 pregnancies during the study period. None of the pregnancies treated with antenatal IVIg resulted in FNAIT complicated by ICH.

Figure 5. Neonatal outcome of untreated pregnancies - ICH.



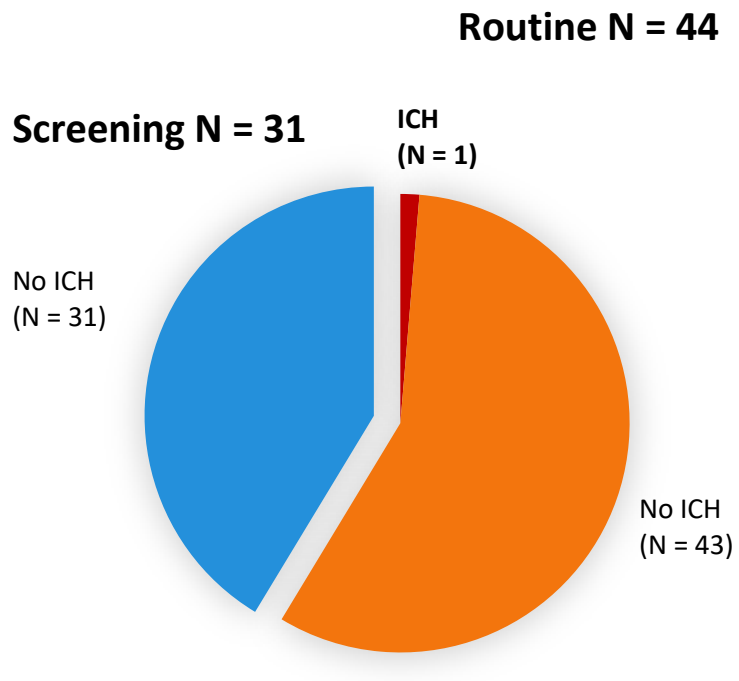
Among the 247 untreated pregnancies with FNAIT, ICH due to FNAIT occurred in 17 pregnancies from 15 women. This gives an overall occurrence risk of 6.9 % for ICH due to FNAIT. The risk of FNAIT was known before delivery in only 2 pregnancies. In the remaining 15 of these pregnancies, the risk of FNAIT was not known before delivery. 12 of these women were nullipara, while 3 were multiparous.

Figure 6. Risk of ICH in subsequent untreated pregnancy when a previous child had FNAIT complicated by ICH.



The Norwegian recurrence risk is 1/7 = 28,6 %. Only including routine 2/6 = 33.4 %.

Figure 7. Risk of ICH in subsequent untreated pregnancy when a previous child had FNAIT not complicated by ICH.



Overall 1/75 = 1.3 %. Only including pregnancies from the routine 1/44 = 2.3 %.

9 Appendix

9.1 Written Consent

UNN – Generell forskningsbiobank for immunologisk betinget blodplatemangel hos foster og nyfødt
Informasjon og samtykkeerklæring
Tidligere gjennomgått svangerskap
Godkjent av REK: 11.05.15

Forespørsel om å overføre biologisk materiale til "Generell forskningsbiobank for immunologisk betinget blodplatemangel hos foster og nyfødt"

Bakgrunn og hensikt
Dette er en forespørsel til deg som tidligere har vært utredet for blodplatemangel hos nyfødt/ føtal og neonatal alloimmun trombocytopeni (FNAIT) om å overføre blod og/ eller vevsprøve til en forskningsbiobank ved Universitetssykehuset Nord- Norge HF (UNN-HF).

Biobanken er en generell forskningsbiobank der biologisk materiale oppbevares for fremtidig forskning. Formålet med biobanken er å drive forskning på immunresponser og svangerskapsforhold i forbindelse med blodplateantistoffer og FNAIT, som i sin tur kan bidra til å bedre forebygging, diagnostikk og behandling. Materialet oppbevares på ubestemt tid.

1) Vi ber nå om din tillatelse til å bruke eksisterende biologisk materiale og relevante opplysninger om deg og ditt/dine barn som er innsamlet i forbindelse med klinisk utredning til forskning, og at blodprøvemateriale kan lagres i forskningsbiobanken. I noen tilfeller er aktuelt å gjøre utvidete analyser av prøvematerialet som ble innsamlet i forbindelse med utredning.

2) Vi ber samtidig om din tillatelse til å kunne kontakte deg på et senere tidspunkt for å be om nye prøver dersom det er relevant for forskningsprosjektet. Du kan bli kontaktet på et senere tidspunkt med forespørsel om deltakelse i forskningsprosjekter som ikke dekkes av avgitt samtykke.

Selv om du samtykker til å delta i forskningsbiobanken nå, trenger du ikke å avgi ny prøve eller samtykke til nye forskningsprosjekter senere dersom du skulle bli forespurt.

Biobankens ansvarshavende er fag- og forskningssjefen ved UNN-HF. Administrerende direktør ved UNN-HF er databehandlingsansvarlig for opplysningene som registreres.

Innsamling og bruk av helseopplysninger

Biobanken vil inneholde noen opplysninger om deg og ditt/dine barn (mors navn og fødselsnummer, svangerskaps termin, barnets kjønn og fødselsdato, barnets fødselsvekt, barnets blodplatetall ved fødsel, antistoffpåvisning hos mor). Disse opplysningene er imidlertid kun tilgjengelige gjennom en koblingsnøkkel som skal beskytte din identitet men

samtidig gjøre det mulig å knytte dine opplysninger til dine prøver gjennom en kodeliste. Institusjonen er ansvarlig for at koblingsnøkkel oppbevares og forvaltes forsvarlig. Det er kun autorisert personell tilknyttet biobanken som har adgang til navnelisten og som kan finne tilbake til dine data. Opplysninger lagres permanent og vil analyseres i forbindelse med spesifiserte forskningsprosjekter. Det vil ikke være mulig å identifisere deg eller dine barn i resultatene av studiene når disse publiseres.

Genetiske undersøkelser

Det kan være aktuelt å gjøre genetiske analyser på innsamlet materiale. Det vil dreie seg om analyser som er relevante for utvikling av antistoffer i svangerskap/ FNAIT/blodplatemangel hos nyfødte. Aktuelle genetiske analyser vil være enkeltgen-analyser (for eksempel din blodtype, vevstype, blodplatetype) eller andre typer analyser (genomvide assosiasjonsstudier). Resultatene av disse analysene vil ikke kunne si noe om din fremtidige generelle helse, men dersom resultatene avdekker funn som vil kunne ha betydning for eventuelle fremtidige svangerskap hos deg, vil du bli kontakten med tilbud om informasjon og veiledning.

Sammenstilling av data fra biobanken med andre opplysninger

I enkelte forskningsprosjekter kan det være aktuelt å sammenstille informasjon fra biobanken med opplysninger fra pasientjournalen (mor og barn), opplysninger knyttet til den diagnostiske biobanken eller helseregistre (for eksempel Medisinsk fødselsregister).

Bredt samtykke
Når du avgir biologisk materiale til denne generelle forskningsbiobanken, avgir du også et bredt samtykke til at prøver og relevante helseopplysninger kan benyttes til fremtidig forskning om antistoffer i svangerskap/ FNAIT/blodplatemangel hos nyfødte.

Godkjenning av fremtidige forskningsprosjekter
Alle fremtidige forskningsprosjekter skal forhåndsgodkjennes av en regional komité for medisinsk og helsefaglig forskningsetikk. Den enkelte avgiver vil ikke bli forespurt om bruk av sitt eget materiale i spesifikke forskningsprosjekter som er dekket av dette samtykket.

Informasjon om fremtidige prosjekter

Avgiver av materiale til biobanken har krav på generell informasjon om hva biobankens materiale brukes til. Således har vi en offentlig nettside http://uit.no/forskning/forskningsgrupper/gruppe?p_document_id=340545, der vi legger ut informasjon om hvilke forskningsprosjekter som har fått utlevert materiale fra biobanken.

Resultatene av forskningsprosjektene vil presenteres i form av publikasjoner i vitenskapelige tidsskrifter.

Utlevering av prøvemateriale

Det kan være aktuelt at biologisk materiale utleveres til andre forskere i forbindelse med samarbeidende prosjekter. Materialet vil kun utleveres uten navn, fødselsnummer eller andre direkte gjenkjennende opplysninger. Det kan bli aktuelt å sende prøver til analyser i utlandet til samarbeidende forskningsinstitusjoner. Navnelisten vil uansett forbli i Norge.

Det er frivillig å delta

Å avgi biologisk materiale til Generell forskningsbiobank for immunologisk betinget blodplatemangel hos foster og nyfødt er frivillig og krever samtykke. Det vil ikke ha noen betydning for din behandling dersom du velger å ikke samtykke, eller dersom du senere ønsker å trekke deg.

Mulighet for å trekke sin deltakelse/innsynsrett, endring og sletting av opplysninger
Du kan til enhver tid få innsyn i hvilke prøver og opplysinger som er registrert om deg. Du har også rett til å få korrigert eventuelle feil i disse opplysningene. Du kan når som helst og uten å oppgi noen grunn, trekke tilbake ditt samtykke. I så fall kan du kreve sletting av opplysningene og destruksjon av materialet, med mindre opplysningene og materialet allerede er brukt i analyser eller i vitenskapelige publikasjoner. Opplysningene og eventuelt restmateriale vil da ikke brukes i senere forskningsprosjekter. Dette vil ikke få konsekvenser for din videre behandling.

Dersom du senere ønsker å trekke deg eller har spørsmål knyttet til biobanken, kan du kontakte biobankkoordinator i UNN-HF på e-post: biobank@unn.no, tlf. 77 66 91 17. Se også nettsiden www.unn.no/biobank for mer informasjon om biobanker i UNN-HF.

Ansvarlig for biobanken/kontaktperson:

Professor Bjørn Skogen

Telefonnummer: 95 10 36 05

E-post adresse: Bjorn.R.Skogen@unn.no

Samtykke til lagring av biologisk materiale

Jeg er villig til å avgi bredt samtykke for at mine prøver kan oppbevares varig i Generell forskningsbiobank for immunologisk betinget blodplatemangel hos foster og nyfødt og bli benyttet i fremtidig forskning. (tidligere gjennomgått svangerskap).

Navn med store bokstaver (Signert av deltaker, dato)

9.2 Approval REK

Tiller Heidi
Heidi.Tiller@unn.no

Deres ref.: Vår ref.: 200801545-10/MRO/400 Dato: 20.01.2009

P REK NORD 57/2008 IMMUNRESPONS VED HPA 1A ANTIGEN STIMULERING I SVANGERSKAPET - UTVIDELSE AV STUDIEN MED DATA FRA EN DIAGNOSTISK BIOBANK - PROSJEKTET GODKJENNES

Vi viser til tidligere kontakt, senes prosjektleders e-post av 12.01.2009 og reviderte informasjonsskriv datert .....

Det planlegges ikke å hente inn samtykke for bruk av materiale som allerede finnes i diagnostisk biobank og/eller journaler. I utgangspunktet gjelder dette kvinner som tidligere hadde født barn med NAIT eller hvor det har vært mistanke om NAIT. Det er i utgangspunktet snakk om materiale fra ca 600 personer og opplysningene strekker seg tilbake til 1994. Ved senere diagnostisering har det vist seg at ved ca 200 tilfeller har det faktisk vært konstatert NAIT. Det er dette som er den aktuelle gruppen. Det vil antagelig heller ikke være nødvendig å innhente opplysninger om alle.

Komiteen vil påpeke at det som hovedregel skal innhentes samtykke til bruk av data til forskning. Dersom det skal gjøres unntak fra hovedreglen må dette begrunnes særskilt, og man må søke Helsedirektoratet om dispensasjon fra taushetsplikten.

Prosjektleder begrunner at det ikke skal innhentes samtykke men at man anser det som potensielt psykisk belastende for kvinnene og bli bedt om å ta ny stilling til større negative livshendelser som ligger langt tilbake i tid. I de fleste tilfellene dreier seg om utredning av alvorlig blodplatemangel ved fødsel hos barn, med eller uten hjerneblødning, eller intrauterine fosterdød/dødfødsler uten kjent årsak. Prosjektleder mener også at det er grunn til å tro at informasjonen til kvinnene som ble utredet i rutinen for 15 år siden ikke var like god og detaljert som den informasjonen som ble gitt til kvinnene som deltok i screeningstudien eller kvinner som utredes i rutinen i dag. Man har bl.a. mye mer kunnskap om disse blodplateantistoffene nå enn da utredningene startet i 1994. Det er derfor ikke usannsynlig at kvinnen ikke har oppfattet hva som ble utredet i sin tid og at en forespørsel i dag vil kunne gjøre henne forvirret og gi henne mange nye spørsmål - som igjen vil bidra til å at hun på ny må ta stilling til det som skjedde for flere år tilbake. De opplysningene det er aktuelt å hente fra journal er opplysninger om tidligere svangerskap og fødsler, trombocytt-tall hos barnet ved fødsel, blødningstegn hos barnet (spesielt hjerneblødning). Det kan også være aktuelt å koble dataene med opplysninger fra Medisinsk fødselsregister.

REGIONAL KOMITÉ FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK, NORD-NORGE
REK NORD
Postadresse:TANN-bygget, Universitetet i Tromsø, N-9037 Tromsø
telefon sentralbord 77 64 40 00 telefon ekspedisjon 77 62 07 58 e-post rek-nord@fagmed.uit.no
www.etikkom.no

Komiteen finner å kunne tilrå at studien gjennomføres uten at det innhentes informert samtykke. Komiteen finner prosjektleders begrunnelse vektig og legger samtidig avgjørende vekt på at forskningen er av vesentlig interesse for samfunnet og hensynet til deltakerens velferd og integritet er ivaretatt. Prosjektleder må likevel forelegge spørsmålet om dispensasjon fra taushetsplikt for Helsedirektoratet.

Etter fullmakt har komiteens leder fattet slikt

vedtak: *prosjektet godkjennes.*

Det forutsettes at prosjektet er godkjent av andre aktuelle instanser før det settes i gang. Prosjektet må forelegges komiteen på nytt, dersom det under gjennomføringen skjer komplikasjoner eller endringer i de forutsetninger komiteen har basert sin avgjørelse på. Komiteen ber om å få melding dersom prosjektet ikke blir sluttført.

Vedtaket oversendes til Helsedirektoratet for behandling av søknad om oppretting av forskningsbiobank.

Vedtaket kan påklages av en part eller annen med rettslig klageinteresse i saken jf. fvl. §28. Klagefristen er tre uker fra det tidspunkt underretning om vedtaket er kommet fram til vedkommende part, jf. fvl. § 29. Klageinstans er Den nasjonale forskningsetiske komité for medisin og helsefag, men en eventuell klage skal rettes til Regional komité for medisinsk og helsefaglig forskningsetikk, Nord Norge. Det følger av fvl. § 18 at en part har rett til å gjøre seg kjent med sakens dokumenter, med mindre annet følger av de unntak loven oppstiller i §§ 18 og 19. Se også http://www.etikkom.no/REK/klage.

Vennlig hilsen

May Britt Rossvoll
rådgiver/sekretariatsleder

9.3 Assessment of main articles

1) Kjeldsen-Kragh J, Killie MK, Tomter G, Golebiowska E, Randen I, Hauge R, et al. A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. Blood. 2007;110(3):833-9.

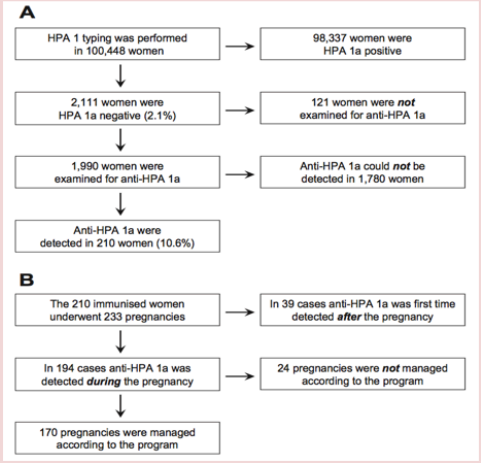
2) Tiller H, Kamphuis MM, Flodmark O, Papadogiannakis N, David AL, Sainio S, et al. Fetal intracranial haemorrhages caused by fetal and neonatal alloimmune thrombocytopenia: an observational cohort study of 43 cases from an international multicentre registry. BMJ Open. 2013;3(3).

3) Brussel JB, Berkowitz RL, Hung C, Kolb EA, Wissert M, Primiani A, et al. Intracranial hemorrhage in alloimmune thrombocytopenia: stratified management to prevent recurrence in the subsequent affected fetus. Am J Obstet Gynecol. 2010;203:135.e1-14.

4) Sainio S, Jarvenpaa AL, Renlund M, Riikonen S, Teramo K, Kekomaki R. Thrombocytopenia in term infants: a population-based study. Obstetrics and gynecology. 2000;95(3):441-6.

5) Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion. 2004;44(8):1220-5.

Reference: Kjeldsen-Kragh J, Killie MK, Tomter G, Golebiowska E, Randen I, Hauge R, et al. A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. Blood. 2007;110(3):833-9.	**Design: Prospective screening/intervention study**	
	Level of scientific evidence:	IIb-III
	Grade:	3

Objective	Materials and method	Results	Discussion
Identify HPA 1a-negative women and to offer them an intervention program aimed to reduce morbidity and mortality of neonatal alloimmune thrombocytopenia (NAIT). **Conclusion** Acknowledging the limitation of comparing with historic controls, implementation of our screening and intervention program may reduce mortality and serious morbidity associated with severe NAIT. **Country** Norway **Years Data Collection** 1995-2004	**Included cases:** Pregnant women recruited from North Norway and from Health Regions South and East in the southern part of Norway had their HPA 1 allotype determined, and HPA 1a-negative pregnant women were enrolled in the intervention program via their GP (a letter sent to the women's GP). *Excluded:* No exclusion criteria were applied. **Intervention program:** - Every fourth week during pregnancy a blood sample was examined for anti-HPA 1a antibodies and quantified when present. - Immunized women were referred to the Department of Obstetrics and Gynaecology. - Genomic platelet typing of the neonate was performed in samples from cord blood or from buccal swabs. - The number of clinical visits before CS was decided by the obstetrician. - Delivery by CS 2-4 weeks prior to term. - If the neonate had petechiae or platelet count was less than 35 X 10^9/L, platelets were transfused immediately. - Measured after birth: Platelet counts, clinical outcome (Severe: ICH, death) and adverse effects of the program. **Confounding:** Not considered. **Literature search:** Pubmed was used to search for studies on NAIT with focus on the total number of cases with severe NAIT defined as intrauterine death or severe thrombocytopenia in the fetus or newborn. **Statistical analyses:** X^2-test, Fisher exact test.	 Of the women screened, 2.1% were HPA 1a negative, and anti-HPA 1a was detected in 10.6 % of these. 55 of 161 HPA 1a-positive children had severe thrombocytopenia, including 2 with intracranial haemorrhage. One women with a twin pregnancy missed the follow-up and had one stillborn and one severely thrombocytopenic alive child. The number of severe NAIT-related complications were 3 of 57. In 15 previous prospective studies (n=136 814) there were 51 cases of severe NAIT (3 intrauterine deaths and 7 with ICH). The risk of serious complications to severe NAIT in our study was app. ¼ (p=0,034). 37 neonates suffered from adverse effects associated with the intervention program and required treatment at the neonatal intensive care unit (related to the time of delivery), but none of the neonates suffered any sequelae.	**Checklist:** - *Are the groups comparable in relation to important background factors?* Not two groups of cases. - *Are the groups recruited from the same section of the population?* Yes. - *Were the exposed individuals representative for a defined section of the population?* Yes. - *Was the study prospective? Yes.* - *Were exposure and outcome measured equal and reliable? Yes.* - *Were sufficient number of persons in the cohort followed up?* Yes. - *Is it performed drop out analyses?* Yes. - *Was the follow up time lengthy enough to prove positive and/or negative outcomes?* Yes. - *Are important confounding factors in design/implementation considered?* No. - *Was the person who evaluated the results (end points) blinded group identification?* Not relevant. **Strengths:** - Relatively great number of cases - Minimal missing data **Weaknesses:** - Comparison with previously published studies with different methodology - Some noncompliance - Confounding? - No control group consisting of planned vaginal deliveries (however, difficult to implement)

Reference: Tiller H, Kamphuis MM, Flodmark O, Papadogiannakis N, David AL, Sainio S, et al. Fetal intracranial haemorrhages caused by fetal and neonatal alloimmune thrombocytopenia: an observational cohort study of 43 cases from an international multicentre registry. BMJ Open. 2013;3(3).	**Design: Observational cohort study**	
	Level of scientific evidence:	IIb
	Grade:	(2-)3

Objective	**Materials and methods**	**Results**	**Discussion**
To characterize pregnancies where the fetus or neonate was diagnosed with fetal and neonatal alloimmune thrombocytopenia (FNAIT) and suffered from intracranial haemorrhage (ICH), with special focus on time of bleeding onset. **Conclusion** ICH caused by FNAIT often occurs during second trimester and the clinical outcome is poor. In order to prevent ICH caused by FNAIT, at-risk pregnancies must be identified and prevention and/or intervention should start early in the second trimester. **Country** Netherland, Finland, Sweden, Norway and UK **Years Data Collection** 2001-2010	**Data source:** - Laboratory data: NOICH registry, but for maternal anti-HPA-1a antibody levels, measured at the National reference laboratory of clinical platelet immunology in Tromsø by MAIPA, 4 cases in Finland (reproducibility secured by double analysis of some sera samples from both countries). - Clinical data: NOICH registry and from original medical records by each country coordinator. **Included:** Pregnancies where both the diagnosis of FNAIT and ICH were confirmed (n=43). *Cases of FNAIT*: 1) Incompatibility between maternal and paternal/fetal HPA type was confirmed and maternal anti-HPA antibodies were detected, 2) HPA-incompatibility between the mother and the father was confirmed and the fetus/neonate suffered ICH and 3) anti-HPA antibodies were detected in the mother but data on fetal/paternal HPA genotype was missing. *Cases of ICH*: 1) Neuroradiological images or 2) autopsy reports. All available neuroradiological images were re-evaluated by an experienced independent paediatric neuroradiologist. Not available images: Written reports of the imaging evaluations were used to evaluate if the diagnosis was correct. **Excluded:** All cases where ICH og FNAIT diagnosis could not be confirmed (n=23). **Outcome**: ICH due to FNAIT. **Main exposure:** - Gestational age at onset of ICH (based on well-recognised imaging principles). - Type of ICH: Intraventricular, periventricular or parenchymal haemorrhage. - Clinical outcome: Died, survived with severe neurological disabilities and alive and well at time of discharge. **Confounding:** Not considered. **Statistical analyses:** T-test, Kruskal-Wallis-test.	The majority of bleedings occurred before 28 gestational weeks, by the end of second trimester. One-third of the children died within 4 days after delivery. 53 % of the children survived with severe neurological disabilities and only 5 were alive and well at time of discharge. Antenatal treatment was not given in most (91 %) cases of fetal/neonatal ICH.	**Checklist:** - *Are the groups comparable in relation to important background factors?* Not relevant. - *Are the groups recruited from the same section of the population?* No, but all included cases evaluated to be FNAIT-pregnancies. - *Were the exposed individuals representative for a defined section of the population?* Uncertain. - *Was the study prospective?* No. - *Were exposure and outcome measured equal and reliable in the two groups?* No, both exposure and outcome were measured differently among the cases. - *Were sufficient number of persons in the cohort followed up?* Yes. - *Is it performed drop out analyses?* Not relevant. - *Was the follow up time lengthy enough to prove positive and/or negative outcomes?* Not relevant (retrospective). - *Are important confounding factors in design/implementation considered*? No. - *Was the person who evaluated the results (end points) blinded group identification?* Uncertain. **Strengths:** - Relatively high number of cases when the study outcome is taken into account - Competent professionals to evaluate the outcome **Weaknesses:** - Missing cases (cases not registered in the population)? Minimal information about the NOICH registry. - Both exposure and outcome measured differently among cases - Included uncertain FNAIT-cases (point 3) - Confounding? - Missing data (information bias)

Reference: Brussel JB, Berkowitz RL, Hung C, Kolb EA, Wissert M, Primiani A, et al. Intracranial hemorrhage in alloimmune thrombocytopenia: stratified management to prevent recurrence in the subsequent affected fetus. Am J Obstet Gynecol. 2010;203:135.e1-14.			**Design: Observational cohort study**
			Level of scientific evidence: IIb
			Grade: (2-)3
Objective	**Materials and methods**	**Results**	**Discussion**
Prevent intracranial haemorrhage (ICH) trough antenatal management of alloimmune thrombocytopenia (AIT). **Conclusion** The findings demonstrate the success of stratified treatment in "high risk" patients, which tailored interventions according to the timing of the sibling´s ICH. **Country** US, Nederland **Years Data Collection** 1994-2008	**Data source:** A specified subset of those who were recruited and treated in 2 consecutive studies of antenatal management of AIT: - 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 steroids to intravenous gamma-globulin. Am J Obstet Gynecol 1996;174:1414-23 - Berkowitz RL, Kolb EA, McFarland JG, et al. Parallel randomized trials of risk-based therapy for fetal alloimmune thrombocytopenia. Obstet Gynecol 2006;107:91-6. **Patients:** Maternal-fetal pairs were considered for the study if the following conditions were met: There was a history of well- documented AIT in a previous sibling who experienced an antenatal or perinatal ICH, and the fetus in the current pregnancy was affected of AIT. Totally 33 women with 37 separate pregnancies. Patients were subdivided into 3 stratification groups based on the timing of the previous sibling's haemorrhage: - Extremely HR (EHR) patients (n=8) were those in whom the ICH in a previous pregnancy had occurred in utero < 28 weeks. - Very HR (VHR) patients (n=17) were those in whom the ICH had occurred in utero between 28-36 weeks. - HR patients (n=12) were those with a perinatal haemorrhage occurring > 36 weeks in utero or during the first weeks after birth. **Outcome**: ICH (grade I-III) or not, and platelet counts. **Main exposure:** - Timing of ICH in the previous child: Antenatal, maternally administered treatment with IVIG and steroids was initiated according to protocol. Treatment was initiated at 12 weeks with iv. Immunoglobulin 1 or 2 g/kg/wk, and if the fetal platelet count by cordocentesis was < 30 000/mL despite treatment, prednisone and/or more iv. Immunoglobulin were added. **Statistical analyses:** Data were analysed descriptively using means and SD, medians and ranges.	5 of 37 foetuses suffered ICHs. Two ICHs had platelet counts > 100/mL (not due to thrombocytopenia), and 1 was grade I. The other 2 ICHs were un-equivocal treatment failures; both were grade III-IV and resulted in fetal demise.	**Checklist:** - *Are the groups comparable in relation to important background factors?* Not relevant. - *Are the groups recruited from the same section of the population?* Yes. - *Were the exposed individuals representative for a defined section of the population?* Uncertain. - *Was the study prospective?* Yes. - *Were exposure and outcome measured equal and reliable in the groups?* Yes. - *Were sufficient number of persons in the cohort followed up?* Yes. - *Is it performed drop out analyses?* Not relevant. - *Was the follow up time lengthy enough to prove positive and/or negative outcomes?* Yes. - *Are important confounding factors in design/implementation considered*? No. - *Was the person who evaluated the results (end points) blinded group identification?* Uncertain. **Strengths:** - Relatively high number of cases when the study outcome is taken into account - Minimal missing data **Weaknesses:** - Missing cases (cases not registered in the two studies used as a source)? - Confounding?

Reference: Sainio S, Jarvenpaa AL, Renlund M, Riikonen S, Teramo K, Kekomaki R. Thrombocytopenia in term infants: a population-based study. Obstetrics and gynecology. 2000;95(3):441-6.			**Design: Observational cohort study** Level of scientific evidence: IIb-III Grade: (2-)3
Objective To assess the prevalence and causes of thrombocytopenia among full-term infants. **Conclusion** Immunologic studies should be considered in all cases of severe neonatal thrombocytopenia for careful monitoring and prevention of potentially severe complications in subsequent pregnancies. **Country** Finland **Years Data Collection** 1997-1998	**Data source:** A 1-year, population-based surveillance study in all full-term infants (at least 37 weeks' gestation) born to native Finnish women from Helsinki. From August 1997 to July 1998, 5227 women delivered 5285 infants at term (six stillbirths). **Included:** - Platelet counts were done in cord blood from 4489 infants, 84.9% of the study population. **Excluded:** - In 99 cases (2,2 %), samples were clotted, or microscopy showed aggregation. - In 691 infants (13,1 %), samples were not collected because of refusal of mothers or inability of midwives to collect samples owing to technical reasons. - 6 stillbirths **Outcome**: - Platelet count - Platelet typing in cases of thrombocytopenia **Confounding:** To exclude any possible bias in the selection of cord-blood samples, infants from whom no sample was collected or whose samples were clotted were compared for maternal age, maternal parity, birth weight, and Apgar scores with infants from whom counts were collected, and there were no significant differences between groups. **Statistical analyses:** Confidence intervals (CIs) (95%) were done based on binomial distribution. One- way analysis of variance and x^2 test were used to determine differences between groups.	Eighty-nine infants had platelet counts below 150 x 10^9/L (2.0%; 95% CI 1.5, 2.3) in cord blood and 11 were less than 50 x 10^9/L (0.24%; 95% CI 0.10, 0.38). All causes of clinically important thrombocytopenia, those presenting with bleeding and requiring treatment, were related to fetomaternal alloimmune thrombocytopenia. An immunologic mechanism was involved in ten of 65 (15.4%; 95% CI 6.6, 24.2) infants studied and in four of 15 (26.7%; 95% CI 4.3, 49.1) cases of severe thrombocytopenia.	**Checklist:** *- Are the groups comparable in relation to important background factors?* Not relevant. *- Are the groups recruited from the same section of the population?* Yes. *- Were the exposed individuals representative for a defined section of the population?* Yes. *- Was the study prospective?* Yes. *- Were exposure and outcome measured equal and reliable?* Yes. *- Were sufficient number of persons in the cohort followed up?* Yes. *- Is it performed drop out analyses?* Not relevant. *- Was the follow up time lengthy enough to prove positive and/or negative outcomes?* Yes. *- Are important confounding factors in design/implementation considered*? Yes. *- Was the person who evaluated the results (end points) blinded group identification?* Not relevant. **Strengths:** - Relatively great number of cases - Have taken confounding in consideration **Weaknesses:** - Missing cases - Underestimation of fetomaternal alloimmune thrombocytopenia because diagnosis was confirmed only when the specific antibody was detected in the mother of an antigen-incompatible infant. HPA-1a antibodies have been reported to be detectable in less than 80% of clinically suspected cases and even fewer cases when other HPA systems are concerned.

Reference: Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion. 2004;44(8):1220-5.			**Design: Observational cohort study**
			Level of scientific evidence: IIb
			Grade: (2-)3
Objective	**Materials and methods**	**Results**	**Discussion**
To investigate the following 1) the number of clinically suspect NATP cases referred for evaluation annually, 2) the proportion of serologically confirmed cases, 3) changes in referral patterns over time, and 4) the spectrum of HPA-specific alloantibody specificities identified. **Conclusion** Although, as with the earlier series, maternal HPA-1a alloimmunization was the dominant cause of NATP, the identification of an increasing number of cases due to alternative HPA polymorphisms suggests that investigation for HPA-1 incompatibility alone is no longer sufficient to fully evaluate clinically suspect NATP cases. **Country** US **Years Data Collection** 1990-2002	**Data source:** The Platelet and Neutrophil Immunology Laboratory of the Blood Center of Southeastern Wisconsin is a reference laboratory for NATP investigations, receiving samples from throughout the US. **Included:** Laboratory records were analysed for the period from January 1, 1990, to December 31, 2002. **Outcome:** - Number of clinically suspected NATP cases - Serological confirmed cases - HPA-specific alloantibodies **Confounding:** Not considered. **Statistical analyses:** Data were analysed descriptively.	HPA-specific alloantibodies were identified in 1162 (31%) of 3743 sera of mothers of infants with clinically suspected NATP. Maternal HPA-1a alloimmunization accounted for the majority (79%) of confirmed NATP cases, with HPA-5b, HPA-3a, and HPA-1b alloantibodies accounting for 9, 2, and 4 percent of cases, respectively. In addition, an increase in the number of cases in which multiple HPA-specific alloantibodies were present in maternal sera was observed during the study period.	**Checklist:** - *Are the groups comparable in relation to important background factors?* Not relevant. - *Are the groups recruited from the same section of the population?* Yes. - *Were the exposed individuals representative for a defined section of the population?* Yes. - *Was the study prospective?* No. - *Were exposure and outcome measured equal and reliable?* Yes. - *Were sufficient number of persons in the cohort followed up?* Yes. - *Is it performed drop out analyses? Not relevant.* - *Was the follow up time lengthy enough to prove positive and/or negative outcomes?* Not relevant (retrospective). - *Are important confounding factors in design/implementation considered*? No. - *Was the person who evaluated the results (end points) blinded group identification?* Not relevant. **Strengths:** - Relatively high number of cases, also when the study outcome is taken into account - Minimal undetermined data - Comparison with earlier reported series **Weaknesses:** - Missing cases (cases not registered in the population)? - Confounding?