Research protocol

Towards Routine HPA-screening in Pregnancy to prevent FNAIT: Assessing Disease Burden and Optimising Risk Group Selection

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Laboratory sites	Sanquin Amsterdam

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

CRP	C-reactive Protein
GA	Gestational Age
GP	Glycoprotein
HPA	Human Platelet Antigen
IC	Informed Consent
ICH	Intracranial Haemorrhage
IvIG	Intravenous Immunoglobulin
FNAIT	Fetal and Neonatal Alloimmune Thrombocytopenia
Rhc	Rhesus c
RhD	Rhesus D
RIVM	National Institute of Public Health and the Environment (in Dutch: Rijksinstituut voor Volksgezondheid en Mileu)

SUMMARY

Rationale: Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) is the most common cause of severe thrombocytopenia in neonates. It is an immunological process, in which Human Platelet Antigen (HPA) alloantibodies produced by the mother can cross the placenta and target fetal platelets. The most frequent alloantigen to elicit platelet-reactive antibody responses is HPA-1a. The resulting low platelet count in the fetus or neonate correlates with an increased risk of bleeding complications and severe adverse outcome, defined as perinatal death or intracranial haemorrhage (ICH). This can lead to life-long handicaps, cerebral palsy, cortical blindness and mental retardation. One in 50 pregnancies is at risk for FNAIT, since 2,1% of the Caucasian population is HPA-1a negative. Alloantibodies are calculated to be present in 1:400 pregnancies, leading to FNAIT-related severe adverse outcome in at least 1:1300 fetuses or neonates, and this is likely an underestimation. There is a highly effective antenatal treatment available for preventing these severe adverse outcomes, consisting of weekly injection of intravenous immunoglobulins (IvIG). Unfortunately, in the current state of practice, this treatment can only be given in subsequent pregnancies because the disease is diagnosed after birth of a symptomatic child. In the presence of an antenatal screening program for HPA-alloantibodies all pregnancies at risk can be identified in time, to start IvIG treatment and reduce severe adverse outcomes. However, before such a program can be realised detailed information about incidence and natural course of the disease is needed. Furthermore laboratory tests are needed to identify fetuses at high risk to prevent overtreatment, since approximately 10-30% of the HPA alloimmunised cases result in severe thrombocytopenia and clinically relevant disease.

Objectives:

1. The main objective of this study is to assess the incidence and severity of FNAIT and bleeding complications (including ICH) among neonates.
2. To develop a screening platform, including diagnostic assay(s) to identify fetuses at high risk for bleeding complications due to FNAIT.

Study design: Prospective observational cohort

Study population: Pregnant women

Main study parameters/endpoints: The main study parameters are HPA-1a alloantibodies, clinically relevant FNAIT. Secondary parameters include: neonatal outcome (bleeding signs other than ICH, treatment for thrombocytopenia, morbidity).

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: These pregnant women participate in the national antenatal screening programme for Prevention and Screening of Infectious diseases and Erythrocyte Immunisation (PSIE) and have a routine blood sampling at 27th week of gestation. We will use this blood sample to perform all necessary tests, so no additional (medical) procedures will be performed. Additionally, after delivery we collect clinical data concerning the pregnancy, delivery and the health of the child in the first postnatal period by means of questioning the obstetric health care provider.

1. INTRODUCTION AND RATIONALE

Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) is the most common cause of severe thrombocytopenia in neonates. In pregnancies complicated by FNAIT, Human Platelet Antigen (HPA) incompatibility between mother and fetus can lead to production of alloantibodies. In Caucasians, the most frequent alloantigen to elicit platelet-reactive antibody responses is the HPA-1a.[1] These antibodies can cross the placenta and lead to destruction of fetal platelets causing FNAIT. Consequently, this can cause bleeding complications in fetus and neonates, of which the most severe complication is intracranial haemorrhage (ICH), leading to perinatal mortality in 7%-10% and severe neurological sequelae including mental retardation, cerebral palsy and cortical blindness 10-20% of FNAIT pregnancies.[2-4]
In the Netherlands, an estimated one in 50 pregnancies is at risk for FNAIT, since 2,1% of the Caucasian population is HPA-1a negative.[5] Alloimmunisation occurs in approximately 12% of these cases, with development of severe fetal thrombocytopenia (platelet count < $50x10^9$/L) occurring in about 150 cases each year. This leads to severe adverse outcomes in 20-25 pregnancies, with approximately 13-17 children born with an ICH each year, who are at serious risk for life-long neurodevelopmental handicaps, and this is likely to be an underestimation.[1] The disease has close similarities to haemolytic disease of the fetus or newborn due to red cell (mainly Rh) alloimmunisation in pregnancy, for which all western countries have implemented screening programs to start timely treatment. Screening for FNAIT has an international interest. Recently, the National Institute of Public Health and the Environment in the Netherlands (in Dutch: Rijksinstituut voor Volksgezondheid en Mileu; RIVM) recognised FNAIT as a significant disease, and advised to obtain more knowledge on detection and treatment of screen-detected FNAIT-risk cases, to support possible national implementation of screening. Results from a large prospective screening study in Norway amongst 100,000 pregnant women, suggest that screening all pregnant women for FNAIT could significantly reduce adverse outcome and might be cost-effective.[6, 7] Their interventions however, were restricted to offering a near-term caesarean section and availability of matched platelets at birth. As expected, their policy did not prevent several cases of neonates born with ICH of antenatal origin. We hypothesise that a further reduction of this serious adverse outcome might be possible by providing antenatal treatment in the screen-positive pregnancies.
In the last decade, our insights in the pathophysiology, laboratory methods for detection of pregnancies at risk and treatment options have vastly increased. Until now, affected fetuses and neonates are only diagnosed in the presence of, invariably totally unexpected, clinical symptoms, when treatment is often too late. In a subsequent pregnancy the current management consists of offering non- invasive preventive measures including maternal administration of intravenous immunoglobulin (IvIG). Research from our group suggests that antenatal treatment of the mother with IvIG is highly effective in the prevention of FNAIT related ICH.[8] It is likely that in presence of an antenatal screening and intervention program, perinatal death and severe handicap due to FNAIT can be significantly reduced in our country**.** To realise such a screening program adequate information about the incidence and prevalence of both the HPA-1a alloimmunised pregnant population and the subset of cases showing clinically relevant disease is necessary. This information is not retrievable from publications, because these involve a selected population or provide antenatal treatment.
Calculations suggest that screening pregnant women for HPA antibodies, and start IvIG treatment for those is cost-effective.[6] However, since in approximately 10-30% of the alloimmunised cases a severe thrombocytopenia is found and the antibody titres alone do not seem to correlate with the severity of the disease, a screening program using current methods might result in unnecessary anxiety and “over-treatment” in the screen positive cases.[9, 10] Therefore further laboratory tests to identify fetuses at high risk need to be defined. In this light we recently found that HPA-1a alloantibody characteristics such as a low Fc-glycosylation correlate with disease severity.[11] Furthermore, we have demonstrated that HPA1a

alloantibodies induce heterogeneous levels of e.g. phagocytosis of HPA-1a opsonized platelets, reduction of integrity of endothelial cell layers and platelet aggregation; the clinical relevance, in terms of thrombocytopenia and bleeding tendency, of these observations needs to be further confirmed.
This protocol describes the outline and background of a prospective study providing information about the natural course of this disease necessary to ultimately develop a nationwide screening program. Furthermore it enables us to further validate several assays, which could be used to further analyse the characteristics of the screen-detected cases.

2. OBJECTIVES

Primary Objectives:

- To assess the incidence of HPA-1a alloantibodies and the incidence of clinically relevant FNAIT in the Netherlands.

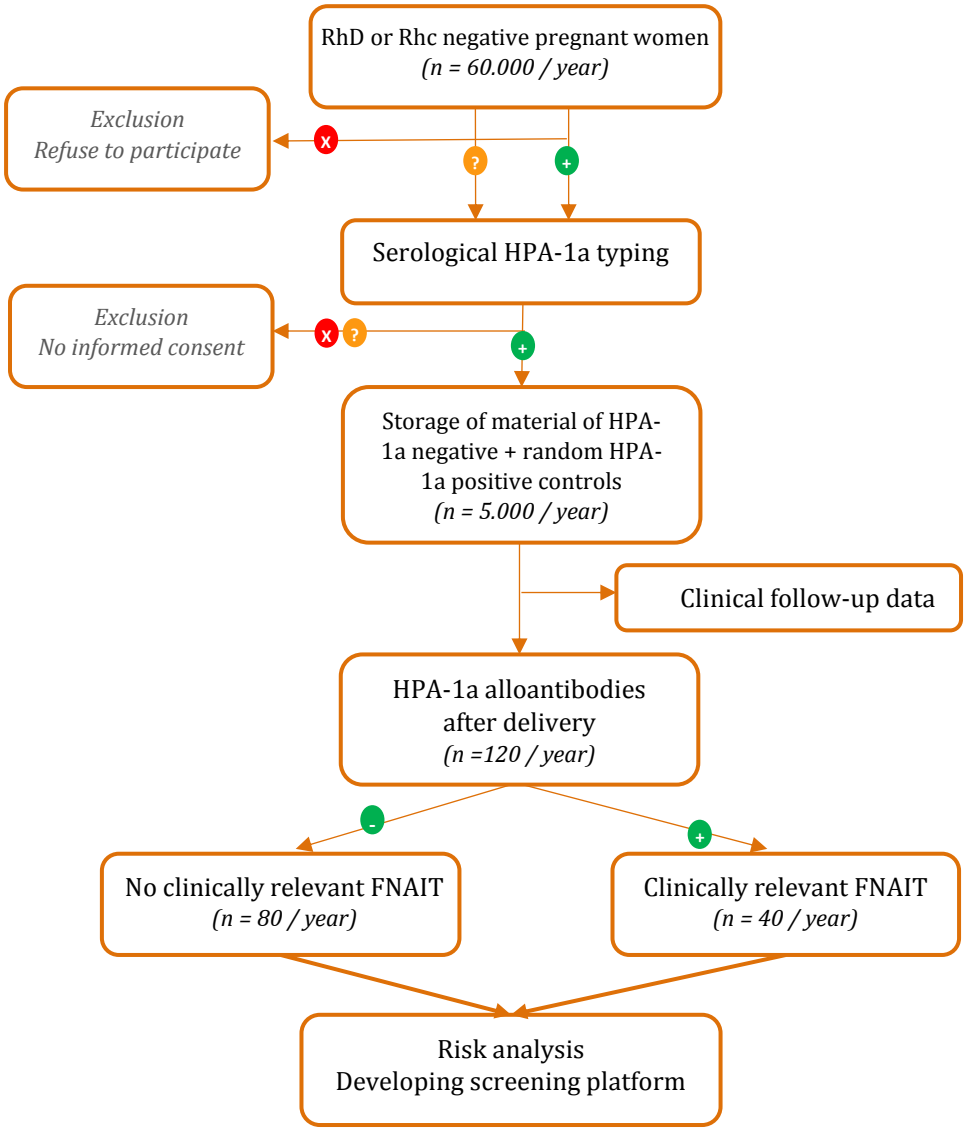
Secondary Objective(s):

- To develop a screening platform, including diagnostic assay(s) to identify fetuses at high risk.
- To determine the number of pregnant women whose children would benefit from treatment and the number who will receive treatment unnecessarily.
- To assess costs and effectiveness of implementing a nationwide screening program for FNAIT.

3. STUDY DESIGN

We will perform an observational prospective cohort study, during a period of two years. All pregnant women, participating in the nationwide prevention program organized by the RIVM to screen for red cell alloimmunisation in Rhesus D (RhD) and Rhesus c (Rhc) negative pregnant women, will be informed about participation in our study. The RIVM was consulted during the development of the study design and consented under certain conditions. An informed consent procedure will be applied, in which the RhD and Rhc negative pregnant women will be informed by their obstetric caregivers using our information flyers and are subsequently given the opportunity to either accept or object to participation. See chapter 11.2 for further specification. Regardless, all blood samples of RhD and Rhc negative pregnant women will be tested, because the blood sample for HPA-1a typing is needed to be fresh. These data will be quarantined. The HPA-1a typing will be performed using the blood sample that is already send to Sanquin.

***Figure 1 -** Flow chart of study design*



Nothing will be changed about the procedure as it currently is, regarding to the logistics as well as the volume of blood drawing itself. Blood samples from the expected 2.1% HPA-1a negative women and a control group consisting of 3 random HPA-1a positive women for each HPA-1a negative woman will be stored.

When receiving an objection to participate these samples will be deleted from storage as well from the database. After delivery, the stored blood sample will be used to test for the presence of HPA-1a alloantibodies. Clinical follow up data concerning the first postnatal period will be obtained by directly contacting the obstetric and paediatric care givers. After obtaining clinical information the depicted samples can be used to determine the diagnostic accuracy of several experimental assays that may be used to discriminate high risk cases, who will develop clinically relevant disease, from low-risk cases. An overview of the study design is displayed in **Figure 1**.

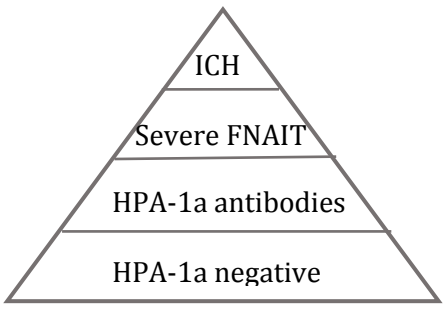
4. STUDY POPULATION

4.1 Population (base)
Pregnant women.

4.2 Inclusion criteria
All pregnant women, of whom routine blood samples are taken at 27 weeks gestational age (GA).

4.3 Exclusion criteria
There are no predefined exclusion criteria, since we are aiming to determine the incidence in the complete pregnant population in the Netherlands.

4.4 Sample size calculation
Approximately 2.1% of pregnant women are HPA-1a negative, thus 1:50 pregnancies.[1, 12] In about 12% of these women HPA-1a alloimmunisation will occur and in circa one third of these cases this will cause severe FNAIT (platelet count <50 x10^9/l), of which 10% will be suffering from ICH, or 1:12.500 pregnancies.[1] In the Netherlands we thus expect a minimum of 16 affected children annually, who could possibly suffer life-long handicaps and neurological damage.



%	Incidence	NL per year *Total n = 180.000*	Study period *Total n = 96.000**
10%	1:12.500	12	7
30%	1:1.300	120	75
10%	1:400	360	240
2%	1:50	3.600	2.016

** based on an enrolment of 80%, for a study period of 2 years*

Each year there are approximately 60.000 RhD and/or Rhc negative pregnant women, to be screened for red cell alloimmunisation at 27 weeks gestation. We expect a 80% enrolment, based on our experience during the OPZI-study.[13] Therefore our sample size will consist of approximately 240 new cases of HPA-1a alloimmunisation, with at least 7 and more likely 10 neonates suffering from ICH.

However, the numbers above are based on published cohorts that did not describe the natural history of HPA-1a-mediated FNAIT.[1] Identified cases of FNAIT were treated with various modalities; therefore, the true prevalence would likely be higher. A second systematic review, using data from postnatal screening studies, confirmed that attempts to assess the prevalence of neonatal FNAIT purely based on clinical evaluation of symptomatic bleeding leads to significant underestimation of the prevalence.[14] Many cases had been missed. We therefore expect to find an even higher incidence of FNAIT with adverse perinatal outcomes, possibly between 1 in 5.000 and 1 in 10.000 pregnancies. Obviously, solid estimates of these numbers are essential elements for the development and implementation of a national HPA-screening program.

5. TREATMENT OF SUBJECTS

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The main study parameter is the incidence of HPA-1a alloantibodies and the incidence of clinically relevant FNAIT. Clinically relevant FNAIT will be defined as perinatal death, ICH or internal organ haemorrhage.

8.1.2 Secondary study parameters/endpoints

- Laboratory platform for antibody screening and assay(s) for identification of cases, who need treatment.
- Neonatal outcome:
    - Laboratory findings: platelet count, haemoglobin, CRP, other
    - Neonatal bleeding signs other than ICH or internal organ haemorrhages: petechiae, hematoma, purpura, mucosal bleeding, other.
    - Neonatal treatment of thrombocytopenia: platelet transfusion (random versus matched), IvIG, red blood cell transfusion, other.
    - Neonatal morbidity: infections, hours/days in hospital (NICU versus Medium Care), need for additional treatments, congenital abnormalities, other causes causing increased bleeding tendency.

8.1.3 Other study parameters

- Baseline characteristics
    - Demographic: age, ethnicity
    - Medical history: idiopathic thrombocytopenic purpura, bloodtype (ABO and RhD and Rhc), body-mass index
    - Obstetric history: live births, spontaneous abortions, intrauterine fetal demise, mode and GA of previous deliveries.
    - Pregnancy related: gravidity, parity, pre-eclamspsia, gestational diabetes, medication, smoking, obstetric care giver
    - Delivery related: GA at birth, mode of delivery, Apgar Scores, perinatal asphyxia/fetal distress, birth weight, male/female sex, place of delivery (home, hospital primary care practice, hospital secondary care practice, tertiary hospital)

8.2 Randomisation, blinding and treatment allocation

For every HPA-1a negative sample, the independent technicians performing the serological HPA-1a typing will select the three consecutive HPA-1a positive samples with an adequate amount of sample available. Those will be included as positive controls and material will be stored next to the HPA-1a negative samples as well. The researchers, when collecting clinical informative, will be blinded for the results of the serological HPA-1a typing as well as the HPA-1a alloantibody detection.

8.3 Study procedures

<u>1. Serological HPA-1a typing</u>

To determine the incidence of HPA-1a alloimmunisation and clinically relevant FNAIT, we will be performing a serological HPA-1a typing using routine 27th week blood samples sent to Sanquin. We want to emphasize that there will be no extra blood volume taken from the patients. There will be no change in current practice for obstetric care givers. We will only need approximately 20ul of plasma and use an HPA-1a specific monoclonal antibody for serological HPA-1a typing using a flowcytometer.
Optimal assay conditions for a no lyse no wash approach will be determined. Analysis will be automated.

2. HPA-1a alloantibody screening

More than two weeks after estimated due date based on the GA at submission of the blood samples, these samples will be tested for HPA-1a antibodies. Plasma samples of the HPA-1a negative women will be tested for the presence of anti-HPA-1a antibodies by the monoclonal antibody immobilisation of platelets. We will compare this assay with at least two other approaches:

- Luminex assay with glycoprotein (GP) IIIa coated beads as described by Chong *et al* [15]
- Surface Plasmon Resonance analysis of antibody binding to GPIIIa immobilised on a sensor chip.

3. Clinical follow-up data

Clinical data, of both HPA-1a negative women and HPA-1a positive controls, concerning the pregnancy, delivery and the health of the child in the first postnatal period will be obtained by contacting the obstetric and paediatric care givers without informing them on the screening result. For retrieving these clinical data the researcher(s) will receive a list of names of included HPA-1a negative and HPA-1a positive pregnant women and their obstetric care givers without disclosing the results of HPA-1a typing or antibody screening. An exception will be made for cases in which the obstetric or paediatric care giver has a suspicion of FNAIT, resulting in blood samples being sent to the diagnostic platelet serology laboratory for complete FNAIT diagnostics. In these cases the researcher will be acquainted with the test results as well. Resulting in a more fluent contact between the researcher and health care professional, when obtaining the necessary clinical information for the study.

4. Identifying high-risk cases

In the past years we have developed several assays, which might enhance the clinical prediction. In the present project this will be possible for the first time. We will evaluate the added diagnostic value of the following assays:

- Platelet respiratory burst assay. The interaction of opsonized platelets with phagocytic cells using the respiratory burst as read out.
- Fucosylation level of anti-HPA1a antibody.
- Interaction of anti-HPA-1a with FcRIIIa. The interaction of the antibodies with the FcRIIIa is highly dependent on the fucosylation level of the antibody. Directly measuring this interaction would be a relative simple approach to measure the level of fucosylation as well as the avidity of the antibody.
- Effect on the integrity of endothelial layer.
- Effect on platelet aggregation. Alloantibodies against GPIIIa can interfere with its function. The inhibiton of platelet aggregation will be measured over time.
- C-reactive protein (CRP) concentration in the maternal plasma. Since we found that CRP enhances antibody induced phagocytosis, and CRP levels are slightly elevated in some FNAIT cases, maternal CRP levels will be determined.

8.4 Withdrawal and replacement of individual subjects

Subjects can leave the study at any time for any reason. After withdrawal individual subjects will not be replaced.

8.5 Follow-up of subjects withdrawn from treatment

Not applicable

8.6 Premature termination of the study

There are no predefined criteria for terminating the study prematurely.

9 SAFETY REPORTING

9.1 Section 10 WMO event
In accordance to section 10, subsection 1, of the the Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen; WMO), the investigator will inform the subjects and the reviewing accredited Medical research ethics committee (in Dutch: medisch ethische toetsing commissie; METC) if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs
Not applicable

9.3 Annual safety report
Not applicable

9;.4 Follow-up of adverse events
Not applicable

9.5 Data Safety Monitoring Board / Safety Committee
Not applicable

10 STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

- HPA-1a alloantibodies categoric variable quantitative
- Clinically relevant FNAIT categoric variable quantitative
  - Perinatal death
  - ICH
  - Internal organ haemorrhage other than ICH

10.2 Secondary study parameter(s) and other study parameter(s)

Categorical data:

- Neonatal bleeding signs
- Neonatal treatment of thrombocytopenia
- Neonatal morbidity: need for other treatments, congenital abnormalities, causes of increased bleeding tendency
- Demographic: ethnicity
- Pregnancy related
- Delivery related: Apgar scores, mode and place of delivery, fetal distress, sex
- Obstetric and medical history

Continuous variables:

- Laboratory findings neonate: platelet count, haemoglobin level, CRP
- Neonatal morbidity: days in hospital
- Maternal characteristics: age
- Delivery related: GA at birth, birth weight
- Medical history: BMI

Further specification of variables can be found in chapter 8.1.

Statistical analysis

For comparison of the secondary study parameters to a control group we will be using the HPA-1a negative women without the formation of alloantibodies as well as HPA-1a positive cases. For categorical variables we will be using Pearson's chi-square tests and Fisher's exact tests when applicable and for continuous variables we will be using Student's t-test or Mann-Whitney test, as appropriate.

We expect perinatal death or ICH due to FNAIT to be the most infrequent outcome. As described before we expect a total of 10 cases of ICH in our study population. In the controls we expect 0-1 ICH will develop. This will lead to a significant difference between the study group and the controls (P<0.0001).

	Case (As+)	Control (As-)	
ICH +	10 (a)	1 (c)	11
ICH-	110 (b)	359 (d)	469
	120	360	480

Relative risk: a/(a+b)/(c/(c+d)= (10/120)/(1/360)= 0,0833/0,00278=30
Fisher exact test: p < 0.0001

10.3 Interim analysis

Not applicable

11 ETHICAL CONSIDERATIONS

11.1 Regulation statement
The design of this study, not involving any additional or invasive tests, ensures that it is not subject to the Medical Research Involving Human Subjects Act (In Dutch: Wet Medisch Wetenschappelijk onderzoek met Mensen; WMO). Therefore, the legal framework of our study is laid out in the Medical Treatment Agreement Act (in Dutch: Wet Geneeskundige Behandelingsovereenkomst). The study will be conducted in accordance with this Medical Treatment Agreement Act, expanded in the Codes for Good Behaviour and Good Use (in Dutch: Code Goed Gebruik en Code Goed Gedrag), developed by the Federation of Medical research Committee (In Dutch: Federatie van Medisch Wetenschappelijke Verenigingen (FMWV)).

11.2 Recruitment and consent
As stated above the legal framework for our specific study design is presented in the Medical Treatment Agreement Act. This act states that passive consent can only be applied in case of completely not retraceable material. Because we need to contact obstetric caregivers to obtain clinical information after performing tests, this procedure does not apply to our study design. We will therefore be obtaining active informed consent, using the same application form that is already been used for consenting to an requesting for the regular RhD and Rhc screening performed by Sanquin. Because our research is not subject to the WMO, we will not be obtaining a separate signed consent form.
To make sure all pregnant women are adequately informed about the study, and can therefore be making a well informed choice, RhD negative and Rhc negative pregnant women in the Netherlands will be receiving an information flyer, provided by their obstetric care givers.
On the application form for the regular RhD and Rhc screening at 27th weeks gestation, there will be two additional boxes, one for declining to participate in our study and one for consenting to participate in our study.
RhDRhc

11.3 Objection by minors or incapacitated subjects
RhD negative and Rhc negative pregnant women will be informed by their obstetric care givers about the HIP study as well as being asked to participate. Obstetric care givers will be making sure that pregnant women that are underage or evidently unable to understand what it means to participate in our study will be excluded. All obstetric care givers will be informed about this procedure before starting the study.

11.4 Benefits and risks assessment, group relatedness
Screening programs require a careful balance between benefit and potential harm. Women receiving the message that they are among the 2% HPA-1a negative women, and therefore at risk for bleeding in their child, obviously will worry about adverse outcome until they are reassured by other testing, or ultimately by the birth of a healthy child. Therefore nor the participating pregnant women, nor the obstetric caregiver will be informed about the serological HPA-1a typing results. Also, we decided to perform the antibody detection test after birth. Thus no additional information can be known that would otherwise change the management of the current pregnancy, according to the Dutch Guidelines. Consequently, there will be no direct benefit for the pregnant women during this pregnancy.

11.5 Compensation for injury
Not applicable

12 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents
Data of participants will be handled confidentially. When samples of participants are being tested these have already been given a unique (bar)code, which is not directly retraceable to the patient. Once included in the study (the HPA-1a negative women as well as the HPA-1a positive controls) the unique (bar)code will be linked to an unique study-code. The results of the serological HPA-1a typing, as well as the HPA-1a alloantibody screening, will be linked to this unique study-code. The key to these codes (from barcode to study-code) will be safeguarded by the head of the laboratory for red blood cell diagnostics (C.C. Folman, PhD). For collecting the clinical data concerning the pregnancy, delivery and first postnatal period as well as the baseline characteristics, the analysts will supply the researcher with a list of included patients (barcodes) and their obstetric care givers without disclosing the results of HPA-1a typing or antibody screening. This process is also described in chapter 8.2 and 8.3. All personal data will be handled according to the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, Wbp).

12.2 Monitoring and Quality Assurance
Not applicable

12.3 Amendments
Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All substantial amendments will be notified to the METC that gave a favourable opinion. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report
We will be submitting a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 End of study report
We will be notifying the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's delivery. In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, we will be submitting a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy
There is no prearranged policy with the sponsor about publication. However in general we prefer to publish in an open access journal.

13 STRUCTURED RISK ANALYSIS

Not applicable

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