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Anti-HPA-1a antibodies in pregnancy and fetal growth – an analysis of registry data from a previous prospective screening study

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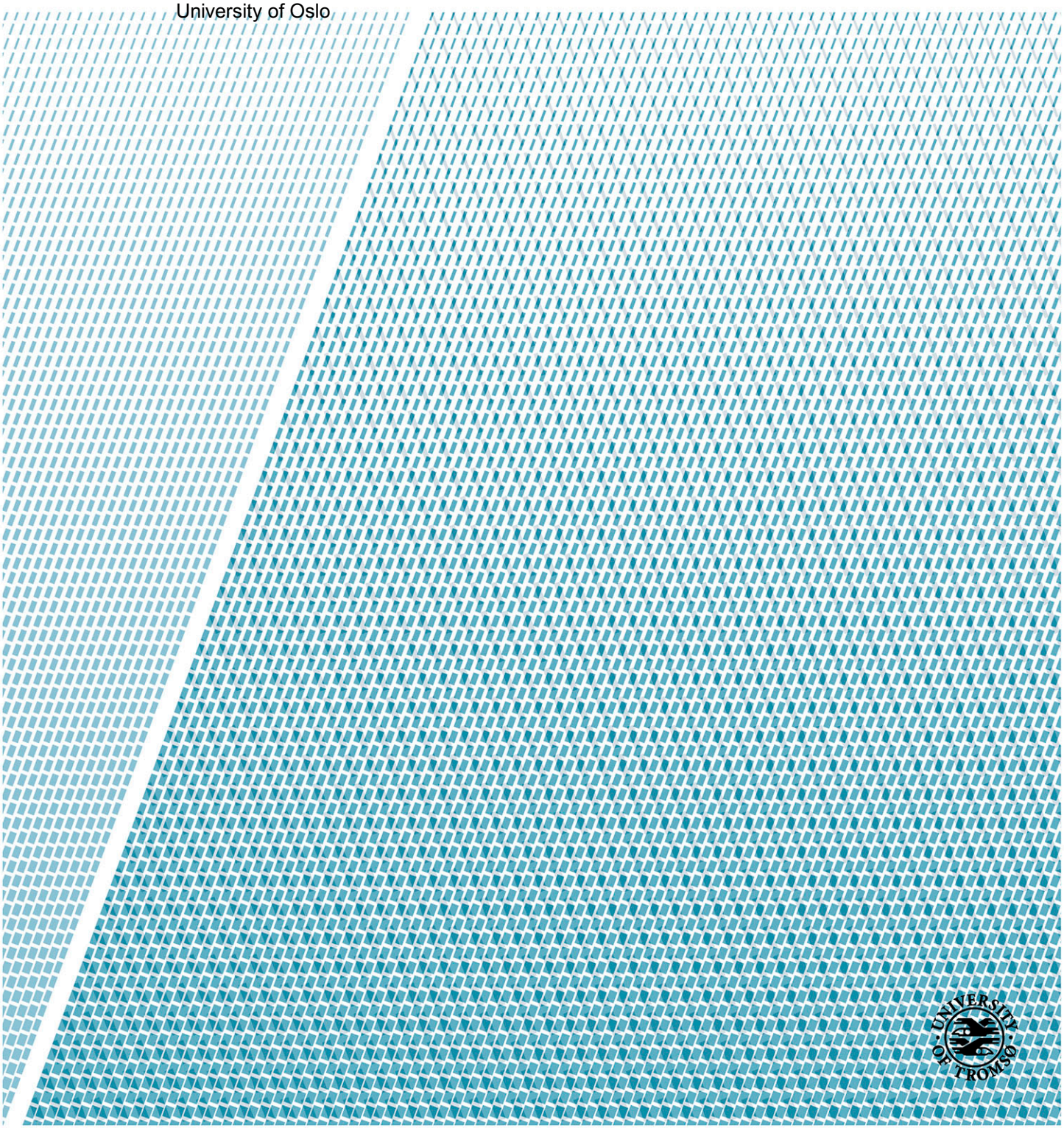

Preface

The aim of this master thesis was to assess influence of maternal anti-HPA-1a antibodies during pregnancy on fetal growth and placental function during pregnancy. The project uses data collected through a previous screening study in Norway, led by the Immunology research group at the UiT The Arctic University of Norway. The initial plan for this thesis was to include a statistical analysis of the most common fetal growth ultrasound parameters from the medical records of HPA-1a alloimmunized pregnancies from the screening study. However, we were not able to manage all the medical records in time for submission of this master thesis. We therefore chose to focus the thesis more toward a detailed literature overview of the relevant research on the theme as well as building the study database and assessing baseline obstetrical and neonatal characteristics of the participants.

My interest for immunology and the condition fetal neonatal alloimmune thrombocytopenia started in 2014, when I got accepted for the medical research program at the University of Tromsø. I had a full year of research in 2016/2017 as a part of the Immunology research group. My project as a student in the research group was to investigate the association between HPA-1a alloimmunization and lower birth weight in neonates in a large Polish prospective screening study (PREVFNAIT).

The work of this thesis has been conducted during the fifth year of medical studies. The work of the thesis started in June 2018, and was continued during the clinical rotation on the fifth year of my medical studies. However, the main part of the work was done from March 2019 to June 2019, in the time designated to the master thesis.

I would like to express my deepest gratitude to my main supervisor Heidi Tiller for invaluable feedback in all aspects of the process, from statistics to writing the thesis. Without her, I would not easily have completed this thesis. I also want to thank my co-supervisor, professor Guttorm Haugen, for suggesting the project idea in the first place, and for valuable feed-back during the writing of the thesis. I also want to thank my family and friends for their encouragement and support. Thank you for being patient and loving throughout this period.

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Summary

Background: A previous retrospective study found a strong association between increasing levels of maternal anti-HPA-1a antibodies and reduced birth weight in boys, and demonstrated increased frequency of small for gestational age (SGA) newborns among mothers with high levels of anti-HPA-1a antibodies during pregnancy. We aimed to use prospective data from a previous HPA-1a screening study to assess if the maternal anti-HPA-1a antibody level affects fetal growth and placental function using various ultrasound parameters and also to study the birth weight and frequency of SGA among HPA-1a immunized women.

Methods: We studied various parameters of fetal growth and placenta function in HPA-1bb alloimmunized pregnant women identified through a previous large Norwegian prospective screening study. Pregnancies from non-immunized HPA-1bb women were not included. Repetitive fetal ultrasound data (parameters of fetal growth and placental function) were collected from the mothers' medical journals. We also assessed birth weight and frequency of SGA among boys and girls.

Results: 135 women with detectable anti-HPA-1a antibodies were identified and included in the study. These 135 women completed a total of 154 pregnancies. 31.1% of the women included were nulliparous. The mean birth weight in the study population was 2995 grams and the frequency of SGA was 11.4%. Preliminary results demonstrates a non-significantly higher frequency of SGA among boys (20%) than girls (5.0%) (chi-square test, p=0.092).

Conclusion: A higher frequency of SGA among boys than girls was demonstrated (not significant). Reduced birth weight and SGA should be considered a possible complication of maternal anti-HPA-1a antibodies detected during pregnancy.

Abbreviations

AC: abdominal circumference

AD: abdominal diameter

AFI: amniotic fluid index

BPD: biparietal diameter

CPR ratio: cerebro placental ratio

DVP: deepest vertical pocket

EFW: estimated fetal weight

FL: femur length

FNAIT: Fetal and neonatal alloimmune thrombocytopenia

HC: head circumference

MAD: mean abdominal diameter

MCA: middle cerebral artery

PI: pulsatile index

PW/BW ratio: Placental weight/ birth weight ratio

SGA: Small for gestational age

SNP: Single nucleotide polymorphism

UA: umbilical artery

1. Introduction

1.1. Physiology

The main function of the blood is to deliver necessary substances as oxygen and nutrients to the cells and also to transport metabolic waste products (1). The blood consists of blood plasma that contains proteins, glucose, mineral ions, hormones, oxygen, carbon dioxide and different blood cells. The blood cells are traditionally divided into three types of cells: red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes).

1.1.1. Thrombocytes

Thrombocytes are the smallest cells of the blood, averaging about 2 to 4 µm in diameter (2). The normal platelet count in humans ranges from $150x10^9$/L to $400x10^9$/L (3). Activation of the platelets and an elevated platelet count (thrombocytosis) are associated with increased risk of thrombotic complications, while a low platelet count (thrombocytopenia) and several platelet function disorders increases the risk of bleeding (4).

The thrombocytes play an important role in the hemostasis where they through their adhesive and cohesive function leads to the formation of a hemostatic plug (5). In the absence of platelets, this important defense reaction cannot occur, and protracted bleeding from small wounds results (2). However, the platelets are also involved in other functional processes, such as inflammation and immune responses. The platelets express several immune receptors and do also store and release several substances, including growth factors, cytokines and regulators of angiogenesis (6). This makes the platelets able to detect pathogens or damage (7), regulate the immune and inflammatory responses.

1.1.2. Integrins

Integrins are transmembrane receptors, and represent adhesion and signaling molecules that are present on many types of cells including platelets (5). Integrins are heterodimers and consists of an α and a β chain (5). There are 18 α chains and 8 β chains known today that

together can assemble into 24 different integrins (8).

1.1.3. β3-integrin

The β3 integrin is only included in two of the possible 24 integrins, the αIIβ3 integrin and the αVβ3 integrin. The αIIβ3 integrin, better known as the fibrinogen receptor, is highly expressed on platelets. Up to 80-100.000 β3 integrins can be present on one thrombocyte. The β3-integrin, is also associated with the αV integrin, forming the αVβ3 integrin, the vitronectin receptor, which is expressed on several cell types, including vascular endothelial cells and invasive trophoblasts (9, 10).

1.1.4. SNP in β3 integrin

Integrin β3 is encoded by the ITGB3 gene that has a single nucleotide polymorphism (SNP). A SNP is a variation at a single position in a DNA sequence among individuals. This variation is localized to position 33 in the β3 integrin, where either a leucine or a proline amino acid can be positioned. This polymorphism gives rise to the human platelet antigen (HPA)-1 (11). The leu33 version is named HPA-1a as it is the more common allele, whereas the pro33 version is named HPA-1b and represents the more rare variant. Approximately 2% of Caucasian women are HPA-1a negative (HPA-1bb) (12, 13).

1.2. Definition of fetal/neonatal alloimmune thrombocytopenia (FNAIT)

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by incompatibility for HPA between the mother and fetus (figure 1). Fetomaternal incompatibility in the HPA-1 system is responsible for around 85% of all FNAIT cases (14, 15). The association between maternal platelet specific alloantibodies and thrombocytopenia in the fetus and newborn is well established and may result in the disease FNAIT. Clinically, FNAIT is important because of its potential to cause intracranial hemorrhage (ICH) in the fetus or newborn, which may result in lifelong disability or fetal death (16, 17). FNAIT is the most common cause of severe thrombocytopenia (platelet count < $50x10^9$/L) in otherwise healthy newborns (18). The condition FNAIT occurs in one per 1,100 births in Caucasian populations and will result in ICH in approximately 10% of cases (18). The immune response against HPA-1a is strongly

associated with MHC class II allele DRB3*01:01 (12, 15, 19). 90% of the HPA-1a immunized women in the Norwegian screening study were DRB3*01:01 positive (12).

1.3. Clinical presentation and diagnosis

In lack of a screening program, the condition FNAIT is typically detected after birth (12). The most common reason for suspecting FNAIT is when newborns develop widespread skin petechia shortly after birth and blood tests show severe thrombocytopenia (platelet count < $50x10^9$/L) (12, 17). The diagnosis is confirmed by detection of anti-HPA-1a antibodies in the mother's plasma and demonstration of fetomaternal incompatibility. However, the symptoms may vary from no symptoms to symptoms of extra- or intracranial hemorrhage (15).

Sometimes the risk of FNAIT is known before birth, usually because the woman previously has given birth to a child with FNAIT. If maternal anti-HPA-1a antibodies in maternal plasma are detected during pregnancy, intervention can be initiated before delivery. Also non-invasive fetal HPA genotyping in maternal plasma (NIPT) can be used to confirm fetalmaternal HPA-1 incompatibility already during pregnancy, but this method is not fully implemented in routine clinical practice in most countries (20).

Detection of anti-HPA-1a antibodies is most often done using the Monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay (21). MAIPA is a highly sensitive and specific test, and is considered the gold standard for detection of maternal antibodies (22, 23). In Norway, MAIPA is used to detect antibodies against glycoproteins gpIIb/IIIa, gpIa and CD109. This will identify the most common anti-HPA antibodies in the HPA systems 1-5 and 15. If anti-HPA-1a antibodies are detected, the antibody level may also be quantified using quantitative MAIPA with an internationally defined standard (IU/ml) (24).

The debate regarding an implementation of a routine screening program to identify all HPA-1a negative pregnant women at risk of having a fetus/ neonate with FNAIT have been discussed in several countries (12, 13, 25). A Norwegian screening study showed that implementation of a screening and intervention program could reduce morbidity and

mortality related to FNAIT with up to 75%, and also proved that it was likely to be cost-effective (12). Also, a cross-sectional study compared the detection rate of NAIT in a non-screened population vs. a screened population, and demonstrated that without a screening program the detection rate of neonatal alloimmune thrombocytopenia (NAIT) is poor (26). Studies have also demonstrated that the majority of ICH cases in relation to FNAIT are reported to occur in the first-born child (16) and that severe FNAIT can occur in the first-born child (15, 17). A prospective observational follow-up study found that the neonatal platelet count was increased or unchanged in the majority of subsequent pregnancies of HPA-1a-immunized women (27), which points out the importance of detecting the first pregnancy affected of FNAIT. Without an antenatal screening program, pregnancies at risk of FNAIT, will have no clinical follow up and management of the first FNAIT-affected pregnancy. However, no country has yet implemented such a screening program because of the lack of consensus on how to manage HPA-1a alloimmunized pregnancies once identified.

1.4. Management

Without antenatal screening for HPA-1a negative women, primary prevention is not possible. Clinical follow up and intervention of FNAIT during pregnancy, is therefore only possible if the woman previously has given birth to a child diagnosed with FNAIT. If anti-HPA-1a antibodies are detected during pregnancy and the risk of FNAIT is considered to be high, the current first-line therapy in most Western countries is weekly injections of intravenous immunoglobulin (IVIg) alone (25, 28, 29) or combined with corticosteroids.

Cordocentesis, also known as percutaneous umbilical cord blood sampling, has also been used as treatment of FNAIT. Cordocentesis is a prenatal diagnostic test where a blood sample of the fetal blood from the umbilical cord is extracted to measure fetal platelet count. In cases of severe fetal thrombocytopenia, the procedure can be followed by intrauterine platelet transfusions. Cordocentesis due do FNAIT-suspicion, however, is highly controversial due to the high risk of complications, and is therefore not practiced in many countries (30).

In Norway, the Norwegian screening and intervention study has formed the basis of the FNAIT management strategy. Women who previously have had a child with FNAIT are followed with frequently clinical examinations, including ultrasonographic examination of the fetal brain and anti-HPA-1a quantifications. In cases where the woman has a high level of anti-HPA-1a antibody level (defined as > 3IU/ml) during pregnancy, delivery by elective caesarean section is recommended 1-2 weeks prior to term. The National clinical guidelines only recommends weekly administration of IVIg if the woman previously has given birth to a child with ICH caused by FNAIT (31).

1.5. Birth weight

Birth weight (BW) is the first weight recorded after birth. Birth weight in a fully completed pregnancy is usually between 2500-4500 grams (32). In Norway, the mean birth weight is around 3500 kg according to the Medical Birth Registry of Norway (32). Several factors are known to affect birth weight, hereunder gestational age at delivery, multi pregnancy, smoking habits during pregnancy, the mothers health status, heritability and ethnicity (33).

Low birth weight is often defined as birth weight less than 2500g (34). Low birth weight is one of the major causes of morbidity and mortality in early infancy and childhood throughout the world (35). The primary cause of low birth weight is premature birth (being born before 37 weeks gestation). Small for gestational age (SGA) is lower fetal weight than expected in relation to gestational age and gender. It is typically defined as BW below the 10^{th} percentile, but some use BW below 3^{rd} percentile as definition (36).

Fetal growth restriction, or intrauterine growth restriction, has been defined as the rate of fetal growth below the normal growth potential of a specific fetus as per ethnicity and gender (37). Defining what is normal growth potential for the individual fetus is important to distinguishing small but healthy fetuses from fetuses with growth restriction. Several models that adjusts for maternal and fetal factors have been developed (38), and are shown to be useful in screening for intrauterine growth restriction (39).

The size of the fetus is influenced by a number of factors; maternal, placental and fetal. Maternal factors includes the age of the mother, maternal health and behavioral habits (37). Fetal malformations, chromosomal abnormalities and sex of the fetus (40) are fetal factors related to development of IUGR. Any mismatch between the supply of nutrient by the placenta and the demand of the fetus can also lead to IUGR (37). Intrauterine growth restriction and growth restriction/lower birth weight entails an increased risk for disease and death throughout life (41) and is therefore important to detect.

1.6. FNAIT and birth weight

A previous retrospective observational cohort study on FNAIT found a strong association between maternal anti-HPA-1a antibodies and reduced birth weight in boys (42). In this study birth outcome of 165 pregnancies from 88 HPA-1 alloimmunized mothers was analyzed. The study found a linear relation between maternal anti-HPA-1a antibody level and birth weight, demonstrating that the birth weight in boys decreased as the anti-HPA-1a antibody level increased ($p<0.001$). The study also found that very high levels of anti-HPA-1a antibodies (>15IU/ml) during pregnancy, resulted in an adjusted mean birth weight in boys that was 530g lower compared with anti-HPA-1a antibody negative pregnancies ($p<0.001$). Another interesting finding was the highly significant association between anti-HPA-1a antibody level and the risk of having a SGA neonate. This finding was also significant when the anti-HPA-1a antibody level was treated as a categorical variable with four groups. The risk of having an SGA neonate increased by 500% when the anti-HPA-1a antibody level increased from one antibody level group to the next ($p=0.002$). The mean maternal anti-HPA-1a antibody level for the SGA pregnancies was significantly higher than the mean antibody level for the non-SGA pregnancies (42). Also, the mean neonatal platelet count was significantly lower in the SGA-pregnancies. An association between anti-HPA-1a antibodies and birthweight was also found, however the risk for SGA was significantly only for boys.

An observational cohort study of all recorded cases of ICH caused by FNAIT from the international No IntraCranial Haemorrhage registry during the period 2001-2010 had a similar observation, showing that 23% of neonates with ICH were below the 10^{th} percentile for birth weight and defined as small for gestational age (16). This study was based on 13

tertiary referral centers from nine countries across the world, and studied 37 mothers and 43 children of FNAIT pregnancies complicated by fetal or neonatal ICH. They found that the majority of bleedings (54%) occurred before 28 gestational weeks and affected the first born child in 63% of the cases. 35% of the children died within 4 days after delivery, while 53% survived with severe neurological disabilities. At time of discharge only 13% were alive and well. In the majority (65%) of ICH cases, the fetuses/neonates were male and perinatal death occurred in 14% of the girls, and 46% in boys.

A retrospective study by Dahl et al (43) on maternal anti-HLA class I antibodies had an interesting finding on an association between the presence of maternal anti-HLA class I antibodies and reduced birth weight in children who were thrombocytopenic at birth (43). They studied studied data from 50 cases over a period of 11 years, and found that the thrombocytopenic neonates had a significantly higher odds of being small for gestational age ($p<0.001$). Increasing anti-HLA class I antibody levels in the mother were significantly associated with lower birth weight and placental weight among thrombocytopenic neonates.

The project I have been working on as a part of the medical research program, involved investigation of possible associations between maternal HPA-1a alloimmunization and birth weight. This project was part of a large prospective screening study, PREVFNAIT – prevention of foetal/neonatal alloimmune thrombocytopenia in Polish fetuses and newborns, conducted in Poland in the period December 2013 to March 2016. The project involved screening of 25.000 Polish pregnant women where the main outcome data has not been published yet. We demonstrated that the birth weight was significantly lower if the mother had detectable anti-HPA-1a antibodies during pregnancy. We also reproduced the previous finding with the birth weight being significantly lower if the neonate was a boy, but interestingly the birth weight among girls was also significantly affected in this cohort, although to a lesser degree. Another interesting finding was that there was significantly more boys than girls among HPA-1a immunized mothers compared to non-immunized mothers. Strength of this study was the large prospective population and the fact that non-immunized women (HPA-1a negative) were included as a control group. This is, to our

knowledge, the first study of birth weight in relation to FNAIT where HPA-1a negative women have served as controls.

1.7. Placenta

The placenta is a temporary organ that connects the developing fetus via the umbilical cord to the uterine wall during pregnancy. The placenta is a highly specialized organ of pregnancy that supports the normal growth and development of the fetus (44). The main functions of the placenta are to supply the fetus with nutrition and oxygen, and remove the wastes. The placenta also has a crucial function in the production of hormones, and to protect the fetus from bacteria and infections.

The main functional units of the placenta are the chorionic villi (44). A chorionic villus transports oxygen and nutrients between the fetus and the mother (45). A well-formed placenta is crucial for a healthy pregnancy. Placental insufficiency is a process that decreases the transfer of oxygen and nutrients to the fetus (46). The most common causes of placental insufficiency are preeclampsia, hypertension, diabetes, smoking, drug abuse, use of blood thinner medication and maternal blood clotting disorders (46, 47). Early placental dysfunction can lead to adverse outcomes in pregnancy, such as fetal growth restriction, fetal distress or stillbirth (48).

Intrauterine growth restriction is reported to be associated with several placental lesions (49). Placental lesions are often divided into groups of placental vascular processes and placental inflammatory-immune processes (50). Chronic villitis is a placental lesion of the inflammatory-immune process group, affecting the chorionic villi. Chronic villitis is reported to affect 5 to 15% of all placentas and is also the placental lesion with significantly highest recurrence risk in subsequent pregnancies (49). Chronic villitis of unknown etiology (VUE) is associated with intrauterine growth restriction and have also been reported as a finding in relation to FNAIT (51).

1.8. FNAIT and placenta

A retrospective cohort study from the southeast of France identified 147 cases of pregnancies between 1998 and 2014 in which the mother carried anti-HPA alloantibodies, with the aim to assess placental histological findings (52). 21 placentas from FNAIT-affected pregnancies were included and 42 control cases were selected to match for gestational age. The study demonstrated a significantly higher frequency of several placental lesions, including chronic intervillositis in FNAIT pregnancies (52). They also found that chronic villitis was almost four times more frequent in FNAIT pregnancies compared to the control group, suggesting that anti-HPA alloimmunization may trigger an immunological response. However, the population was of small size and accurate comparisons of the type of HPA antibodies and the nature or severity of placental lesions was therefore not possible to conduct.

Chronic intervillositis is a rare inflammatory placental lesion that is associated with poor outcome (53). Chronic intervillositis has been suggested to be linked with alloimmunization, but the condition is not fully understood (54). A case report from 2015 was the first report to describe a case of FNAIT associated with massive chronic intervillositis (55). A recent study conducted by the Immunology research group at the University of Tromsø found that chronic intervillositis was detected significantly more frequent in pregnancies where the mother was alloimmunized towards HPA-1a than in the non-immunized control group (56). These results are preliminary, but are based on a large prospective study group were placentas from a group of HPA-1bb women served as control group.

Our research group has also showed that anti-HPA-1a antibodies can bind to the vitronectin receptor expressed on extravillous throphoblasts and preliminary data has shown that trophoblast functions crucial for early placental development may be affected by such binding (9), indicating that maternal anti-HPA-1a antibodies may affect the fetal growth during pregnancy, perhaps by affecting the placental function. This, however, was a pilot study and future studies are needed to confirm and further explore these results.

1.9. Ultrasound parameters on fetal growth and placental function

All pregnant women in Norway are offered an ultrasound examination around gestational weeks 18-20. The primary purpose of this ultrasound examination is to determine the number of fetuses, assess the placental location in the uterus and perform ultrasound measurements to evaluate fetal growth and development, determine ultrasound based due date and assess for structural fetal anomalies. Several ultrasound parameters are used to measure and study the growth of the fetus during pregnancy: measurements of the fetal head (biparietal diameter BPD, head circumference HC), measurements of the fetal abdomen (abdominal circumference AC, abdominal diameter AD, mean abdominal diameter MAD) and femur length (FL). The size and growth of the fetus can be assessed based on individual biometric measurements, or by calculating fetal weight (estimated fetal weight). Estimated fetal weight may be calculated using various formulas, but typically include measurements of the fetal head, abdomen and femur (57). BPD and HC are the most commonly used biometric targets from the fetal head. HC is less affected by maternal and fetal variables, such as head shape and position of the fetus than BPD (58-60).

The measurements are plotted in a fetal growth chart (figure 2) to follow the development visually and to see where in the population distribution the measurements are. A single measurement of fetal size tells us at which percentile a fetus is in relation to the reference population. If the fetus is on a low percentile, this may indicate poor fetal growth. However, to evaluate if the fetal growth is adequate, repetitive measurement are required.

The amount of amniotic fluid is also widely used to assess fetal wellbeing. Reduced amniotic fluid is one of the parameters studied when looking at fetal growth and placental function. Quantitation of amniotic fluid can be evaluated by calculating the amniotic fluid index (AFI) or measurement of the single deepest vertical pocket (DVP) (61). The AFI is the sum of the maximum vertical amniotic fluid pocket diameter in the four quadrants of the uterus, while DVP is a measurement of the maximal depth of a pocket of amniotic fluid which is free from an umbilical cord and fetal parts (61).

Doppler sonography is a diagnostic technique used for noninvasive evaluation of the vascular circulation (62). To provide measurement of the blood velocity, the Doppler is transmitting a frequency of a certain wavelength towards the blood vessel. The difference in frequency between the ultrasound signals that is transmitted and reflected is correlated to the blood flow velocity. An abnormal blood flow velocity pattern may indicate poor fetal prognosis (63). Doppler signals from the uterine arteries, the umbilical arteries and the fetal middle cerebral artery are of great importance in predicting pregnancies at risk of preeclampsia, fetal growth restriction, intrauterine death and placental abruption(63). Pregnancies with preeclampsia or fetal growth restriction may have altered blood flow in these arteries (63). Doppler blood flow velocimetry can be used to evaluate the pulsatile index (PI) in arteria umbilicalis, pulsatile index (PI) in arteria cerebri media, and calculate the cerebroplacental ratio (CPR). Normal reference ranges are available for all these parameters. CPR is a ratio of the PI in the middle cerebral artery and the PI in the umbilical artery (MCA PI/UA PI) and is established to be an important predictor of adverse pregnancy outcome in diagnosis of SGA (64, 65). CPR >1 is considered normal, and a CPR <1 indicates a fetal circulatory redistribution in order to achieve increased blood flow to the heart, brain and adrenal glands in situations where the oxygen supply to the fetus is reduced (66).

1.10. Objective

The main objective of this master thesis was to assess possible associations between maternal anti-HPA-1a antibodies and fetal growth and/or placental function by studying various ultrasound parameters. We used data from the large prospective Norwegian FNAIT-screening study where the main results from the study already have been published (12). However, data on birth weight, fetal growth and placental function during pregnancy have not been studied before in this cohort.

Since we were not able to obtain all the medical journals, and thereby ultrasound parameters, from the immunized women in time for submission of this master thesis, we chose to focus the thesis toward a detailed literature overview of the relevant research on the theme as well as building the study database and assessing baseline obstetrical maternal and neonatal characteristics.

2. Methods

2.1. Study population

Participants were identified from a previous Norwegian screening and intervention study that was conducted from December 1995 to March 2004 (12). In the original study, all pregnant mothers where maternal anti-HPA-1a antibodies were detected were followed up with regular ultrasound examinations and blood tests.

All the women who met the following criteria were included in the study: 1) maternal genotype HPA-1bb, 2) HPA-1a alloimmunization during pregnancy confirmed, 3) available data on gestational age at delivery, parity, maternal age, birth weight, sex of the newborn and eventual comorbidity in the mother relevant to fetal growth/ birth weight (diabetes mellitus, preeclampsia, smoking) and 4) available fetal ultrasound parameters as described above. HPA-1a compatible pregnancies were excluded. Pregnancies where maternal anti-HPA-1a antibodies were only detected in the postpartum sample, and not during pregnancy, were excluded.

2.2. The Norwegian Screening and Intervention program

This project involved HPA-1a genotyping for a total of 100 488 pregnant women (12). The participants were recruited from North Norway (December 1995 until March 2004) and from health Regions South and East in the southern part of Norway (September 2001 until March 2004) (12).

All the pregnant women screened were tested for HPA-1 genotype. All HPA-1a negative women were tested for development of anti-HPA-1a antibodies. There was no further follow-up for the HPA-1bb women who did not develop anti-HPA 1a antibodies, and ultrasound measurements and Doppler findings from this part of the study population was therefore not available.

In pregnancies where anti-HPA 1a antibodies were detected during pregnancy, a blood sample was examined approximately every fourth week during pregnancy with

quantification of the anti-HPA-1a antibodies. The immunized women were also referred to the Department of Obstetrics and Gynecology at 1 of the 3 university hospitals (Ullevål University Hospital, Rikshospitalet-Radiumhospitalet Medical Center, or University Hospital of North Norway) for clinical follow-up (12). An important part of the clinical follow-up was serial fetal ultrasound examinations, primarily aimed to examine for ICH. Fetal growth was also assessed and data documented in the clinical records.

The intervention program included the following clinical interventions: 1) Delivery was performed by caesarean section 2-4 weeks prior to term, 2) 1-2 days before Caesarean section, HPA 1a-negative platelets compatible with the plasma from the immunized women were harvested by platelet apheresis, 3) If the platelet count was less than $35x10^9$/L and/or if the neonate had petechia, platelets were transfused immediately at a dosage of $60x10^9$ to $120x10^9$. Genomic platelet typing of the neonate was performed in samples from cord blood or from buccal swabs (12).

2.3. Clinical data

Some clinical data were obtained during the screening study: maternal age, ultrasound determined due date, date of delivery, sex of the newborn, anti-HPA-1a antibody levels during pregnancy, platelet count at birth, placental weight, parity and some data on maternal comorbidity relevant to fetal growth/ birth weight (diabetes mellitus, preeclampsia and smoking). Medical records including information from the prenatal care had to be obtained for the current study. These medical records contained information about the ultrasound measurements during pregnancy, hereunder: amount of amniotic fluid (AFI, single DVP or reported as normal/ reduced/ increased by the examiner), BPD, HC, AC, MAD, FL, PI in the UA, PI in MCA, CPR and arteria uterine Doppler signals.

2.4. Database

The work on the database included obtaining relevant medical records from the hospitals, extracting relevant data and plotting them into a SPSS database. All the women were given an ID-number and the participants in the database were therefore non-identifiable.

We created an SPSS database consisting of variables that included maternal data such as maternal age at delivery, obstetric history (gravida status, parity, previous children born with FNAIT), clinical observations during pregnancy, estimated due date, mode of delivery and placental weight. Clinical data of the child included the following variables: date of birth, gestational age at delivery, sex of the child, birth weight, platelet count at birth, and genotype of the child. Finally, some laboratory data (anti-HPA-1a antibody levels during pregnancy and at birth), data on the father (genotype) and data on which hospital the delivery took place were included.

2.5. Definitions

Gestational age a time of delivery was calculated from ultrasonographically determined pregnancy due date using the formula: 280 – (pregnancy due date – delivery date (difference in days))/7.

Based on gestational age at time for delivery, birth weight and sex of the neonates, a z-score was calculated based on the standard of Skjaerven (67). Small for gestational age (SGA) was defined as birthweight less than the 10^{th} percentile. Birth weight < 10 percentile was defined as a z-score < -1.285 and a birth weight < 2.5 percentile was defined as a z-score <-1.96.

2.6. Ethics

The study was approved by the Regional Committee for Medical Research Ethics, North Norway, Approval no: 2018/1665. All participants had previously given written consent that included using relevant clinical information from the pregnancy.

2.7. Statistics

All statistical analysis was performed using SPSS Statistics (Version 25.0 SPSS, SPSS Inc. Chicago, IL, USA). For all the statistical analysis, each neonate was included as one case, including twins and all children to women who gave birth to more than one child during the study period.

3. Results

A total of 100,448 pregnant women were included in the original screening study. 2,111 were found to be HPA-1a negative. Of these, 1990 were followed regarding the development of anti-HPA-1a antibodies. In 210 women (10,6%) anti-HPA-1a antibodies were detected. These 210 women completed a total of 233 pregnancies during the study period. In 39 of the pregnancies antibodies were only detected six weeks postpartum. Of the 194 remaining, 170 women completed the intervention program. Of these 170 women, we excluded all the women that did not meet the inclusion criteria, resulting in a total of 135 women and 154 pregnancies (two twin pregnancies, 18 women gave birth to two children each and one woman gave birth to three children). Within the time for submission of this thesis, we had obtained medical records including ultrasound and Doppler parameters on 16 women and 16 pregnancies.

Main obstetrical and neonatal characteristics are given in table 1. 31.3% of the women included in the study were nulliparous. In almost all cases (97%), the neonates were delivered by caesarean section. There were 54% boys vs. 46% girls among the immunized women (binominal test, p=0.53).

The mean birth weight in the study population was 2995 grams (range 2492g-3498g). The unadjusted mean birth weight was 2999 grams (range 2499g-3499g) among boys compared to 2926 grams (range 2458g-3394g) among girls (t-test, p=0.48). The frequency of SGA in the study population was 11.4%. Among boys, the frequency of SGA was 20% vs. 5.0% among girls (chi-square test, p=0.086). Table 2 gives an overview of perinatal outcome in boys and girls.

4. Discussion

4.1. Findings

In this large screening study, 154 pregnancies from HPA-1a alloimmunized mothers were included. The preliminary results of the baseline characteristics demonstrates a higher percentage of male fetuses among the alloimmunized women, suggesting that the risk of

being immunized is higher if the fetus is a boy (not significant). This finding supports a previous finding from the PREVFNAIT screening study, where 67% of the neonates of HPA-1a immunized women were boys vs. 50% among the non-immunized women (t-test, p=0.032, data not published) (68). The results from this thesis, however, include only 89 of 154 pregnancies, and may change when all the pregnancies are included in the statistical analyses.

The frequency of SGA in our study population was higher among boys than girls (not significant). This finding has also been described previously in a retrospective study where the birth weight was lower among immunized women, and also the frequency of SGA was higher among boys than girls (42). Also, the PREVFNAIT study demonstrated a lower birth weight among boys of HPA-1a immunized women (linear regression, p=0.003, data not published)(68).

The relation between male fetuses and higher risk of growth restriction and bad outcomes in pregnancy, in general, is frequently reported (69, 70). Also, it is well established that male fetuses are particularly affected by maternal alloimmunization to D antigen than female fetuses (71). It is therefore not unlikely that also in relation to FNAIT, there are possible additive negative effects of being pregnant with a boy.

4.2. Strengths and limitations

The study has several strengths, primarily that is was conducted prospectively. The study includes a sufficient number of cases and the participants were recruited from all over the country. The ultrasound examinations were performed by experienced obstetricians/ fetal medicine specialists and for most pregnancies repetitive measurements were obtained.

However, the study also has some limitations. The results are entirely observational and no assessments of causality can be made. We have no control group of HPA-1a negative pregnant women with ultrasound measurements during pregnancy who would serve as an optimal control group. During the screening study, non-immunized pregnant women were not followed, and therefore few outcome data from this important group are available.

However, all maternity units in Norway must notify births to the Medical Birth Registry of Norway, including information about complications during pregnancy or at birth, labour interventions and birth weight. We plan to obtain medical information regarding the pregnancies for the non-immunized group, and use the non-immunized group as a control group for the future planned statistical analyses.

There are also some missing data on ultrasound measurements and Doppler findings, despite the fact that these are few. From the 16 pregnancies where we so far have obtained medical records we have repetitive ultrasound and Doppler measurements available for almost all cases (n=14/16).

Writing the REK application and to get approval took longer than we expected. We also had to make changes and clarifications to get it approved. Following approval, we started the work on obtaining the medical records. We sent an information letter along with the consent to all the hospitals from where the women were included. In this information letter we described the aim of the study and asked for all the medical records related to pregnancies for all the women included in the study. For some of the cases we received the medical records immediately. However, some of the women did not give birth at the hospital where they were followed during pregnancy. In these cases, we had to find out at which hospital the woman had the delivery, and send a request to the given hospital. In other cases the hospital did not send the medical records we needed, and we had to make another request. Also, in some cases the hospital wanted more information about the study before they sent us the medical records. This resulted in a much longer process of obtaining data than expected, and is the main reason why we were not able to finish the planned project before deadline.

4.3. Future perspectives

Despite the fact that we did not manage to obtain all the planned medical records in time for submission of this thesis, we plan to complete the study. When all the medical records have been obtained, data extracted and plotted into the SPSS-file, we will do the planned statistical analysis on anti-HPA-1a antibodies and fetal growth.

To compare means (birth weight, placental weight, anti-HPA-1a antibody level) we will use an independent sample t-test. We plan to use a general linear model to assess possible associations between maternal anti-HPA-1a antibody level during pregnancy and ultrasound parameters and Doppler findings. We will use ultrasound parameters and Doppler findings as both continuous and categorical variables (divide the results into groups of low/normal/high levels and normal/abnormal levels). Based on the preliminary results on birth weight in this study, is it not unlikely that also the future analysis on fetal growth that includes ultrasound parameters, will not be significantly affected by maternal alloimmunization. However, only 89 pregnancies are included in the preliminary results, and therefore the results may change when including all cases.

In a FNAIT perspective, the results of the study are interesting as they are prospective and from a large population group. It is also of interest because this will be the first study to evaluate the growth during pregnancy, and not only at birth in the form of birth weight. We plan to publish the findings of the study in an international peer-reviewed medical journal.

Several studies indicate that HPA-1a alloimmunization affects more than neonatal platelet counts, hereunder fetal growth, birth weight and placental findings (9, 16, 42, 43). However, more studies are needed to confirm this hypothesis. It would be of great importance to conduct a prospective screening study of HPA-1a negative women with focus on anti-HPA-1a antibody level during pregnancy combined with ultrasound measurement and Doppler findings. In such a study it would be of great importance to include the non-immunized women in the intervention program, and use these women as a control group.

5. Conclusion

In this study we found that the frequency of male fetuses among HPA-1a immunized pregnancies was higher than female fetuses (not significant). Also, the frequency of SGA was non-significantly higher among boys than girls. Further studies of alloimmunized pregnancies are needed to disclose whether maternal anti-HPA-1a antibodies occurring during pregnancy can affect fetal growth and placental function, and also if being pregnant with a boy affects the pregnancy negatively in relation to FNAIT.

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7. Tables

Table 1. Description of the study population

Characteristics	Total population (n=154)
Maternal age in years, mean (SD)	31.1 (4.4)
Parity, nullipara %	31
Sex of the newborn, boys %	54*
Birth weight, grams (SD)	2995 g (503)
Small for gestational age (SA), %	11.4*
Mode of delivery, cesarean section %	97.4

*Data available for 89 cases

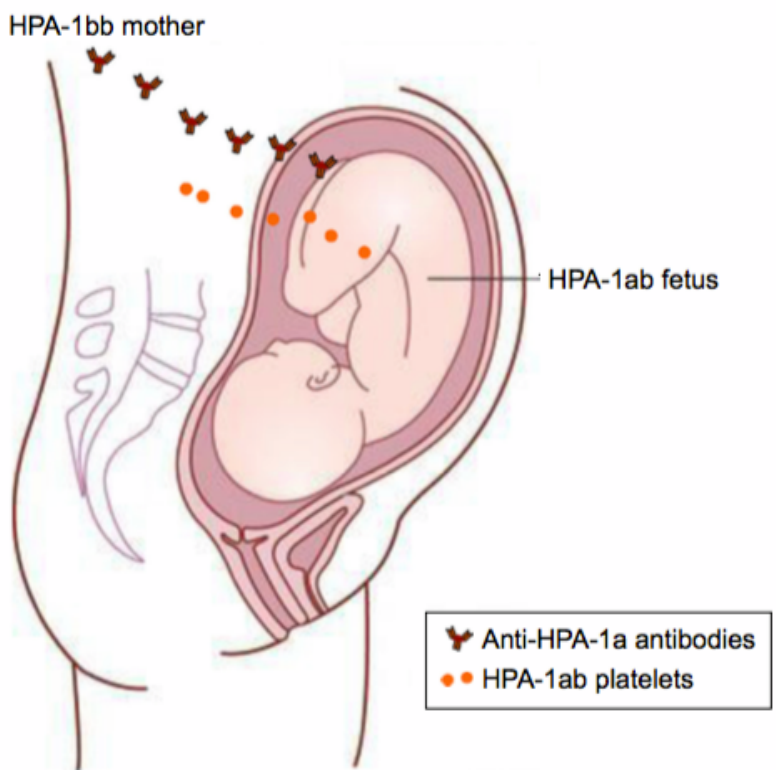
Table 2. Perinatal outcome in boys and girls

Characteristics	Boys (n=48)	Girls (n=41)	p-value
Gestational age at time for delivery, mean weeks (95% CI)	37.1 (35.5-38.6)	36.5 (34.9-38.2)	0.13
Birth weight in grams, mean (95% CI)	2999 (2499-3499)	2927 (2459-3395)	0.48
Small for gestational age (SGA), %	20	5	0.086

8. Figures

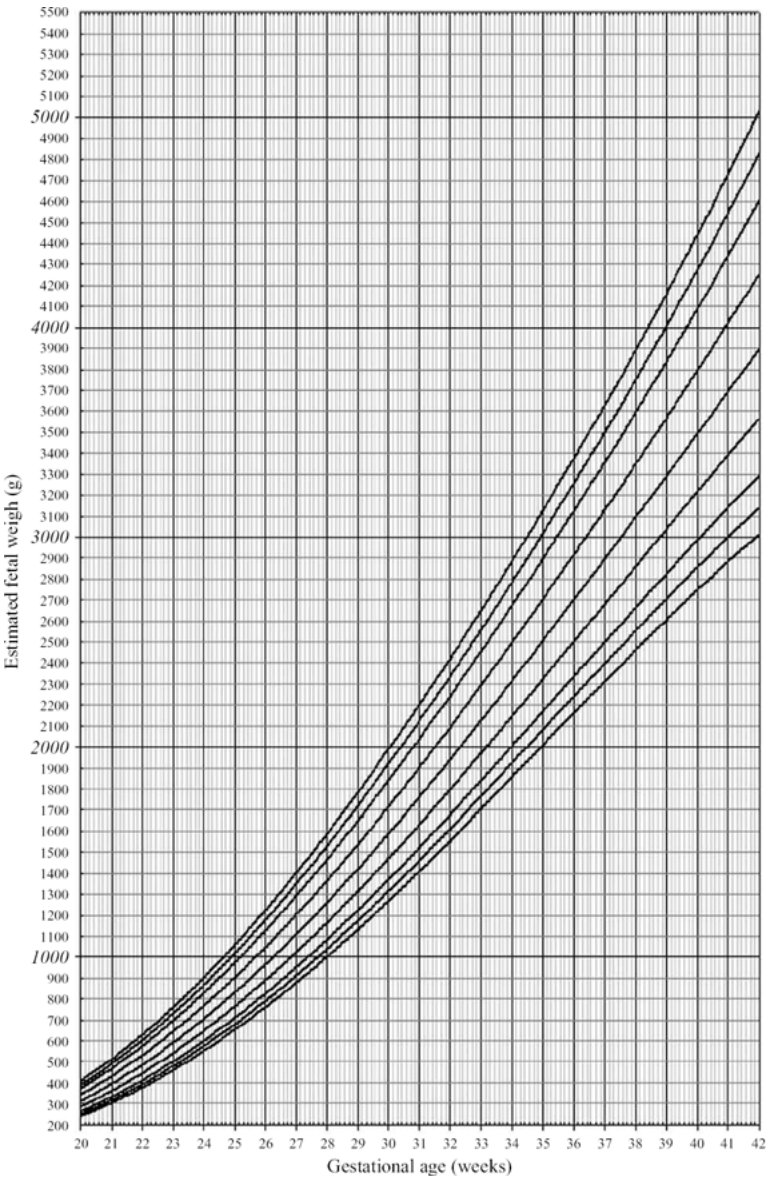
Figure 1. Illustration of the pathophysiology of FNAIT.

Reproduced from Heier HE, Berge LN, Hervig, et al. Immunisering i svangerskapet. (Immunization during pregnancy). *Tidsskr Nor Laegeforen*. 2009;129(19):2016-2018.



In an HPA-1a incompatible pregnancy, fetal platelet antigen may enter the maternal circulation resulting in production of anti-HPA-1a antibodies.

Figure 2 is an example of a growth curve and represents the percentiles suitable for serial assessment of estimated fetal weight (EFW) (72)



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 avgi 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 avgi 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.

Navn med store bokstaver (Signert av deltaker, dato)

9.2. REK approval

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord			02.10.2018	2018/1665/REK nord
			Deres dato:	Deres referanse:
			14.08.2018	

Vår referanse må oppgis ved alle henvendelser

Heidi Tiller
Breivika

2018/1665 Blodplateantistoff i svangerskap og føtal vekst – analyse av registerdata fra tidligere prospektiv screening studie

Forskningsansvarlig institusjon: UiT - Norges arktiske universitet
Prosjektleder: Heidi Tiller

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK nord) i møtet 13.09.2018. Vurderingen er gjort med hjemmel i helseforskningsloven (hforsknl) § 10.

Prosjektleders prosjektomtale
Tilstanden føtal og neonatal alloimmun trombocytopeni (FNAIT) er en sykdom som rammer foster og nyfødt, og som karakterises ved at den nyfødte har lavt blodplatetall ved fødsel, og noen få får også hjerneblødning. Sykdommen skyldes at mor danner antistoffer mot fosterets blodplater. Antistoffene kan krysse morkaken og binde seg til fosterets blodplater slik at disse destrueres. Nye studier tyder på at slike antistoffer også kan påvirke fosterets vekst, fordi vi har sett sammenheng mellom antistoffene og barnets fødselsvekt. Vi tror at dette skyldes at antistoffene påvirker morkakeutvikling. Vi ønsker å studere data og journalopplysninger som ble innhentet i forbindelse med en tidligere stor norsk FNAIT screening studie, der vi spesielt vil se på sammenhenger mellom antistoffene hos mor og ultralydfunn ila svangerskapet som er sier noe om fostervekst og morkakefunksjon. Dette vil bidra til økt forståelse, samt vise om slike ultralyd-undersøkelser kan være klinisk nyttig ved FNAIT.

Data
Data fra pasientjournal av 170 utvalgte screeningstudie om FNAIT, av mor og barn.

Data behandles avidentifisert.

Samtykke
Studien er samtykkebasert. Det framgår av søknaden at: *«Alle kvinnene har tidligere samtykket til deltakelse i screeningstudien der de journalopplysningene vi nå ønsker å bruke ble innhentet (vedlagt samtykkeskjema nr 1). I dette samtykket er det ikke nevnt noe om journaldata fra barnets journal. Flere av disse kvinnene er imidlertid i ettertid tilsendt et oppdatert og utvidet samtykkeskjema der barnets relevante journaldata også er inkludert (vedlagt samtykkeskjema nr 2). Vi vil derfor kun innhente samtykke (samtykkeskjema 2) for de av de 170 kvinnene der dette mangler, og da med tanke på relevante og manglende journalopplysninger fra barnet.»*

Videre framgår det at*: «For de pasientene vi mangler samtykke for (se over) vil disse kontaktes per brev med et kort skriv (vedlegg) der vi forteller hvorfor vi nå kontakter dem påny der det nye utvidete*

samtykkeskjemaet (samtykkeskjema 2) også ligger ved.»

De nye skrivene må innsendes REK for vurdering og arkiv.

Komiteen forutsetter at de nye informasjonsskrivene som skal knyttes til studien revideres i tråd med ny mal på REKs nettsider, slik at informasjonen som gis til deltakerne også er forenlig med ny personopplysningslov.

Vedtak
REK har gjort en helhetlig forskningsetisk vurdering av alle prosjektets sider og godkjenner det med hjemmel i helseforskningsloven § 10.
Vi gjør samtidig oppmerksom på at etter ny personopplysningslov må det også foreligge et behandlingsgrunnlag etter personvernforordningen. Det må forankres i egen institusjon.
Før prosjektet kan igangsettes må det sendes inn revidert informasjonsskriv. Skrivet sendes som vedlegg i e-post til post@helseforskning.etikkom.no.

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK nord på eget skjema senest 07.12.2022, jf. hfl. § 12. Prosjektleder skal sende søknad om prosjektendring til REK nord dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes til REK nord. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK nord, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Kopi til:gunbjorg.svineng@uit.no;postmottak@uit.no

9.3. GRADE tables

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) Tiller H, Husebekk A, Skogen B, Kjeldsen-Kragh J, Kjaer, MM. True risk of fetal/neonatal alloimmune thrombocytopenia in subsequent pregnancies: a prospective observational follow-up study. BJOG: an international journal of obstetrics and gynaecology. 2016;123(5):738-44.
4) Ghevaert C, Campbell K, Walton J, Smith GA, Allen D, Williamson LM, et al. Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. Transfusion. 2007;47(5):901-10.
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:	Design: Prospective screening/intervention study	
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.	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** Implementation of a screening and intervention program may reduce mortality and serious morbidity associated with severe NAIT. **Country** Norway **Years Data Collection** 1995-2004	**Population:** HPA 1a typing was performed in 100 448 pregnant women recruited from North Norway (December 1995 until March 2004) and from Health regions South and East in the southern part of Norway (September 2001 until March 2004). **Exclusion criteria:** 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 reffered to the Department of Obstetrics and Gynecology at 1 of 3 university hospitals (Ullevål University Hospital, Rikshospitalet-Radiumhospitalet Medical Center or University Hospital of North Norway) for clinical follow-up -Genomic platelet typing of the neonate was performed in samples from cord blood or from buccal swabs -Delivery was performed by Cesarean section 2 to 4 weeks prior to term -One or two days before CS, HPA-1a negative platelets compatible with the plasma from the immunized mother were harvested by platelet apheresis -If the platelet count was less than $35x10^9$/L and/ or if the neonate had petechiae, platelets were transfused immediately after birth. **Confounding:** Not considered. **Statistical analyses:** X^2-test, Fisher exact test.	Of the women screened, 2.1% were HPA 1a negative, and anti-HPA was detected in 10.6% of these. 170 pregnancies were managed according to the intervention program, resulting in 161 HPA-1a positive children. 55 had severe thrombocytopenia (<50 x 10^9/L), including 2 with intracranial haemorrhage (ICH). One women with a twin pregnancy missed the follow-up and has one stillborn and one severely thrombocytopenic live child. In 15 previous prospective studies there were 51 cases of severe NAIT (3 intrauterine deaths and 7 with ICH). 37 (21.5%) of the neonates suffered from adcerse effects associated with the intervention program and were treated at the neonatal intensive care unit. No sequelae were observed in any of the 37 neonates.	**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 historic controls - Some noncompliance - No control group consisting of planned vaginal deliveries

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

Objective	Materials and method	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:** -All laboratory data except maternal anti-HPA-1a antibody levels were collected from the NOICH registry database. -Maternal anti-HPA-1a antibody levels, except for the Finnish cases (n=4), were measured at the National reference laboratory of clinical platelet immunology in Tromsø by MAIPA. -Reproducibility between Norwegian and Finnish quantitation was secured by double analysis of some sera samples from both countries. - Clinical data were collected from the NOICH registry database. Additional clinical information was retrieved from original medical records by each country coordinator. **Included:** Pregnancies where both the diagnosis of FNAIT and ICH were confirmed (n=43). A case was defined as FNAIT if 1) Incompatibility between maternal and paternal/fetal HPA type was confirmed and maternal anti-HPA antibodies were detected, 2) HPA-incompatibility between the mother and 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. A case was defined as ICH by: 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:** 23 cases where ICH and FNAIT diagnosis could not be confirmed **Outcome**: ICH due to FNAIT. **Main exposure:** - Gestational age at onset of ICH (- Type of ICH: Intraventricular, periventricular or parenchymal haemorrhage. - Clinical outcome: Died, survived with severe neurological disabilities and alive and well at time of discharge **Statistical analyses:** T-test, Kruskal-Wallis-test.	From a total of 592 FNAIT cases in the registry, 43 confirmed cases of ICH due to FNAIT were included in the study. The majority of bleedings (54%) occurred before 28 gestational weeks and affected the first born child (63%). 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 - Competent professionals to evaluate 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:	Design: Prospective observational follow-up	
Tiller H, Husebekk A, Skogen B, Kjeldsen-Kragh J, Kjaer, MM. True risk of fetal/neonatal alloimmune thrombocytopenia in subsequent pregnancies: a prospective observational follow-up study. BJOG: an international journal of obstetrics and gynaecology. 2016;123(5):738-44.	Level of scientific evidence:	IIb-III
	Grade:	3

Objective	Materials and method	Results	Discussion
The aim of the study was to assess neonatal platelet count by comparing alloimmunized pregnancies from a Norwegian screening and intervention study with subsequent pregnancies from the same women	**Population:** Prospective observational follow-up study were all HPA-1a immunised women from the Norwegian screening and intervention study who gave birth to one or more children from 2004 until August 2012 were identified. **Exclusion criteria:** Non-immunised women (no detectable anti-HPA-1a antibodies), HPA-1bb women from the screening study, HPA-1a compatible pregnancies (HPA-1bb children) were excluded. If the neonatal platelet type was missing, the pregnancy was also excluded. **Follow-up:** Maternal anti-HPA-1a antibody levels were measured around 22 and 34 weeks of gestation and at 6 weeks postpartum. Neonatal platelet count were measured at birth. The anti-HPA-1a antibody level and the neonatal platelet count were compared to a subsequent pregnancy from the same woman included in the screening study. Neonatal platelet count were categorized in three groups according to the severity of thrombocytopenia: severe (1-49x10^9/l) or moderate (50-149x10^9/l, and normal platelet count (150x10^9/l. **Statistical analyses:** t-test, Mann-Whitney test, ANOVA, linear mixed model	-The premature delivery rate was 36% and the Caesarean section delivery rate was 82% (comparable with the index pregnancy) -The platelet count in the index pregnancy was not significantly different from the subsequent pregnancy (neither in unadjusted or adjusted analyses) -The individual pattern of neonatal platelet counts in 29 women were studied: the neonatal platelet count in the subsequent pregnancy was increased in 18% of cases, unchanged in 52% of cases and worse in 30% of cases copared with the corresponding index pregnancy -Significant association between maternal anti-HPA1-1a antibody level and neonatal platelet counts after adjusting for confounding factors (linear mixed model, p <0.001)	**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 in the two 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*? Yes - *Was the person who evaluated the results (end points) blinded group identification?* No **Strengths:** - Prospective study - The women are compared to themselves -Adjusted for multiple variables (parity, gravida status, maternal age at delivery, sex of the fetus) **Weaknesses:** - Few cases - CS 2 weeks prior to term, the platelet count could have been different at term
Conclusion The neonatal platelet count was increased or unchanged in the majority (2/3) of subsequent pregnancies of HPA-1a-immmunised women.			
Country Norway			
Years Data Collection 2004-2012			

Reference: Ghevaert C, Campbell K, Walton J, Smith GA, Allen D, Williamson LM, et al. Management and outcome of 200 cases of fetomaternal alloimmune thrombocytopenia. Transfusion. 2007;47(5):901-10.	**Design: Prospective observational study**	
	Level of scientific evidence:	IIb-III
	Grade:	3

Objective	Materials and method	Results	Discussion
To evaluate the relationship between human platelet antigen (HPA) antibody specificity, clinical presentation, morbidity, mortality, and therapeutic interventions in the antenatal and postnatal period, with long-term follow-up of neonates with intracranial hemorrhage (ICH) **Conclusion** HPA-1a antibodies are most commonly implicated in severe thrombocytopenia but HPA-5b and HPA-15b antibodies can also result in poor outcome. Postnatal transfusion management is extremely variable, and fetal transfusion are associated with significant morbidity and mortality. **Country** England **Years Data Collection** 1998-2005	**Background:** Referrals to the Platelet Immunology Laboratory at the National Blood Service (NBS; Cambridge, UK) are from the southeast of England, including London, and cover a population of 16 million with birth rate of approximately 240.000 per year. There is no screening for FMAIT in England, so referrals for investigation are purely on clinical grounds and NBS guidance which recommends referrals of all cases of isolated thrombocytopenia in an otherwise healthy and term neonate and of all pregnancies in women known to be a HPA-immunized. **Included:** All 1148 referrals between 1998 and 2005 were included for study. **Data collection:** Preliminary clinical information was obtained by telephone from clinician at the time of referral. After the immmunoserological investigations confirmed HPA immunization, two different questionnaires were sent to the consultants in obstetrics and neonatology to obtain information about the obstetric history and the fetal and neonatal care. For each surviving infant with ICH, a follow-up questionnaire was sent on a yearly basis to their paediatrician to obtain information on long-term neurological sequelae. **Outcome:** -HPA antibody specificities -Clinical data (frequency of severe thrombocytopenia, ICH frequency, neurological sequelae, mortality) -Therapeutic intentions in the antenatal and postnatal period **Exclusion criteria:** No exclusion criteria were applied. **Laboratory analyses:** Flow cytometry, MAIPA, PCR	-HPA antibodies were confirmed in 200 (17%) of 1148 cases -The most common specificities were anti-HPA-1a (75%), anti-HPA-5b (15.5%) and anti-HPA-15b (4%) -Of 123 (62%) cases with no previous history of FMAIT, intrauterine deaths occurred in 5: anti-HPA-1a alone, 3, in combination with anti-HPA-5b,1, and anti-HPA-15b,1. -Of the 120 liv neonates, 103 had severe thrombocytopenia and 17 (14%) developed ICH -Of the remaining 77 cases with a history of FMAIT, 40 received intrauterine transfusions. Six (15%) of these foetuses dies in utero and an additional developed ICH postnatally -Of the 19 children with ICH, one died on day +1 and neurological sequelae persist in 13 (mean follow-up, 2.5 years)	**Checklist:** - *Are the groups comparable in relation to important background factors? Yes* - *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 in the two groups?* Not relevant - *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?* No **Strengths:** -Prospective study -Relatively high number of cases **Weaknesses:** -Missing cases (estimated referrals should be almost ten times more)

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
Objective	**Materials and method**	**Results**	**Discussion**
The aims of the study were to investigate the following 1) the number of clinical suspect NAPT cases referred for evaluation annually, 2) the proportions 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 earlier series, maternal HPA-1a alloimmunization was the dominant cause of NAPT, 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** USA **Years Data Collection** 1990-2002	**Background:** The Platelet and Neutrophil Immunology Laboratory of the Blood Center of Southeastern Wisconsin is a reference laboratory for NAPT investigations, receiving samples from throughout the US. **Included:** The laboratory records of all cases of suspected NAPT referred for evaluation from January 1, 1990, to December 31, 2002, were analysed. **Laboratory analysis:** Flow cytometry, ELISA, ACE **Outcome:** -Number of clinically suspected NAPT cases -Analysis of HPA-specific alloantibodies identified in the sera of mothers of infants with NAPT **Exclusion criteria:** No exclusion criteria were applied. **Statistical analyses:** Descriptive analyzes only.	-A laboratory diagnosis of NAPT was confirmed in 1162 (31%) of 3743 suspected cases referred for evaluation -Anti-HPA-1a was the dominant alloantibody identified, occurring alone in 79% -Anti-HPA-5b was the second most commonly implicated antibody (9%) -Maternal alloantibodies against HPA-3a and HPA-1b were detected in 2% and 4% of NAPT cases, respectively -An increase from 11% to 24% in the number of serologically confirmed cases of NAPT due to HPA-specific alloantibodies other than anti-HPA-1a	**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 in the two 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? Not relevant* - *Are important confounding factors in design/implementation considered*? No (no clinical data) - *Was the person who evaluated the results (end points) blinded group identification?* Not relevant **Strengths:** - High number of cases -Comparison with earlier reported series **Weaknesses:** -No clinical data available -Confounding?