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EXPERT REVIEWS

Reconsidering fetal and neonatal alloimmune thrombocytopenia with a focus on screening and prevention

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Uncertainty regarding the pathophysiology of fetal and neonatal alloimmune thrombocytopenia (FNAIT) has hampered the decision regarding how to identify, follow-up and treat the women and children with this potentially serious condition. Since knowledge of the condition is derived mainly from retrospective studies, understanding of the natural history of this condition remains incomplete. General screening programs for FNAIT have still not been introduced, mainly because of a lack of reliable risk factors and effective treatment. Now, several prospective screening studies involving up to 100,000 pregnant women have been published and the results have changed the understanding of the pathophysiology of FNAIT and, thereby, the approach toward diagnostics, prevention and treatment in a more appropriate way.

Keywords: anti-HPA-1a antibodies • fetal and neonatal alloimmune thrombocytopenia • FNAIT • pregnancy • thrombocytopenia

Current knowledge of FNAIT

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare (one in 1250 pregnancies) but potentially serious condition. Incompatibility between the mother and fetus regarding human platelet antigen 1 (HPA-1) is the most frequent cause of FNAIT. A Leu/Pro single amino acid polymorphism at residue 33 of glycoprotein IIIa is responsible for the antigen disparity [1]. The HPA-1a-negative woman may become immunized against HPA-1a during pregnancy if the fetus is HPA-1a positive, or as a result of an HPA-1-incompatible transfusion. As the glycoprotein IIIa (GPIIIa) is also present as part of the vitronectin receptor on invading trophoblasts, we cannot exclude the possibility that those cells may be responsible for immunization at a very early stage of pregnancy [2]. Antibodies to the HPA-1a antigen can be detected in approximately 10% of HPA-1a negative women who have been pregnant with an HPA-1a positive child [3]. Anti-HPA-1a IgG antibodies can traverse the placental barrier and opsonize fetal platelets, rendering the fetus thrombocytopenic and at risk of bleeding.

Usually, no clinically significant bleeding is associated with FNAIT, but intracranial hemorrhage (ICH) has been found in 7–26% of newborn children with this condition. Fatal outcome was reported in approximately one third of the cases [4–7], but the significance of these results can be debated, as they are derived mainly from retrospective studies.

Alloimmunization to HPA-1a is associated with the *HLA-DRB3*0101* allele, as approximately 90% of women with HPA-1a antibodies have this allele [3,8–10]. On the other hand, only approximately 30% of HPA-1a-negative women who are positive for this allele are immunized [11]. Except for the incompatibility regarding platelet antigens and HLA, other factors that may influence the immune response to HPA-1a have been identified. Recently, it was observed that the maternal ABO phenotype, and *ABO* genotype, was correlated with the severity of thrombocytopenia of the newborn [12].

Information derived from retrospective studies

The current understanding of FNAIT and its pathophysiology is, to a large extent, derived from retrospective studies. When a woman gives birth to a child with petechiae or other obvious signs of bleeding and the mother has antibodies to a platelet antigen that she is lacking but is

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present in the child, FNAIT can be diagnosed. In forthcoming pregnancies, the mother and child will be given the necessary follow-up. This procedure will secure the follow-up of the woman who have had an index case. However, in women with thrombocytopenic newborns without obvious signs of bleeding, no diagnosis will be made, no proper follow-up will be carried out in the next pregnancy, and they will not be included in studies or databases concerning FNAIT.

Therefore, the complete understanding of the natural history of FNAIT cannot be achieved by retrospective studies because asymptomatic cases will not be detected; the first-born with symptomatic thrombocytopenia is born without anyone being aware of the risk of bleeding. Subsequent pregnancies of women with children affected by FNAIT only represent a selection of pregnancies, not necessarily giving the complete picture of the disease. It is known that petechiae is not always present in FNAIT, even if the child has severe bleeding [3,13,14].

The Norwegian screening program included more than 100,000 pregnancies. Since all routine laboratory investigations of FNAIT cases in Norway are performed in one single reference institution, we have been able to compare the detection rate of FNAIT based on index pregnancies with the rate based on general screening. Only 14% of the FNAIT cases are referred to the national reference laboratory for investigation [15]. Hence, it is unlikely that information derived from this 14% is representative of the total group of HPA-1a-immunized women.

Information derived from prospective studies

The prospect of introducing screening programs to identify women at risk of giving birth to a child with FNAIT has been discussed by others [10,16–18]. The main reasons for the hesitation to introduce such screening programs have included uncertainty regarding reliable parameters to identify children who are severely affected, the guidance and intervention offered to immunized women and finally, the cost of screening programs. A few randomized controlled trials comparing any two prenatal intervention categories – for example, steroids versus intravenous immunoglobulin (IVIG) alone, or IVIG plus steroids versus IVIG alone – have been conducted. No statistically significant differences in predefined outcomes were found between the treatment arms. Randomized controlled trials comparing any intervention with no treatment have not been performed [19,20].

Current research in the field is hopeful of a solution to these questions [11,21]. A large prospective screening and intervention study carried out in Norway from 1995 to 2004, including 154 HPA-1a-immunized women in 170 pregnancies, indicated that it is possible to reduce the risk for morbidity and mortality associated with FNAIT [3]. In addition, owing to the size and prospective feature of this study, some observations were made that challenge the current opinion regarding the natural history of FNAIT [11]. The most interesting observation was that immunization very often occurs after delivery of an incompatible child and not during the first pregnancy.

Time of immunization

The glycoprotein IIIa is present in the platelet membrane from week 16 of gestation [22], and immunization may occur as early as at week 16–20 [23,24]. In several aspects, FNAIT is the platelet counterpart to hemolytic disease of the newborn (HDN). However, FNAIT has not been considered for any prophylactic efforts similar to those of HDN because it is generally believed that immunization against HPA-1a frequently takes place during the first incompatible pregnancy [25]. The design of our screening program enabled us to scrutinize this assumption. We found only 13 primigravidae among 154 women with anti-HPA-1a antibodies who were immunized during pregnancy [11]. Turner *et al.* conducted a large prospective study of antenatal screening for anti-HPA-1a antibody-induced FNAIT, and they reported that only one out of 25 women was in her first pregnancy, suggesting that FNAIT is very unlikely to occur in a primigravida [17]. Williamson and coworkers reported that eight of 33 immunized women were primigravidae [10]. Therefore, in these three prospective screening studies, the frequency of immunization during the unique first pregnancy seems not to be higher than 8, 4 and 24%, respectively.

In the Norwegian screening study, the presence of antibodies to HPA-1a was analyzed in samples obtained approximately 6 weeks after delivery in those women who were not immunized during pregnancy. To our surprise, we observed that approximately 60 of 1780 HPA-1a-negative women without anti-HPA-1a at the end of the pregnancy had such antibodies 6 weeks postpartum. This shows that a large proportion of the immunizations took place at the time of delivery [11]. Altogether, these results indicate that the frequency of immunization in first pregnancy is much lower than was hitherto believed, and that FNAIT, in this respect, is more similar to HDN than was previously assumed.

Occurrence of intracranial hemorrhage

Intracranial hemorrhage and death are the most severe complications of FNAIT. The clinical outcome for ICH due to FNAIT is often worse compared with neonatal ICH from other causes [26,27]. The main goal for the diagnosis and intervention of FNAIT is to prevent bleeding and, in particular, the consequences of ICH. It has been argued that a screening program for FNAIT has no effect, since the majority of ICH cases seem to occur during pregnancy and can therefore not be prevented.

The detection and handling of ICHs that occur during pregnancy demand different, and more challenging, approaches compared with bleedings in the neonatal period. It is therefore crucial to consider the time of bleeding onset when discussing screening for HPA-1a-negative pregnant women and in the management of FNAIT.

In two prospective screening studies for FNAIT, three out of four cases (75%) of ICH occurred *in utero* [3,10]. In one review of retrospective FNAIT studies and case reports, 81% of ICH cases were detected antenatally [5] while another literature review found that 51% of ICH cases occurred *in utero* [28]. In this last review, *in utero* ICH cases were mainly diagnosed after 30 weeks gestation, and only 14% were detected before 20 weeks of gestation

[5]. The same trend was found in another review study, in which only 15% of *in utero* ICH cases occurred before 24 weeks [28]. We have identified 16 cases of ICH reported in the literature, where the bleeding was diagnosed *in utero* and the gestational age at the time of diagnosis was stated. Four (25%) of the cases were diagnosed before 29 weeks (weeks 20, 21, 24 and 28) [23,29,30]. The majority of cases (75%) were diagnosed after 30 weeks (range: 31–37 weeks; mean: 33.5 weeks) [3,30–37], but the time of diagnosis does not, of course, equate to the time of bleeding onset; the bleeding may occur some time before diagnosis. On the other hand, a publication bias towards very early cases of *in utero* ICH is also conceivable. In summary, although no study specifically addressed the onset of bleeding, available data indicate that 50–80% of ICH cases happen *in utero* and then mainly during the third trimester.

The chance of a favorable clinical outcome may be higher if the ICH is detected as soon as possible after it occurs, and is followed by delivery and compatible platelet transfusion to avoid increment of ICH and permanent parenchymal damage, or the institution of high-dose IVIG with or without steroids. Preterm delivery by caesarean section has been performed in cases of ICH due to FNAIT and with favorable results [3]. Frequent fetal ultrasounds from gestational week 24 in high-risk pregnancies should therefore play a central part of a screening and intervention program. There is reason to believe that many cases of severe neurological complications or intrauterine fetal deaths due to FNAIT could be prevented by early diagnosis of bleeding.

Obstetric history of FNAIT as a predictor of severity

The natural history of FNAIT and associated complications in subsequent pregnancies is still poorly documented and current knowledge is mainly derived from retrospective data.

Kaplan *et al.* reported follow-up studies in nine subsequent pregnancies [38]. Eight of the women gave birth to severely thrombocytopenic children in both of their pregnancies. However, no aggravation in clinical outcome from first to second pregnancy was reported and none of these fetuses/newborns had severe bleeding complications either during gestation or after delivery. One woman gave birth to a child with less-severe FNAIT in her second pregnancy. Bussel *et al.* observed that the severity of thrombocytopenia and the clinical consequences were more pronounced when there was a history of antenatal ICH in the sibling [39].

Single case reports on FNAIT-induced ICH in subsequent pregnancies do not unequivocally demonstrate that the clinical condition in one pregnancy indicates a poorer outcome in the next incompatible pregnancy [40–44].

Two literature studies summarized data on untreated FNAIT cases and found an 80% recurrence rate for ICH [5,28]. However, the authors stated that this might be an overestimate since reports of less-severe cases of FNAIT are likely to be under-represented in the literature.

In 2003, a European collaborative study group was established to determine whether the severity of FNAIT in a current pregnancy could be predicted from the sibling history of FNAIT. They analyzed prospective and retrospective cases from six European countries (UK, France, Germany, Poland, Austria and Finland), including data collected from 56 children, and concluded that both clinical outcome and the degree of thrombocytopenia in previously affected siblings could be used to predict the severity of FNAIT in subsequent pregnancies [45]. Consistent with Bussels' observation [39], it was found that if the former sibling had antenatal ICH, the platelet counts in 92% of the subsequent fetuses were less than 20×10^9/l before treatment was started. In addition, it was found that 66% of the women who had given birth to newborns with platelet counts less than 20×10^9/l (with or without postnatal ICH) had fetuses with severe thrombocytopenia in a subsequent pregnancy. In contrast to this, Gaddipati *et al.* found no relationship between obstetric history and sibling fetal platelet count in a retrospective study of 74 cases [46]. Data analyses that only included prospective cases gave a positive predictive value for obstetric history of 41% [11].

Despite all these results evolving from different study designs (case reports, literature reviews, retrospective or prospective datasets), the emerging consensus seems to be that the highest risk for FNAIT-related complications in subsequent pregnancies is among those infants with siblings that experienced antenatal ICH. Among siblings with severe FNAIT and no ICH (defined as a platelet count of <20 or 50×10^9/l), data are still unclear, ranging from no relationship to a 66% recurrence rate [11,45–47].

The greatest problem in using obstetric history as a risk factor is that the first affected child goes undetected during pregnancy and receives no attention, neither during pregnancy nor immediately after birth.

Antibody level as a predictor of severity

The lack of a robust parameter to predict the fetal platelet count in HPA-1a-incompatible pregnancies has been a problem. Severe thrombocytopenia in a newborn with FNAIT has been used as a risk factor for the next pregnancy. However, the positive predictive value using this predictor has been only approximately 40% [11]. This predictor has been used to decide about follow-up procedures for the next child but does not offer any help to the first affected child in a family.

More recently, maternal anti-HPA-1a antibody level during the pregnancy has been claimed to be a better predictive factor, although the predictive value has been questioned [48]. Williamson *et al.* reported a correlation between antibody titre in the last trimester and severity of thrombocytopenia [10]. This observation has been further substantiated by antibody quantitation performed with the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) technique [49]. In 2000, our group reported that antibody levels in the mothers' plasma at delivery correlated inversely with the neonatal platelet count [50]. More recent studies, also using the MAIPA technique, have revealed similar results. Thus, Killie *et al.* found that the mean antibody level at delivery (23.7 IU/ml) was significantly higher in the serum of women having babies with severe thrombocytopenia compared with those having babies with only mild-to-moderate thrombocytopenia (4.2 IU/ml) [11]. Using 3 IU/ml as the level of antibody concentration to identify cases

at risk of FNAIT, the clinical sensitivity and specificity were 93 and 63%, compared with 13 and 92% when the clinical history of a previous thrombocytopenic child was used as a predictor of risk. The positive predictive value of obstetric history was not significantly different from that of antibody quantitation. However, antibody quantitation is superior to obstetric history as predictive factor since it has a much higher negative predictive value.

Cellular immunity associated with FNAIT

Although the cross-placental transfer of platelet-reactive antibodies is known as the major etiology of FNAIT, little is known regarding the cells and cellular interactions underlying the production of these antibodies in the mother. Still, a number of findings link maternal T-cell responses to FNAIT.

T-cell antigen recognition is dependent on specific MHC molecules (MHC restriction) and most HPA-1a-immunized *HPA-1bb* women carry the MHC allele *HLA-DRB3*0101* [9]. This allele, together with *HLA-DRA*, encodes the heterodimeric MHC class II molecule HLA-DR52a. In Norway, more than 90% of HPA-1a-immunized women carry the *HLA-DRB3*0101* allele [3], while the frequency in the general population is slightly less than 30%. These numbers reflect the situation in other populations as well [9,10]. This clear association between the presence of a particular MHC allele and the production of anti-HPA-1a antibodies provides strong support for the involvement of HLA-DR52a-restricted maternal CD4 T-cell responses in the development of FNAIT. This also suggests that a specific peptide, which fits the binding motif of HLA-DR52a and forms an immunogenic peptide–MHC complex, is required for driving the production of platelet-reactive antibodies. In this respect, it has been demonstrated that peptides derived from GPIIIa and contain the HPA-1a Leu33 residue (here referred to as 'HPA-1a peptides') can bind to recombinant HLA-DR52a molecules [51]. Moreover, it was shown that the Leu33 residue serves as an anchor for stable binding to HLA-DR52a; corresponding peptides containing the alternate polar Pro33 residue (HPA-1b peptides) did not bind. These findings suggest that the Leu33 residue could form the basis for both the B-cell and T-cell epitopes. Shortly before, the same research group demonstrated the proliferation of T cells specifically in response to culturing peripheral blood mononuclear cells from HPA-1a-immunized women with HPA-1a, but not HPA-1b, peptides, supporting the notion that HPA-1a could be a T-cell epitope associated with FNAIT [52]. Later, other groups also demonstrated proliferative responses in peripheral blood mononuclear cells from HPA-1a-immunized women cultured with HPA-1a peptides [53,54]. It was also demonstrated that the proliferating cells were CD4 positive [54]. Furthermore, it has been demonstrated that a naturally processed epitope containing the HPA-1a polymorphism can be isolated from *HLA-DRB3*0101*-positive antigen-presenting cells [55]. Recently, the generation of clonal HPA-1a-specific CD4 T-cell lines was reported in two independent studies [8,56]. In both of these studies, the specificity of the T-cell clones for HPA-1a peptides was demonstrated. In one of the studies, Ahlen *et al.* demonstrated unequivocally that HLA-DR52a is the restricting MHC class II molecule by using MHC-matched antigen-presenting cells [8]. This finding links the *HLA-DRB3*0101* allele association of FNAIT to a function of the molecule encoded by this allele in HPA-1a-specific T-cell recognition. However, this finding does not rule out the possibility that other MHC molecules and other T-cell epitopes may also be associated with FNAIT. In fact, not all *HPA-1bb* women producing HPA-1a-specific antibodies have the *HLA-DRB3*0101* allele. In these women, T-cell responses may be driven by other MHC molecules and may be T-cell epitopes derived from alloantigens different from, but still physically linked to, HPA-1a. In fact, one other MHC allele, *HLA-DQB1*0201*, has also been found to be associated with FNAIT [9,54].

While the aforementioned T-cell studies provide formal evidence for the existence of HPA-1a-specific CD4 T cells in these women [8,56], there is still no formal evidence that these T cells are directly involved in supporting antibody responses, although it would seem likely that they are.

Since most humoral immune responses are dependent on CD4 T cells, manipulation of CD4 T-cell responses could be used in a strategy to prevent or lower the formation of undesired antibodies or other undesired T-cell effector functions. Tolerization of T cells to antigens has been used therapeutically in other settings [57], and the identification of the major T-cell epitope associated with FNAIT and the availability of clonal HPA-1a-specific CD4 T cells are important contributions towards the development of such therapeutic strategies.

Does the current knowledge of FNAIT justify national screening programs for identification of pregnancies at risk?

In the following paragraphs, the introduction of screening for FNAIT is discussed in relation to the revised WHO screening criteria [58] and based on the newly acquired knowledge [11,59].

A screening program should respond to a recognized need

Fetal and neonatal alloimmune thrombocytopenia due to anti-HPA-1a antibodies can be recognized in one out of 1250 pregnancies in the Caucasian population, and with ICH in one out of 12,500 to one out of 25,000 pregnancies [3,10,60].

Objectives of screening should be defined at the outset

The objective of screening for FNAIT should be to reduce morbidity and mortality related to thrombocytopenia-induced hemorrhage in the fetus and newborn. As it is now documented that immunization most often occurs in accordance with delivery, one objective would be to try and prevent immunization using methods already in use for preventing rhesus D-antigen (RhD) immunization [61]. The other objective would be the possibility for follow-up and treatment of the first effected child, antenatal, perinatal and/or postnatal.

There should be a defined target population

The target population in FNAIT is HPA-1a-negative pregnant women who are *HLA-DRB3*0101* positive. Other genetic and phenotypic markers are being studied to define the population more accurately.

There should be scientific evidence of screening program effectiveness

There are strong indications that a screening program would be clinically efficient [3], and also cost effective [21]. This evidence could be created as a result of a randomized study but this is considered unethical to perform. As national screening programs will not be introduced in all countries simultaneously, it will be possible to compare the effectiveness between screening and no screening in countries with equal standards of health care, and where there are no major genetic differences between the populations.

The program should integrate education, testing, clinical services & program management

HPA-1a typing and RhD typing can be performed in the same blood sample and would be easy to introduce. Introduction of the program would create the need for increased clinical activity and follow-up using well-known procedures. In addition, more information would have to be introduced to the general population.

There should be quality assurance, with mechanisms to minimize potential risks of screening

The laboratory tests used for typing, antibody detection and quantification are stable and international validation programs are in use. More knowledge of the pathophysiology may identify the need for more tests that may have to be implemented. The follow-up for pregnant women at risk must be planned in order to reduce the anxiety of, and to care for those identified with, high-risk pregnancies.

The program should ensure informed choice, confidentiality & respect for autonomy

When screening is introduced as part of a general program for the surveillance of pregnant women, the logistic procedure already in place for hemolytic disease of the newborn could be used to inform and obtain consent.

The program should promote equity & access to screening for the entire target population

In countries with already established national screening programs for pregnant women, a new screening test will target the whole population.

Program evaluation should be planned from the outset

The results of a screening program should be followed carefully. In the case of FNAIT, many groups have already followed large cohorts with suitable methods, and end points are easy to create (death or disability due to intracranial hemorrhage or thrombocytopenia in the newborn).

The overall benefits of screening should outweigh the harm

If the screening program reduces fetal and neonatal morbidity and mortality, it is likely that the screening process by far outweighs the anxiety experienced by women identified with high-risk pregnancies. Recently, a study was published about women's attitudes towards prenatal screening for red blood cell antibodies. The results showed that the program was highly accepted although the information regarding the pregnant women subjected to screening for red blood cell antibodies needed to be improved [62].

Until now, it has been argued that screening for FNAIT is not justified owing to the lack of efficient treatment of immunized women. The new knowledge that most immunizations take place at the time of delivery has enabled the opportunity of preventing immunization by the injection of anti-HPA-1a antibodies, using the same principle as prevention of RhD immunization. This still remains to be demonstrated, but animal experiments are currently being conducted to test this hypothesis [61]. In order to study the possible effects of such antibody-mediated immune suppression in humans, screening must be in place to identify the women who are at risk and available for a vaccine trial. In such a program, already immunized women must be offered clinical follow-up. Based on the fulfilment of screening criteria and the new knowledge regarding the pathophysiology of FNAIT, it could be argued that not to introduce screening programs in order to study the effect of the vaccination is unethical.

Expert commentary & conclusion

Although there is no international consensus regarding, or guidelines for, the treatment of FNAIT, large resources are used for the diagnosis, follow-up and treatment of women who have had children with severe thrombocytopenia and ICH. It is a problem that treatment can only be offered to those who have previously had a child with FNAIT. Thus, there are many children that do not have the advantage of early diagnosis and follow-up. Even more serious is the fact that, as FNAIT is underdiagnosed, severe cases may go undetected.

It is a paradox that the index child receives little attention unless the condition is identified at delivery, whereas the next child is the target for great efforts. We know that the first FNAIT-effected child in a family may be affected as severely as the subsequent ones. Even if a child has ICH *in utero*, it may be worthwhile intervening in order to minimize the consequences of bleeding.

For these reasons, national health authorities should seriously consider the introduction of general screening programs for FNAIT.

The size and the prospective nature of the Norwegian screening study and other investigations have made it possible to reveal two important aspects regarding FNAIT [63]. First, the surprising observation that HPA-1a immunization of the mother often occurs at the time of delivery has prepared for the possibility to reduce the frequency of immunization by a prophylactic regimen with passive transfer of anti-HPA-1a antibodies. Second, the massive support of our previous demonstration of an inverse correlation between maternal anti-HPA-1a antibody concentration and fetal platelet count may provide us with a tool to predict the severity of the condition.

The emerging knowledge about the immune response to HPA-1a on the T- and B-cell level, reveal further possibilities to interfere with the process leading to antibody formation.

Five-year view

Although the natural history of FNAIT is well described, several important questions remain to be elucidated. Therefore, it is expected that additional large screening programs will be conducted. Prophylactic treatment to prevent immunization will be tested in a similar way as that for hemolytic disease of the newborn. As the cellular mechanisms responsible for immunization with HPA-1a-positive platelets are being clarified, attempts to induce cellular tolerance against HPA-1a immunization will be tested out. Treatment of FNAIT with IVIG will be further investigated in randomized controlled trials.

Financial & competing interests disclosure

Bjørn Skogen, Mette Kjær Killie, Jens Kjeldsen-Kragh and Anne Husebekk are stock owners in Prophylix Pharma (Tromsø, Norway), a company dedicated to developing a prophylactic regimen for fetal and neonatal alloimmune thrombocytopenia. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Key issues

- Fetal and neonatal alloimmune thrombocytopenia is a condition that is underdiagnosed.
- Immunization seldom occurs in the first pregnancy.
- Immunization takes place in association with delivery in most cases.
- Anti-HPA-1a level is a predictor for the severity of thrombocytopenia.

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