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

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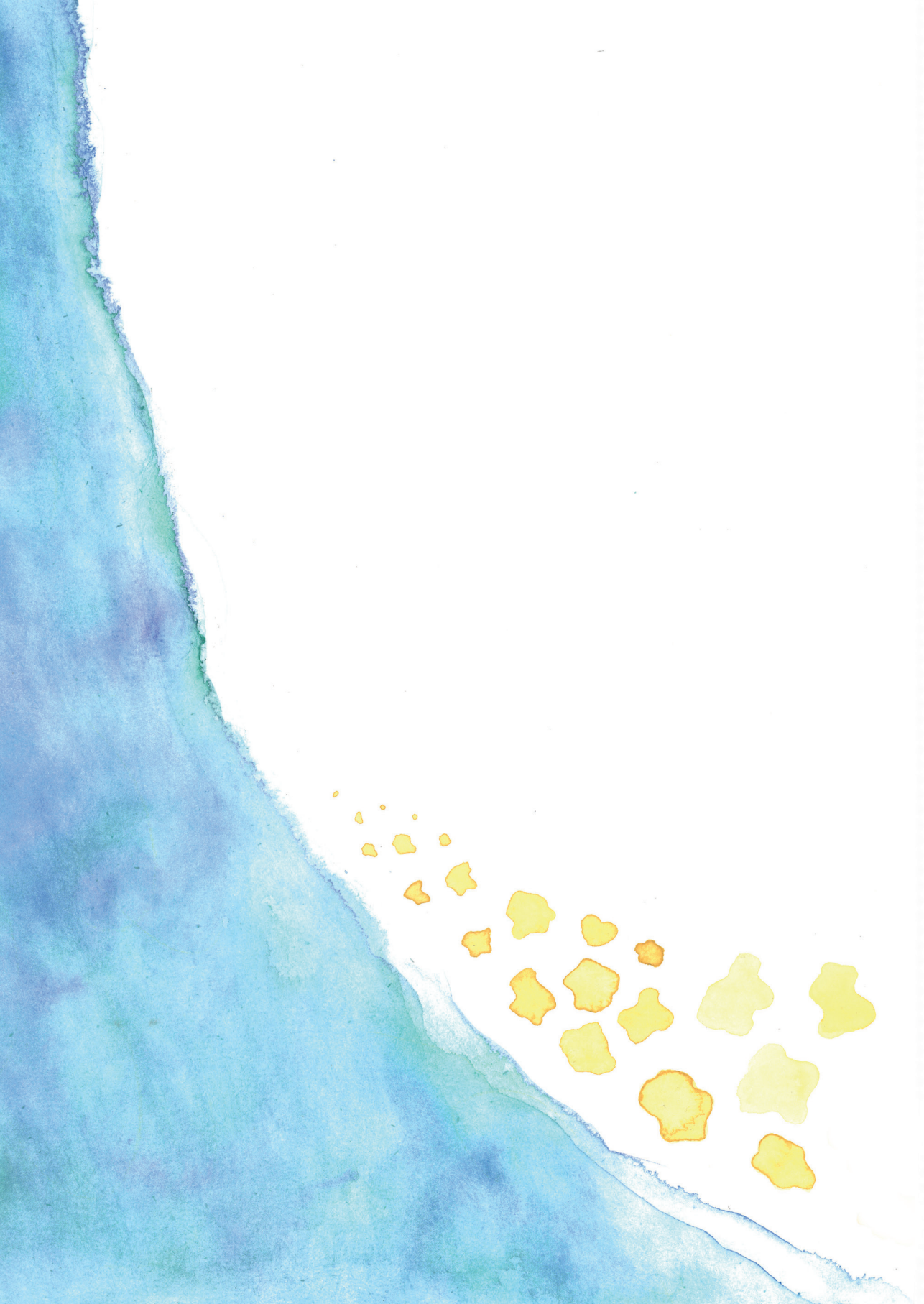
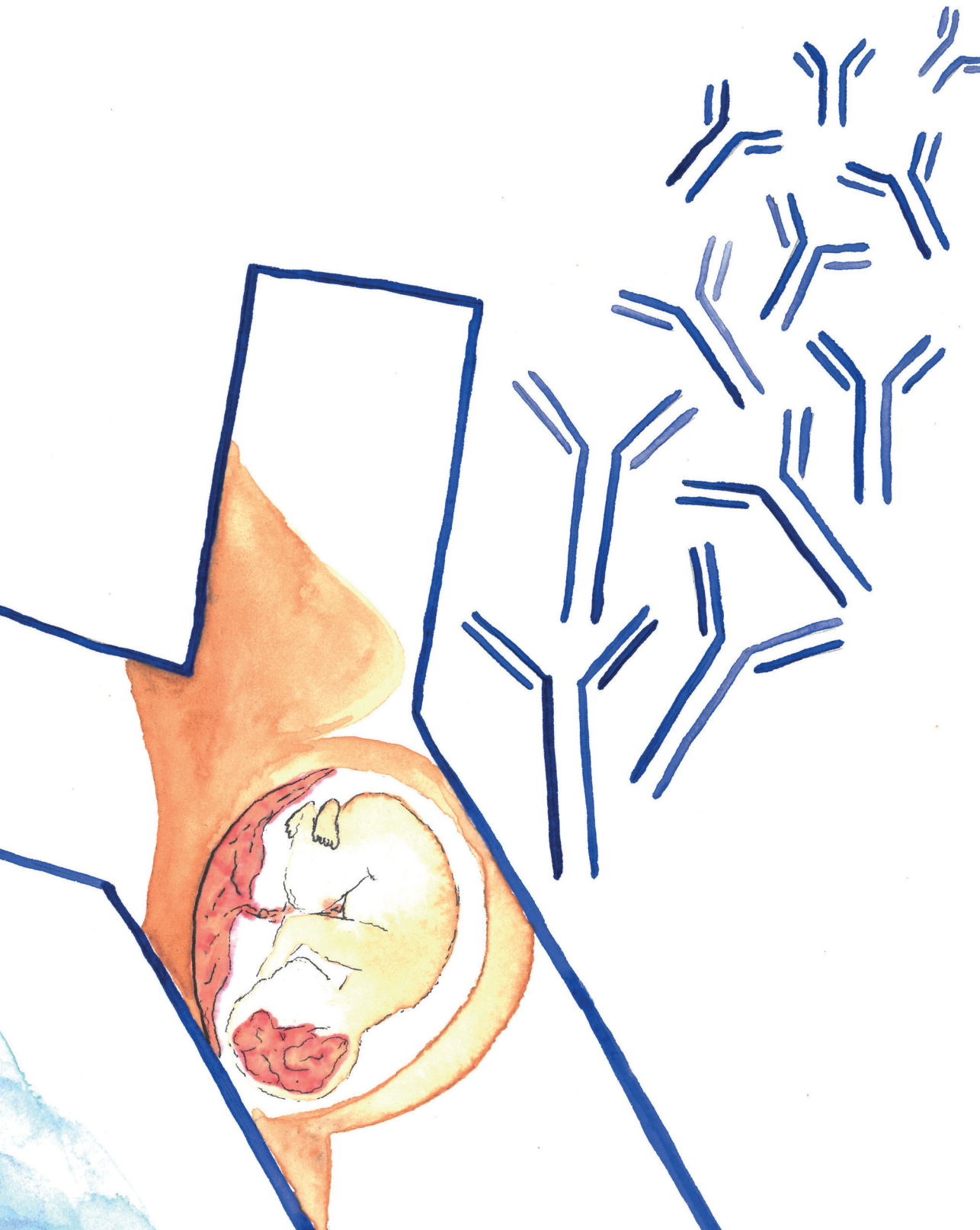


PART ONE

Overview



General introduction and scope of the thesis

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PATHOPHYSIOLOGY OF FETAL AND NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but potentially severe life-threatening disease that can lead to intracranial hemorrhage (ICH) and other severe bleeding symptoms[1] in babies during fetal development and the neonatal period. ICH is associated with perinatal morbidity and mortality and can lead to long-term neurodevelopmental impairment.[2]

In uncomplicated pregnancies, women tolerate their semi-allogenic fetus while maintaining their own immune system to protect themselves from pathogens. In pregnancies affected by FNAIT this miraculous interplay is disrupted. Essential for the development of FNAIT is that there is an incompatibility between the fetal and maternal human platelet antigens (HPA).[3] Exposure of these fetal foreign HPA to the maternal immune system can lead to the formation of HPA directed antibodies of the IgG class. During pregnancy maternal IgG antibodies are actively transported across the placenta to the fetus, therefore HPA antibodies end up in the fetal circulation. In the fetus, these antibodies bind to fetal cells expressing the involved HPA antigen. Binding to platelets and endothelial cells results in platelet phagocytosis,[4] impaired formation of platelets,[5] and for some type of HPA antibodies possibly with interference of the endothelial cell function and even impaired angiogensis.[6, 7] In the placenta, certain subtypes of HPA antibodies may bind to the syncytiotrophoblast cells and involvement of placenta pathology in FNAIT has been suggested.[8-11]

Although the pathophysiology of ICH is not fully understood. It is proposed to result from the combination of thrombocytopenia and antibody interaction with endothelial cells resulting in this severe outcome of the increased bleeding tendency in the fetus and neonate. The clinical outcome of infants from HPA immunized pregnancies can differ from being asymptomatic with normal platelet counts to thrombocytopenia with or without skin bleeding or being severely affected by ICH or organ bleeding (Figure 1).

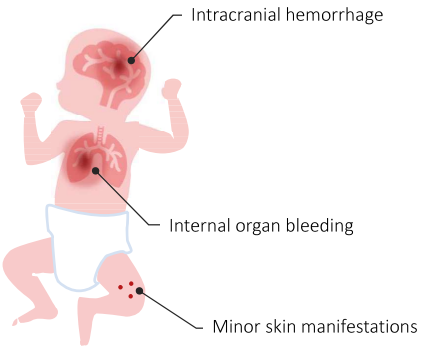

FIGURE 1. Neonatal outcome in FNAIT

HISTORY OF HPA-1a-MEDIATED FNAIT

In 1953 Harrington *et al.*[12] reported on two thrombocytopenic infants from healthy mothers with normal platelet counts. In both cases there was no evidence of sepsis, perinatal asphyxia, metabolic disease or congenital abnormalities. HPAs had not been discovered at that time and serological examinations to prove the presence of maternal alloantibodies were not available back then. However, in their[12] study and other reports that followed,[13, 14] it was suggested that maternal antibodies might play a role in the development of neonatal thrombocytopenia in otherwise healthy born infants. In 1962 Shulman *et al*.[15] implicated that neonatal thrombocytopenia could be explained by maternal alloantibodies directed against a paternally inherited platelet antigen named PL^{A1}. Already in 1959, in the Netherlands, at the Central Laboratory of the Blood Transfusion Service in Amsterdam, this platelet antigen was described as Zw^a by Van Loghem *et al*.[16]. These antigens, PL^{A1} or Zw^a are nowadays better known as HPA-1a.

Currently 41 different HPA have been described.[17] HPAs are small protein-based changes or variations at the glycoproteins expressed on the cell membrane of platelets (Figure 2). These changes, result from single nucleotide polymorphisms (SNPs). HPAs are located on different glycoprotein complexes: GPIb-V-IX (von Willebrand receptor), GPIIb/IIIa (αIIb/β3 integrin, fibrinogen receptor) GPIa/IIa (collagen receptor) and CD109. In addition, women of African or Asian ancestry can have inherited mutations that prevent glycoprotein IV (CD36, Nak) from being expressed on platelets which can result in the formation of so called isoantibodies.[18] Platelets express high levels of the fibrinogen receptor αIIbβ3 carrying HPA-1a which is the most frequently involved antigen in FNAIT in the white population.[19] As with other blood groups, allele frequencies for HPAs involved in FNAIT differ between ethnic populations.

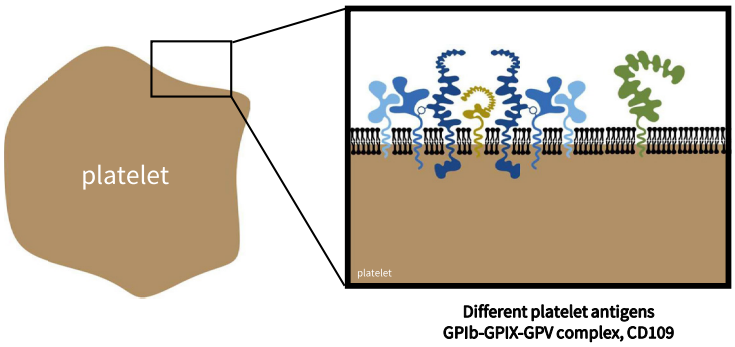

FIGURE 2. Human platelet antigens

Initially, only assays were available with indirect evidence for the presence of platelet antibodies (e.g. platelet aggregation test, complement fixation techniques) in FNAIT suspected cases.[20] Later in the 1970s, more sensitive and specific methods became available with the advantage of fluorescent labelled tools to detect platelet antibodies (e.g. platelet immune fluorescence tests).[20] In the years that followed, many efforts were done to improve assays particularly aimed at preventing non-specific reactions. For years, the monoclonal antibody immobilization of platelet antigens (MAIPA) as published in 1987 by Kiefel *et al*.[21], has been further developed and is now widely used and established as the golden standard for HPA antibody detection. Disadvantages of the MAIPA are that the assay is technically challenging with the necessity of availability of HPA typed platelets, time consuming, and uses relatively large amounts of patient material. More recent assays, such as an assay with platelet GP-coated beads and Luminex technology for antibody detection (e.g. PAK Lx assay from Immucor) can be much more easily implemented and has the advantage to use only low amounts of patient material.[22]

Because the risk of fetal ICH during pregnancy is substantial, if not greatest,[23] during pregnancy, fetal therapy is essential in the treatment of FNAIT. In 1984, Daffos *et al*.[24] were the first to perform an intrauterine platelet transfusion (IUPT) in a pregnancy from a woman of which her previous child was diagnosed with ICH due to FNAIT. IUPTs were successful in raising platelet counts and an ICH did not occur in this child, suggesting that this treatment could prevent the occurrence of severe bleeding in FNAIT. However, in comparison to sequential intrauterine transfusions for fetal anemia in hemolytic disease of the fetus and neonate (HDFN), cordocentesis in thrombocytopenic fetuses is associated with a high risk of fetal bleeding and the short life span of platelets requires weekly transfusions. Therefore, in 1988 Bussel *et al*.[25], studied the effect of administration of intravenous immune globulins (IVIg) to HPA immunized pregnant women. Although IVIg was used regularly after this initial study, the efficacy of this treatment has never been proven in a randomized controlled trial. However, a systematic review[26] concluded that IVIg was effective in preventing ICH in the vast majority (99%) of the cases.

AIM AND OUTLINE OF THE THESIS

FNAIT is currently diagnosed in cases suspected for FNAIT, because of bleeding symptoms or if thrombocytopenia is detected as a finding by chance. Therefore, the larger part of the FNAIT cases is only diagnosed postnatally. In these children, treatment mainly consists of preventing further complications with postnatal platelet transfusions. In subsequent pregnancies, antenatal treatment regimens are used to prevent the occurrence of severe ICH.[27] The successful prevention programs of HDFN, the red cell counterpart of FNAIT, made people postulate that a severe outcome of FNAIT may also be prevented by timely

identification of pregnancies at risk. This would be possible if existing screening programs during pregnancy were supplemented with a screening for platelet antibodies.

To guide the decision on the introduction of a population-based screening program, ten principles (or criteria) of screening on early disease detection formulated by Wilson and Jungner (W&J) were published by the World Health Organization in 1968 (Figure 3). Although these criteria were adapted and supplemented,[28] it was stated that the value of the principles as postulated in 1968 remain preserved.[29] Nationwide population-based screening on platelet antibodies during pregnancy has not been implemented thus far. Missing knowledge on principle 1 important health problem, 2 accepted treatment, 5 suitable test, 7 natural history, 8 whom to treat and 9 costs of case finding hamper the addition of anti-HPA screening to the existing screening programs. The research in this thesis aims to gain new knowledge about FNAIT so that HPA screening during pregnancy can be considered.

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 3. Wilson and Jungner Criteria, World Health Organization, 1968

In the first part we provide an overview of the current knowledge on FNAIT. The narrative review in chapter 1 describes the knowledge available on the epidemiology and management (W&J 1 and 2) of FNAIT.

The second part of this thesis focusses on the natural history of HPA-1a mediated FNAIT. Despite several antenatal screening studies were performed by others,[30-39] knowledge on the natural history of FNAIT (W&J 7) is still uncertain. In the majority of screening studies to date the result of HPA-1a testing and antibody screening was reported to pregnant women and caregivers allowing perinatal medicine, which might have reduced the risk of bleeding in these studies. Chapter 2 describes the study-protocol of the HIP study (HPA-screening in pregnancy study), a large observational prospective study in which caregivers were not

informed about the results of HPA screening tests. In chapter 3 the results of this screening study are reported. Main outcomes in this study are the incidence of severe bleeding, pregnancy outcome and neonatal outcome in HPA-1a immunized pregnancies. (W&J 7) In addition, we explore whether we can select pregnancies at risk for a severe neonatal outcome with characteristics described in the literature (antibody levels[40] and maternal HLA DRB3*01:01 carrier status[41]). (W&J 5 and 8) Evidence emerges that in addition to bleeding symptoms, HPA-1a immunization may be associated with placental damage.[9-11, 42] In chapter 4 we explore this broadening of the clinical spectrum of FNAIT and assess the signs of antibody-mediated placental damage in FNAIT.

The second most frequent involved antibody in FNAIT is directed against HPA-5b.[19] Given the high prevalence of these antibodies in pregnant women (1.8%),[43] thrombocytopenia and/or bleeding may be incidental in HPA-5b immunized pregnancies. In the third part of the thesis (chapter 5) the clinical outcome of anti-HPA-5b cases is described, and the association between HPA-5b and clinical FNAIT is assessed. (W&J 7 and 8)

Evidence on supporting the postnatal treatment in FNAIT is limited. Clinical guidelines on the postnatal management of FNAIT are based on expert opinion and small observational studies.[44] Hence, the fourth part of this thesis (chapter 6) focusses on the postnatal treatment and outcome of FNAIT cases. (W&J 2) We performed an international multicentre study to describe contemporary postnatal treatment and outcome of liveborn FNAIT cases.

To estimate the benefits of a possible screening program, knowledge about the long-term outcome of FNAIT cases is indispensable. The fifth part of this thesis focusses on the long-term outcome of children that were affected by FNAIT. It is known that FNAIT cases with ICH have a high risk of severe neurodevelopmental problems on the long-term.[2, 23] However, knowledge on the long-term outcome of cases newly diagnosed with FNAIT without ICH is limited. In chapter 7 we assess the neurodevelopment at school age, in a cohort of children that were newly diagnosed with FNAIT. (W&J 1) In chapter 8, we describe the long-term outcome of a cohort of children whose mothers were treated with IVIg during pregnancy because a previous child in their family was affected by FNAIT. (W&J 2)

The knowledge of the earlier parts of this dissertation eventually comes together in part six. Chapter 9 we compare the costs of a situation with platelet antibody screening to the situation without antibody screening (W&J 9). Finally, in part seven of this thesis (chapter 10) we discuss the results of the chapters from this thesis and the other available literature guided by the Wilson and Jungner principles.

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