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RECOMMENDATIONS AND GUIDELINES

Investigations for fetal and neonatal alloimmune thrombocytopenia: communication from the SSC of the ISTH

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Introduction

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is an immune-mediated platelet disorder caused by alloantibodies to human platelet antigens (HPA) that can cause severe thrombocytopenia and bleeding in an otherwise healthy infant. Laboratory investigations are needed to confirm a diagnosis of FNAIT for the infant and to assess the risk for future pregnancies. The objective of this report from the Platelet Immunology Scientific Subcommittee (SSC) of the ISTH was to develop a proposal for the standardization of investigations and to highlight challenges with testing and interpretation of results.

Methods

The panel of authors highlighted the following priority domains to be evaluated: clinical definition of FNAIT, sample requirements, and methods for serological investigations and genotyping. A literature search was carried out to address each domain. The panel completed two rounds of consensus discussions during an in-person meeting, by teleconference and by asynchronous e-mail discussions. Where evidence was lacking, recommendations reflect the opinions of the panel. Final recommendations were supported by the chair of the Platelet Immunobiology Working Party of the International Society of Blood Transfusion.

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Recommendation 1: The clinical criteria for suspected FNAIT in the index pregnancy are: (i) nadir platelet count below 100×10^9 L^{-1} at birth or within 7 days after birth of the affected child or (ii) fetal intracranial hemorrhage (ICH), both in the absence of an alternative cause.

A platelet count threshold to identify infants potentially affected by FNAIT in the first affected pregnancy (index pregnancy) was needed to direct investigations appropriately. FNAIT is a rare cause of neonatal thrombocytopenia (platelets $< 150 \times 10^9$ L^{-1}), which can affect up to 5% of all newborns and 35% of neonates admitted to the intensive care unit [1]. Nevertheless, a platelet count $< 100 \times 10^9$ L^{-1} was used as the threshold for FNAIT diagnosis to ensure that even mild cases were not missed, recognizing that most affected infants will have a nadir platelet count that is less than 50×10^9 L^{-1} [2,3]. Rarely, infants with FNAIT can have milder thrombocytopenia (e.g. as a result of anti-HPA-5b antibodies) [4,5]. Before embarking on investigations for FNAIT, other more common causes of thrombocytopenia should be excluded (e.g. hypoxia, infection, etc.).

FNAIT should be considered in an index pregnancy with fetal ICH that occurs in the absence of any apparent cause, including anatomical defects or prematurity [6]. Fetal platelet counts are generally not available; thus fetal thrombocytopenia often cannot be confirmed and the diagnosis of FNAIT is presumptive until the baby is born. The frequency of positive serology for FNAIT in infants with isolated ICH is low [7,8]. There is no confirmed association between FNAIT and isolated fetal brain anomalies such as fetal ventriculomegaly or fetal brain cysts [9].

Recommendation 2: FNAIT testing should include: HPA genotyping from the mother, the neonate or, if not available, the father; alloantibody testing of maternal serum; and a crossmatch with paternal platelets.

The goals are: (i) to determine the genotype of specific HPA alleles to identify a platelet antigen incompatibility between the mother and father, or between the mother and neonate; (ii) to identify a maternal platelet alloantibody; and (iii) to determine the risk of FNAIT in subsequent pregnancies based on the paternal zygosity for the HPA antigen in question. Maternal antibody testing should be carried out on a serum sample, and genotyping and platelet crossmatch could be performed using an EDTA sample. Thus, maternal and paternal DNA are required for platelet genotyping; and maternal serum and paternal platelets are needed for antibody testing and for crossmatch. Neonatal DNA should be used instead of paternal DNA for the initial diagnosis if possible.

In subsequent pregnancies, fetal DNA obtained by amniocentesis or from the maternal circulation may be required if the father is heterozygous for the implicated HPA allele. After delivery, rapid genotyping (within hours) can be performed in infants with suspected FNAIT to select antigen-negative platelet concentrates; however, if severe thrombocytopenia or bleeding is present, random donor platelets should be administered without waiting for results of these tests [10,11]. Serology testing in neonates is less reliable than maternal antibody testing because of binding to fetal tissues that express HPA antigens (e.g. endothelium and placenta). In an international workshop, it was shown that the use of EDTA plasma significantly reduces the sensitivity of the MAIPA assay in the detection of HPA1a antibodies; thus EDTA samples should not be used for alloantibody investigations [12].

Recommendation 3: The mother and father should be tested for a panel of HPA alleles that reflects local genetic diversity.

The panel of antigens should include HPA-1, -2, -3, -4, -5, -6, -9 and -15 alleles and may vary depending on the antigen frequency of the geographical region or the population served by the laboratory. Reference laboratories represented by the panel use the following validated PCR techniques for genotyping: restriction fragment length polymorphism (RFLP), sequence-specific primer (SSP), real-time (RT), next-generation sequencing (NGS) or beadchip techniques. Non-invasive prenatal diagnosis could be performed by NGS, RT PCR or droplet digital PCR [13–15]. Molecular genotyping has mostly replaced platelet phenotyping; however, phenotyping may be required to resolve discrepant or weak results on genotyping.

Recommendation 4: Maternal antibodies should be investigated using two different methods and a crossmatch with paternal platelets.

Testing for maternal alloantibodies should include anti-HPA-1, -2, -3, -4, -5 and -15 alloantibodies, plus anti-HPA-6b and anti-Nak^{a} (CD36 or GPIV), more common in Asian and African populations. Testing can be carried out using bead-based multiplex assays [16], glycoprotein-specific assays such as the monoclonal antibody-specific immobilization of platelet antigen method (MAIPA) [17], antigen capture assays [18], flow-based assays or the platelet adhesion immunofluorescence test using whole (intact) platelets [19,20]. Whole platelet (non-lysed) assays will identify anti-HLA antibodies that can interfere with detection of platelet-specific alloantibodies, which must be interpreted in the clinical context. Moreover, mixed passive hemaglutination can be used to detect anti-HPA-3 alloantibodies that only react with native platelets.

Two serological methods should be used to ensure concordance. Target platelets can be collected fresh from donors (normal platelet count, no concomitant illness and not receiving antiplatelet medication) or frozen (at −80 °C for up to 6 months), especially for rare platelet antigens; however, freezing and thawing can cause the loss or disruption of certain platelet antigens (e.g. cryptic antigens on GPIIbIIIa). Recombinant protein targets may be used for the detection of specific alloantibodies (e.g. anti-HPA-15; CD109) [21].

In addition, alloantibody investigations should include a crossmatch of maternal serum with fresh paternal platelets. This method can detect alloantibodies to low frequency (private) antigens. Serological testing for FNAIT should be carried out in a reference laboratory because of the specialized testing and expertise required for these techniques [22].

Recommendation 5: Consider delayed or low-affinity alloantibodies in mothers with a negative antibody test.

False-negative results of maternal antibody testing can occur as a result of delayed appearance of the antibodies or low-affinity antibodies, which should be considered in serology interpretation.

Delayed alloantibodies (e.g. anti-HPA-1, -3 and -5) may appear several weeks after birth and thus may be missed in the immediate perinatal period [23]. Consequently, when HPA-1, -3 or -5 antigens are implicated because of antigenic incompatibility, the infant genotype should be determined and testing for maternal alloantibodies should be repeated 2–8 weeks later.

Low-affinity antibodies or antibodies to unstable (weak) antigens can also cause false-negative results in some assays. Certain HPA-3 antibodies are not readily detectable using MAIPA but can be detected using fresh whole platelets in the platelet adhesion immunofluorescence test [24]. Anti-HPA-15 (CD 109) alloantibodies can also be missed in the crossmatch with paternal platelets, but can be detected using fresh HPA-15aa or HPA-15bb platelets from high CD109 expressers. Similarly, anti-HPA-9b alloantibodies can be missed in the paternal platelet crossmatch because of inherent characteristics of the antigen [25]. Other low-affinity HPA alloantibodies can be lost during the washing steps of the MAIPA assay [26,27]. Thus, the use of at least two different methods (i.e.

glycoprotein-specific assays such as MAIPA or MACE and a whole-platelet assay such as platelet adhesion or suspension immunofluorescence tests) for alloantibody detection will reduce the risk of false-negative tests. Nevertheless, a discrepancy exists between HPA-1a incompatibility and antibody formation because immunization is dependent on other factors, including the HLA-DRB3*0101 genotype [28].

Anti-HLA antibodies can be detected in up to 30% of multiparous women [29]. In the absence of an HPA incompatibility, anti-HLA antibodies have sometimes been implicated in FNAIT; however, evidence for clinical FNAIT because of anti-HLA is lacking and this is not a major cause. Other antibodies, including anti-A or anti-B antibodies, have also been rarely associated with FNAIT [30].

Conclusion

The clinical criterion for FNAIT is a platelet count below 100×10^9 L^{-1} in an affected infant or fetal ICH, both in the absence of an alternative cause. Laboratory confirmation of FNAIT requires the identification, by genotype, of a platelet antigen incompatibility between the mother and father and the identification of a maternal alloantibody. Several testing strategies are proposed to optimize the accuracy and timeliness of the diagnosis. These tests require specialized skills from a reference laboratory. Results of FNAIT investigations, especially negative results, require careful interpretation that considers the clinical context and the need for treatment.

Addendum

R. Petermann participated in the data collection, summarized the information, drafted the manuscript and approved the final version. T. Bakchoul conceived the research, participated in the data collection, summarized the information, drafted the manuscript and approved the final version. B. R. Curtis participated in the data collection, summarized the information, drafted the manuscript and approved the final version. F. Mullier participated in the data collection, summarized the information and approved the final version of the manuscript. S. Miyata participated in the data collection, summarized the information and approved the final version of the manuscript. D. M. Arnold conceived the research, participated in the data collection, summarized the information, drafted the manuscript and approved the final version.

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Disclosure of Conflict of Interests

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