Fetal and neonatal alloimmune thrombocytopenia: the proof of the pudding is in the eating

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SUMMARY

OVERVIEW AND AIM OF THIS THESIS

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most frequent cause of thrombocytopenia in otherwise healthy term-born neonates. Clinical presentation of FNAIT varies from asymptomatic thrombocytopenia to skin bleeding and, severe organ bleeding. The most feared complication is intracranial hemorrhage (ICH), often leading to irreversible brain damage or death. FNAIT results from fetal maternal incompatibility of the human platelet antigens (HPA). Maternal exposure to foreign, paternally inherited, fetal HPA can lead to the formation of alloantibodies of IgG type. These HPA alloantibodies are transported across the placenta and bind to fetal platelets resulting in platelet destruction by phagocytes. Some HPAs are also expressed by other type of cells than platelets such as endothelial cells and the syncytiotrophoblast cell layer of the placenta. Therefore, some HPA alloantibodies may also induce damage to endothelial cells and placental cells. Possibly the combination of fetal/neonatal thrombocytopenia and HPA alloantibody interference with endothelial cells leads to an increased bleeding risk during fetal development and in the first days after birth. In the white population, alloantibodies directed against HPA-1a are diagnosed in 78% of the FNAIT cases and in 9% of the FNAIT cases alloantibodies directed against HPA-5b are detected. At present, most children affected by FNAIT are diagnosed after birth when (skin) bleeding is observed or thrombocytopenia is detected as a finding by chance. In these children, postnatal platelet transfusions are often administered to reduce the risk of bleeding. However, because the risk of ICH is greatest during fetal development, it is preferable to start treatment already during pregnancy. If pregnancies at risk for FNAIT are identified upon antenatal screening, timely intervention could prevent the occurrence of fetal ICH.

Implementation of population-based screening to prevent FNAIT needs to be a carefully weighted. In 1968, Wilson and Jungner (W&J) published ten principles to guide the discussion on the introduction of population-based screening (Figure 1). Implementation of population based HPA-1a antibody screening is hampered by the lack of knowledge on six principles: W&J 1 important health problem, W&J 2 accepted treatment, W&J 5 suitable test W&J 7 natural history, W&J 8 whom to treat and W&J 9 costs of case finding.

Principles of screening on early disease detection by Wilson and Jungner (1968)

1. The condition 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 phase.
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 case-finding (including a 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 continuous process and not a “once and for all” project.

FIGURE 1. Wilson and Jungner Criteria, World Health Organization, 1968.

THE NATURAL HISTORY OF ANTI-HPA-1a MEDIATED FNAIT

In **chapter 2** we described the study design of the HIP (HPA-screening in pregnancy) study aimed to obtain missing knowledge on the incidence and natural history of FNAIT and to identify pregnancies at high risk of severe outcome. Rh (Rhesus) D-negative or Rhc-negative pregnant women that participate in the nationwide prenatal screening program for erythrocyte immunization at the 27th week of pregnancy were eligible. We performed serological HPA-1a typing on platelets after regular testing and stored all left-over material for subsequent tests to be performed after due date. We included HPA-1a negative women as well as a control group of HPA-1a positive women (ratio 1:3). Pregnant women and caregivers remained blinded for the HPA-1a status and other test results. Researchers requested the caregivers to enter clinical data of mothers and the neonates in an electronic database. After clinical data collection and laboratory assessments, the clinical data were linked to the HPA-1a screening results. Pregnancy and neonatal outcomes of pregnancies from HPA-1a negative immunized women were outcomes of pregnancies of HPA-1a positive women. With this study design, the HIP study provides a unique possibility to assess the incidence of clinical detectable FNAIT and risk factors for HPA immunization or severe bleeding complications without interference of fetal medicine.

The results of the HIP study are described in **chapter 3**. In total, 2.43% (3,722/153,106) of the pregnant women were HPA-1a negative. Anti-HPA-1a was detected in 9.3% of the HPA-1a negative women (85/913 pregnancies, 32 women were included twice). Clinical information was collected of 81 HPA-1a immunised and incompatible pregnancies, 820 HPA-1a negative non-immunised pregnancies and 2704 HPA-1a positive pregnancies. In one (1.2%, 1/81) immunised pregnancy severe ICH was detected at antenatal ultrasound at 29 weeks of gestational age. Magnetic resonance imaging showed extensive damage to the fetal brain with large cysts and after multidisciplinary meeting, late termination of pregnancy was performed at 34 weeks of gestation. Three neonates (3.7%, 3/81) were diagnosed with mild bleeding after birth (two with hematomas and one with mucosal bleeding). In addition, we observed that neonates from HPA-immunized pregnancies were more often born premature (< 37 weeks) (15%, 12/81) compared to neonates of HPA-1a positive women (5%, 132/2749). Mean birthweight in neonates form immunized women was lower (3271 ±631) compared with neonates of HPA-1a positive women (3459 ±545). The proportion of primigravida was highly comparable between immunized, non-immunized and HPA-1a positive pregnancies (32%, 37% and 34% respectively). It was previously argued that the incidence of major bleeding might have been underestimated in other screening studies because of the interventions. However our data strongly suggests that this was not the case, as the proportion with major bleeding found in our study (1.2%) is lower but in line with combined figures from previous studies. We conclude that the incidence of major bleeding in FNAIT is 1 in 913 HPA-1a negative pregnancies, which translates into 11 in 10,000 HPA-1a negative pregnancies.

Besides the classical features of FNAIT such as bleeding complications and thrombocytopenia, it is hypothesized that HPA-1a antibodies can also bind to the placenta, causing placental damage. In **chapter 4** we describe a study that explored signs of placenta damage in FNAIT. We included 23 placentas of which 9 (14 samples) from newly diagnosed FNAIT cases and 14 (21 samples) from FNAIT cases after treatment with intravenous immune globulin (IVIg). As controls, 20 uncomplicated cases were included. Immunohistochemistry was performed, we stained for complement activation markers (C1q, SC5b-9 and mannose-binding lectin). Two blinded observers scored the presence of complement activation markers. Two experienced placenta pathologists scored the histopathology according to the Amsterdam criteria. A higher degree of C4d deposition was present at the syncytiotrophoblast of the newly diagnosed FNAIT cases (10/14 samples) compared to the IVIg treated FNAIT cases (2/21 samples) and healthy controls. Four (44%) placentas of the newly diagnosed FNAIT cases and five (36%) placentas of the IVIg-treated FNAIT cases showed delayed maturation compared to one in the controls. Both C4d deposition and low-grade villitis of unknown etiology was observed in three newly diagnosed FNAIT cases that were born with a birthweight below 10th percentile. In conclusion, we observed a higher rate of classical route complement activation in pregnancies of placentas complicated by HPA immunization that were not antenatally treated. This may impair placental development and fits the observation that HPA-1a immunisation is associated with a reduced birthweight.

CLINICAL RELEVANCE OF HPA-5b ANTIBODIES

Chapter 5 describes a retrospective cohort study aimed to describe the clinical outcome of HPA-1a and HPA-5b associated FNAIT cases. Due to the high prevalence of anti-HPA-5b in pregnant women (1.8%), the detection of anti-HPA-5b in FNAIT suspected cases may in some cases be an incidental finding. In total 1,864 cases were suspected for FNAIT and sent in for platelet antibody detection. In 161 (8.6%) cases, anti-HPA-1a was detected and in 60 (3.2%) anti-HPA-5b. Anti-HPA-5b was detected 1.8 (3.2%/1.8%) times more often in FNAIT suspected cases compared with unselected pregnant women. The percentage of cases affected by severe bleeding if anti-HPA-1a was present (11%, 4/126) was similar to the percentage of severe bleeding in cases with anti-HPA-5b (10%, 4/40). Based on the allele frequencies, we calculated the expected percentage of HPA incompatibility if HPA-1a or HPA-5b was not associated with bleeding: 86% and 52% were expected, respectively. All multigravida women (81/81) with anti-HPA-1a were HPA-1a incompatible with the neonate. In the HPA-5b group, 79% (38/48) of the multigravida women with anti-HPA-5b had an HPA-5b positive child, which is higher than the percentage expected by chance of 52%. Based on the higher proportion of HPA-5b antibodies in the FNAIT suspected population and the higher proportion of HPA-5b incompatibility, we could not exclude that anti-HPA-5b is associated with severe neonatal bleeding. Anti-HPA-5b-mediated FNAIT shows often a less severe clinical course with only moderate thrombocytopenia. However, based on our data, we conclude that anti-HPA-5b is not a coincidental finding in FNAIT suspected cases.

NEONATAL MANAGEMENT

Postnatal treatment aims to reduce the risk of bleeding in neonates affected by FNAIT. Treatment is based on limited evidence and it is currently unknown what the standard of care is in the international perspective. **Chapter 6** describes an international multicenter study on the postnatal management and outcome of liveborn FNAIT cases between 2010 and 2020. In total, 389 liveborn neonates were included from Australia (n = 74), Norway (n = 56), Slovenia (n = 19), Spain (n = 55), Sweden (n = 31), The Netherlands (n = 138), and United States (n = 16). A quarter of the neonates (24%, 92/380) was diagnosed with extreme thrombocytopenia (platelet count < 10×10^9/L). Severe ICH was diagnosed in 6% (22/389) of the FNAIT cases. Platelet transfusions were administered to 53% (207/389) of neonates, either as random donor platelets (43%, 88/207), human platelet antigen (HPA) matched platelets (41%, 85/207), or both (17%, 35/207). Median platelet increment after random and matched platelet transfusions was 59×10^9/L (IQR 35 - 94) and 98×10^9/L (IQR 67 – 134), respectively ($P < 0.0001$). Our data suggest that HPA-matched transfusions lead to higher platelet count increment. However, whether this is also associated with a reduced risk of bleeding remains unknown. The use of HPA-matched transfusions differed between centres from not being used at all to being first choice and used in 62% of cases. Additional postnatal IVIg treatment was given in 29% (110/389) of cases, varying between centres from 12% to 63%. We conclude that the postnatal management varied greatly between the centres highlighting the need of comparative trails.

LONG-TERM OUTCOME

Knowledge on the long-term outcome of children is crucial to provide adequate follow-up care for children affected by FNAIT and to judge the potential benefits of the introduction of an FNAIT screening program. In **chapter 7** we evaluated the neurodevelopmental outcome at school age in children newly diagnosed with FNAIT. Children were invited for cognitive and neurological testing. Behavioral questionnaires and school performance results were obtained. A composite outcome of neurodevelopmental impairment (NDI) was used, subdivided into mild-to-moderate and severe NDI. In total, 44 children were included at a median age of 12 years. Severe ICH was detected in 14% (5/36). Severe NDI was detected in 7% (3/44); in two children with severe ICH and one with low-grade ICH and perinatal asphyxia. Mild-to-moderate NDI was detected in 25% (11/44); in one child with ICH; eight without ICH and in two children neuroimaging was not performed. Adverse outcome (perinatal death or severe NDI) was 16% (8/46). Four children (9%) attended special needs education, of which three with severe NDI and one with mild-to-moderate NDI. Behavioral problem scores were comparable to Dutch norm scores. Based on the results of this study, we concluded that children who are newly diagnosed with FNAIT are at increased risk for long-term neurodevelopmental problems, even those without ICH.

In FNAIT, administration of IVIg to the mother during pregnancy is widely accepted for preventing the occurrence of antenatal or perinatal ICH in the child. However, knowledge about the long-term neurodevelopmental outcome of these children is lacking. The study in **chapter 8** evaluated the long-term neurodevelopmental outcome in children with FNAIT who were treated with IVIg antenatally, using the same methodology as in chapter 7. In total, 82% (41/50) of the eligible cases were included for neurodevelopmental assessment at a median age of 9 years. Severe NDI was not detected. The incidence of mild to moderate NDI was 14% (6/41, 95% confidence interval: 6%–29%). Severe ICH was diagnosed in two cases (5%), one antenatally before the start of IVIg and the other case 1 day after birth. Both cases had a normal neurodevelopmental outcome. The results in this chapter show that the long-term outcome of children whose mothers were treated for FNAIT with antenatal IVIg is comparable to that in the general population.

COST-EFFECTIVENESS

It is postulated that by screening for HPA-1a directed antibodies during pregnancy, timely intervention with antenatal treatment can prevent the occurrence of severe ICH. As the incidence of severe ICH due to FNAIT is low, assessing the cost-effectiveness of adding screening for anti-HPA-1a to the prenatal screening program is relevant for decision making. In **chapter 9**, lifetime costs and effects of antenatal platelet antibody screening were compared to the situation without screening in the Netherlands by developing a decision analysis model. Model parameters were based on literature and expert opinions. The results show that adding of screening for HPA-1a to the current antenatal screening program of the Netherlands will lead to additional costs of 4.7 million euro per year, and a gain of 226 QALY (Quality-adjusted life years) per year. The incremental cost-effectiveness ratio was €20,782 per QALY gained. Based on this model, we conclude that antenatal screening for anti-HPA-1a might be cost effective.

CONCLUSION

In **chapter 10** we consider population-based screening for platelet antibodies by evaluation of the knowledge gained in this thesis and the available literature, according to the principles of Wilson and Junger. Based on this evaluation, we conclude that knowledge is available to all principles and a nationwide screening for anti-HPA-1a during pregnancy seems warranted. FNAIT leads to severe bleeding in 11 in 10,000 HPA-1a negative pregnancies with a high risk of neurodevelopmental impairment. At this time, there are two issues on which additional evidence would be needed for final implementation of screening for HPA antibodies. First, the risk of severe disease can only be estimated with antibody quantification. New diagnostic tests should be developed in the future to narrow the group to be treated. Second, the effectiveness of IVIg in first immunized pregnancies must be proven. To gather knowledge on both topics, a pilot implementation of screening for HPA antibodies is needed. HPA antibody screening will have to prove itself in practice.

The proof of the pudding is in the eating.