Fetal and Neonatal Alloimmune Thrombocytopenia: evidence based screening

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Chapter 11

Summary
Nederlandse samenvatting

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Fetal and neonatal alloimmune thrombocytopenia – evidence based screening

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of thrombocytopenia in otherwise healthy newborns. During pregnancy, fetal blood cells enter the maternal circulation and the mother will be exposed to unknown, paternally derived, antigens. This exposure might result in alloimmunization, the maternal production of antigen-specific alloantibodies. Through active transport across the placenta, these alloantibodies enter the fetal circulation, where they can cause damage. In FNAIT, these alloantibodies are targeted against human platelet antigens (HPAs) that are present on fetal platelets. Alloantibodies against HPA-1a are the most commonly involved in (severe cases of) FNAIT. Clinical presentation can vary from an asymptomatic thrombocytopenia or relatively harmless bruises and petechiae to severe life-threatening and invalidating intracranial hemorrhages (ICHs). Once alloimmunization is detected and diagnosed, subsequent pregnancies can be treated to prevent the recurrence of bleeding complications. Unfortunately, in absence of population-based screening, alloimmunization is virtually only known after an affected fetus or newborn. Affected infants that might have been prevented if only the alloimmunization was known and treated prior to the occurrence of bleeding complications.

1. **The condition sought should be an important health problem**
2. **There should be an accepted treatment for patients with recognized disease**
3. **Facilities for diagnosis and treatment should be available**
4. **There should be a recognizable latent or early symptomatic stage**
5. **There should be a suitable test or examination**
6. **The test should be acceptable to the population**
7. **The natural history of the condition, including development from latent to declared disease, should be adequately understood**
8. **There should be an agreed policy on whom to treat as patients**
9. **The cost of the case-finding program (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole**
10. **Case-finding should be a continuing process and not a "one-time" project**

Figure 11.1 – Principles of early disease detection – Wilson and Jungner screening criteria

Implementation of population-based screening in order to prevent FNAIT needs to be a carefully weighed decision. The benefits of screening need to outweigh the potential harm. To guide careful consideration and decision-making, Wilson and Jungner (W&J) proposed and published ten screening criteria that were adopted by the World Health Organization (Figure 11.1). For FNAIT, some of these criteria are already clearly fulfilled. For example, general

consensus exists on the fact that in current health care facilities for diagnosis and treatment are available and that these facilities are able to detect alloimmunization in pregnancy, before the occurrence of bleeding symptoms, a latent stage of FNAIT. Further, in present health care, a potential population-based screening program would definitely be implemented as a continuing process, until new insights prove otherwise. The remaining seven criteria are in need of additional evidence for fulfillment. This thesis aimed to contribute to this fulfillment and can hopefully enable future decision-making regarding implementation of population-based screening.

Within this consideration of implementation, knowledge on incidence and natural history of FNAIT is indispensable. Whereas anti-HPA-1a is the most commonly involved antibody in FNAIT, screening studies and literature focus primarily on detecting HPA-1a-mediated FNAIT. From prospective screening studies, we know that approximately 2% of the Caucasian population is HPA-1a negative and therefore at risk for alloimmunization. Further, we know that circa 10% of HPA-1a negative pregnant women will produce anti-HPA-1a alloantibodies. Because most of these studies applied a kind of intervention, adequate estimation of the incidence of clinically relevant FNAIT is nearly impossible. It is clear, however, that this only a small proportion of the alloimmunizations. This means that offering preventive treatment for all alloimmunizations will be considerable overtreatment and stipulates that there is need for (a set of) predictive marker(s) that can identify which alloimmunizations will lead to disease. To gather information on both 'natural history' and a 'policy whom to treat', we initiated a nation-wide, prospective, non-interventional cohort study. The design and set-up of this study, called HPA-screening In Pregnancy (HIP-study) are described in *chapter 2*.

In short, for the HIP-study a large group of pregnant women will be serologically screened for HPA-1a on their platelets. Plasma of HPA-1a negative women will be stored, and for every HPA-1a negative case, an HPA-1a positive control will be stored as well. Clinical data on medical history, obstetric history, pregnancy, delivery and neonatal health in the first week of life will be collected of all women of whom samples were stored. An HPA-1a antibody screening will be performed in stored plasma samples from HPA-1a negative women. The complete non-interventional set-up of the study enables an adequate estimation on natural history and incidence of the disease. Further, this approach facilitates the collection of an unique control group, namely alloimmunized pregnancies without clinical disease. Comparing these alloimmunized cases without disease to alloimmunized cases with disease will enable the detection of diagnostic markers that could predict which alloimmunized pregnancies are at high risk of bleeding and would benefit from treatment. At the same time, this would identify alloimmunized pregnancies with a low risk of bleeding, for which we can safely refrain from antenatal treatment.

The disease that screening is implemented for, needs to be an important health problem. This can be either due to its high incidence, or a result of its great clinical impact. In *chapter 3 and 4* we studied the disease burden of FNAIT. Clinically relevant FNAIT is characterized by an increased bleeding tendency, of which the most feared bleeding complication is an ICH. Short-term outcome of these bleedings is highly unfavorable, due to its high risk of perinatal mortality. Long-term outcome is suggested to be unfavorable as well, although no detailed and structured follow-up studies of children suffering from ICH solely due to FNAIT have been described. This is information that is indispensable in the counseling of parents. In *chapter 3*, we have performed an observational cohort study, assessing both short- and long-term outcome of 21 children that suffered an ICH due to FNAIT. We report a high mortality rate (48%) and the suggested poor neurodevelopmental outcome was confirmed as well. Six out of ten surviving children suffered severe neurodevelopmental impairment (NDI), and one child was moderately impaired. All impaired children had a cerebral palsy, a severe cognitive and severe motor delay. In four children there was visual impairment and/or epilepsy as well.

Besides ICH, a hemorrhage caused by FNAIT can occur in every kind of organ. In *chapter 4* the results of a retrospective chart analysis and literature review of cases of FNAIT that presented with severe bleeding other than ICH, are presented. In addition to the pulmonary hemorrhages and gastrointestinal bleeding treated at our center, we found reports of renal, ocular, spinal cord, subgaleal and genitourinary bleedings caused by FNAIT. With this chapter we indicate that although these bleedings are less likely to be associated with FNAIT, these hemorrhages can have severe consequences if not adequately treated. Every thrombocytopenic, otherwise healthy, newborn with an unexpected severe bleeding should be suspected for FNAIT and diagnostic work-up should be easily accessible and performed. That way, the affected infant can be treated adequately, but even more importantly, it enables appropriate follow-up for future pregnancies.

Of the W&J criteria, the WHO stated that perhaps the most important criterion to fulfill is that there needs to be accepted treatment for the disease. We have analyzed both the optimal antenatal and postnatal treatment in *chapters 5 and 6*. In *Chapter 5*, we present a systematic review that analyzed four randomized studies and 22 cohort studies. Antenatal treatment for FNAIT can be divided into invasive and non-invasive treatment. Invasive treatment consists of fetal blood sampling (FBS) with or without an additional intrauterine platelet transfusion (IUPT). Our review confirms that this is a risky procedure; in 11% of pregnancies treated with one or more FBS/IUPTs a complication occurred, of which one in four resulted in perinatal death. Strikingly, of all described fatalities, more than half were related to the procedure itself. Non-invasive treatment consisted of IVIg (mainly at a dose of 1.0 mg / kg maternal body weight / week) with or without corticosteroids. We found no data to support adding corticosteroids to IVIg treatment or the reducing or increasing of IVIg dose (0.5 or 2.0mg/kg/wk). We therefore

advise that antenatal treatment should consist of weekly maternal IVIg infusions at 1.0 mg/kg/wk. Treatment should be started between 20 and 24 weeks' gestation. Except for pregnancies that are at high risk because a sibling suffered an ICH. Here, treatment should start between 12 and 20 weeks' gestation.

In the cohort study, described in *chapter 6*, we analyzed postnatal management strategies and outcomes. We first concluded that, despite national guidelines, many different treatment strategies were applied; no treatment, platelet transfusion (PTx) with compatible or random-donor platelets or both, and IVIg or without PTx. Second, we concluded that in all strategies, a safe platelet count was reached within four days after birth without the occurrence of new hemorrhages. The highest and fastest increment was observed after HPA-compatible PTx and the smallest with IVIg. Treatment with random-donor PTx was not associated with a higher use of additional transfusions, which suggests that if HPA-compatible platelets are not directly available, transfusion with random-donor platelets may be a more appropriate first line therapy in FNAIT.

In *chapter 7*, we describe an enzyme-linked immunosorbent assay (ELISA) that can be used as a suitable test for screening. Whereas, in terms of screening, we focus on FNAIT that is caused by HPA-1a, the first goal is to identify all HPA-1a negative women. Considering that this is approximately 2% of the pregnant population, follow-up testing will only be necessary for 1 in 50 women. Therefore, the HPA-1a typing assay will be a major contributor to the logistical feasibility and cost-effectiveness of the program. In this chapter, we describe the low-cost ELISA that we have designed. The assay was optimized to require no additional handling (swirling or spinning) of stored tubes, which makes it applicable for high-throughput and reduces labor costs. The goal of screening is to identify all HPA-1a negative cases. Our assay reached this 100% sensitivity with still a very high specificity of 99.9% and only a false-HPA-1a negative rate of 0.03 (e.g. 3/100 samples identified as phenotypically HPA-1a negative will have a HPA-1a positive genotype).

Increasingly important in current health care, that is characterized by increased consumerism and individualism, is the acceptability of the screening to the population. Therefore, we performed a cross-sectional questionnaire study among healthy pregnant women that was aimed at assessing women's attitude towards potential future HPA-screening. For this purpose the validated Multidimensional measurement of informed choice model was used, that besides attitude, also measures knowledge and intention to participate. In *chapter 8* we demonstrate that this attitude was very positive, as expressed by 91% of participants, which was based on sufficient knowledge in 94%. Overall, 87% of the choices to intend to participate in the study were informed choices. We have shown that less informed choices were made in non-European women, stipulating the fact that it is important to adapt future counseling to women's ethnicity.

Results of the completed HIP-study, of which the set-up and protocol are described in *chapter 2*, are to be awaited. However, we did perform an interim-analysis after 10-months, which is described in *chapter 9*. During this period 40,945 pregnant women were serologically typed for HPA-1a, of which 986 women (2.4%) were HPA-1a negative. Of the HPA-1a negative cases, 263 (27%) gave informed consent for further testing and storage of material. Within these samples, 24 anti-HPA-1a immunizations (9.2%) were detected, leading to 4 cases of clinically relevant FNAIT. One case of severe FNAIT with a large ICH detected on ultrasound at 29 weeks' gestation that eventually led to a late termination of pregnancy at 34 weeks' gestation. The other three cases expressed minor bleeding symptoms; one cephalic hematoma and two cases with widespread hematomas or petechiae. So far, these numbers are in line with expectations and do not seem to be a potential hitch for the implementation of population-based screening.

With this thesis, important evidence is presented that can be used for the fulfillment of the W&J criteria. A discussion and interpretation of these results is provided in *chapter 10*. Additionally, the potential interaction of anti-HPA-1a antibodies with HPA-1a on integrin αVb3, expressed on placental tissue and endothelial cells, is discussed in this chapter. This interaction might lead to a broadening of the clinical spectrum of FNAIT and might lead to the identification of a new predictor for alloimmunized cases at high risk for bleeding. Further, we propose a potential scenario and address the remaining unanswered questions in the debate towards implementing routine HPA-screening in pregnancy in order to prevent clinically relevant FNAIT.