REVIEW ARTICLE

Approach to Neonatal Alloimmune Thrombocytopenia: The Perspective from a Transfusion Medicine Service

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Abstract

Neonatal alloimmune thrombocytopenia (NAIT) is an important hematological disorder in neonates. The pregnant mother's immune system gets sensitized to antigens expressed on fetal platelets that have been inherited from the father and begins producing specific alloantibodies against these antigens. Some of these antibodies get transported across the placenta into the baby and can damage/destroy platelets to cause fetal/neonatal thrombocytopenia. Many of these fetuses/infants develop major clinical complications such as intracranial hemorrhages. In this article, we describe normal platelet counts in neonates, the pathogenesis and epidemiology of NAIT, specific platelet antigens that have been identified as targets in NAIT, and the approach for laboratory diagnosis of NAIT. From the perspective of a transfusion medicine service, there are two targets as follows: (a) To identify the differences in the antigenic profiles of the platelets of the mother and her fetus/infant and (b) To detect alloantibodies in the maternal serum that may be specifically reactive to these platelet antigens. Early identification of NAIT can help timely institution of appropriate treatment. In this project, we reviewed the laboratory profiles of infants who were diagnosed to have NAIT at our own institution and also mined the literature in the databases EMBASE, PubMed, and Scopus.

Keywords: Alloantibdies, Alloantigens, Antigens capture elisa glycoproteins, Newborn, Platelet genotyping, Platelet specific antigens.

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Key Points

a. In fetal/NAIT, the mother forms antibodies against paternal antigens expressed on the surface of platelets of her fetus/infant. These antibodies cross the placenta and damage the fetal/neonatal platelets.
b. Neonatal alloimmune thrombocytopenia (NAIT) is a major cause of severe, isolated thrombocytopenia in term neonates. The incidence may be as high as 1 in 1,000 live births.
c. Although the term NAIT emphasizes the disease manifestations after birth, the condition can commence *in utero* with serious consequences including intrauterine death or intracerebral hemorrhage during the 20–24 weeks' period of pregnancy.
d. Nearly in 85% of all Caucasian mothers develop some alloimmunization against HPA-1a.
e. We have limited information on the immunogenicity of various platelet antigens in terms of the alloantibody production, the efficacy of various antibodies in terms of transplacental transfer, and the impact of different alloantibodies on platelet function or on the incidence of bleeding complications. Our population data on the distribution of different platelet antigens in various ethnic groups is also limited. Consequently, the development of screening programs for NAIT has been difficult.

Introduction

Thrombocytopenia is a frequently seen hematological abnormality in neonates.[1,2] Platelet counts reach levels of around 150×10^9/L by the late second trimester in fetuses and then plateau at these levels until term gestation.[3] Platelets counts between 100–150 $\times$ 10^9/L have been defined as mild thrombocytopenia, 50–100 $\times$ 10^9/L as moderate, and counts <50 $\times$ 10^9/L as severe thrombocytopenia.[4] Mild thrombocytopenia may be seen in up to 25–30% of term infants and is usually self-limiting and of short duration. Moderate/severe thrombocytopenia occurs less frequently and is seen in 5–10% infants.[5–8]

Neonatal thrombocytopenia with platelet counts less than 30–50 $\times$ 10^6/L has been associated with an increased risk of serious hemorrhages into vital organs.[9,10] There are important associations with intrauterine infections, low Apgar scores, sepsis, and an overall higher acuity of illness even when the etiology is unclear. In premature infants, thrombocytopenia is a stronger predictor of intracranial hemorrhage (ICH) than their birth weight or gestational age.[11]

In this review, we summarized the current definitions of neonatal thrombocytopenia and then focused on NAIT. It is noted that NAIT is an important cause of severe thrombocytopenia in neonates; we present the current evidence on its pathogenesis, clinical manifestations, evaluation, treatment, outcomes, and the

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future directions. This article combines peer-reviewed evidence from our own studies with an extensive literature search in the databases PubMed, EMBASE, and Scopus.

Normal Platelet Counts in Neonates

Existing studies show that 98% of term neonates have platelet counts at or above 150×10^9/L, and thrombocytopenia is usually defined as a number of circulating platelets below these levels. Some extremely premature infants born at 22–24 weeks' gestation may have lower platelet counts at less than 100×10^9/L in the first few days after birth, and most of them are asymptomatic.[3] The timing of presentation of neonatal thrombocytopenia can also be used in diagnostic evaluation. Early-onset thrombocytopenia is noted within the first 72 hours after birth, and it may be caused by intrauterine infections, immune-mediated causes, perinatal asphyxia, and infections. Late-onset thrombocytopenia may be related to more diverse causes including bacterial and viral infections, systemic inflammation, hepatitis, necrotizing enterocolitis, and sometimes, may be iatrogenic due to thrombi in central lines or may develop as adverse drugs of certain drugs.[12–26] Genetic disorders with bone marrow dysfunction are less frequent, but can appear at any age.[27–29]

Baer et al.[23] examined 11281 NICU admissions and identified severe thrombocytopenia in 273 (2.4%). Nearly 30% presented in the first 3 days after birth. Half presented by day 10, 75% by day 27, and 95% by day 100. The prevalence was inversely related to birth weight. Cutaneous bleeding was more common in patients with platelet counts of less than 20×10^9/L; however, there was no statistically significant correlation between platelet counts and pulmonary, gastrointestinal, or intraventricular bleeding. The most common explanations for severe thrombocytopenia were acquired varieties of consumptive thrombocytopenia. Platelet transfusions (median, 5; range, 0–76) were administered to 86% of the patients. No deaths were ascribed to exsanguination. The mortality rates did not correlate with the lowest platelet counts but were proportionate to the number of platelet transfusions.

Wiedmeier et al.[24] examined platelet counts in neonates between the first and the ninetieth day after birth, from 47, 291 neonates delivered at 22–42 weeks gestation. The platelet counts obtained in the first 3 days of life, increased over the range of 22–42 weeks gestation. In those born in less than or 32 weeks gestation, the lower reference range (fifth percentile) was less than 104×10^9/L, but it was less than 123×10^9/L in late-preterm and late-term neonates. Advancing postnatal age affected platelet counts; during the first 9 weeks, the counts showed a sinusoidal pattern with two peaks; one at 2–3 weeks and a second at 6–7 weeks. The upper limit of expected counts (95th percentile) during these peaks were as high as less than 750×10^9/L.

Christensen et al.[25] examined blood counts from extremely-low-birth-weight (ELBW) infants. Multiple platelet counts were obtained in 284, and 208 (73%) had one or more platelet counts less than 150×10^9/L. Most were detected during the first days of life; 80% were detected before postnatal day 7. Thrombocytopenia was seen frequently in the smallest infants; 85% incidence among those born with weights less than or 800 gm, 60% among those 801–900 gm, and 53% among those 901–1000 gm. In 48% of cases, the cause of the thrombocytopenia went undiagnosed. The most common explanations were being small-for-gestational-age (SGA) or delivery to a hypertensive mother, disseminated intravascular coagulation, bacterial infection, fungal infection, and necrotizing enterocolitis, respectively.

The same group of scientists[26] studied a large cohort of SGA infants. A total of 31% (905 of 2,891) showed first-week thrombocytopenia compared to the 10% of matched non-SGA controls (p <0.0001). Of the 905, 102 had a recognized cause of thrombocytopenia (disseminated intravascular coagulation, early-onset sepsis, or extracorporeal membrane oxygenation). The remaining 803 did not have an obvious cause for their thrombocytopenia and were grouped as having "thrombocytopenia of SGA." These infants had a mean nadir count on postnatal day 4 of 93×10^9/L (standard deviation 51.8×10^9/L, tenth percentile 50×10^9/L, ninetieth percentile 175×10^9/L). By postnatal day 14, platelet counts were more than or 150×10^9/L in more than half of the patients. Severely SGA neonates (less than first percentile) had lower counts and longer duration of thrombocytopenia (p <0.001). Thrombocytopenia was more associated with SGA status than with the diagnosis of maternal preeclampsia.

Neonatal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia is a condition in which maternal antibodies are formed against the paternal alloantigen expressed on fetal platelets.[30] The pathogenesis is analogous in some ways to that of the hemolytic disease of the newborn, which affects red blood cells. The fetal platelets carrying paternal antigens cross into the maternal circulation during normal low-grade transplacental cellular exchange or during larger-scale fetal-maternal hemorrhages/transfusions, which may occur during miscarriage or delivery. Antigen-presenting cells in maternal lymph nodes and spleen recognize these fetal antigens and stimulate the production of alloantibodies. The antiplatelet immunoglobulin G (IgG) antibodies are then actively transferred into the fetus and promote phago-immune destruction (Fig. 1).

The fetal platelets express specific human platelet antigens (HPAs) from sixteenth week onward.[31] Platelets carrying HPA epitopes such as HPA-1a present on the glycoprotein (GP) IIIa binding to the syncytiotrophoblasts-derived microparticles (STMPs) increases the likelihood of alloimmunization (Fig. 2). Trophoblasts normally escape allorecognition because of low expression of human leukocyte antigen (HLA) class I and II molecules. There is some expression of HLA-G, which is a non-classical HLA-I molecule and promotes alloantigen tolerance.[32]

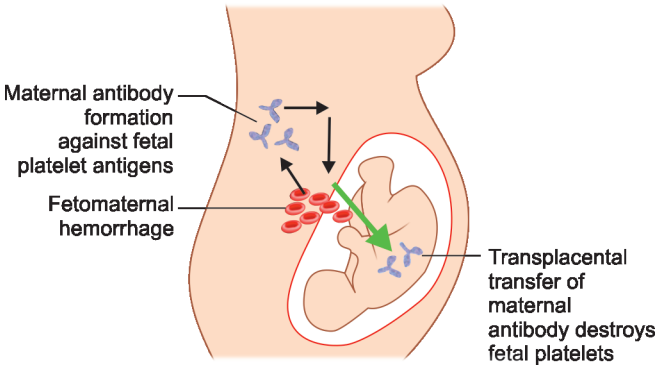

Fig. 1: Pathogenesis of NAIT

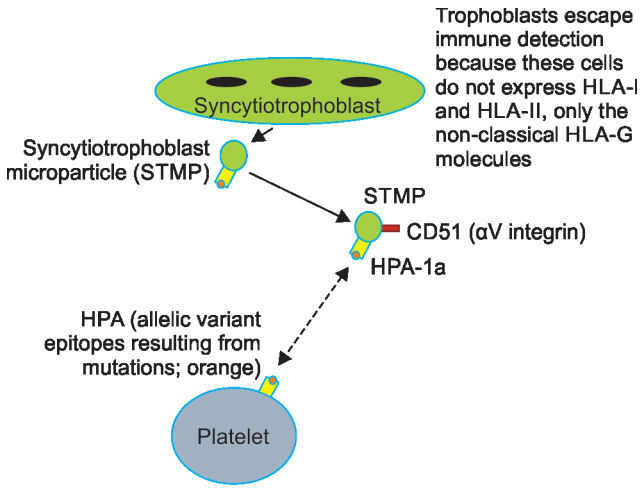

Fig. 2: The β3 integrin (platelet GPIIIa); CD61is expressed on the placental syncytiotrophoblast, the syncytiotrophoblast microparticles (SMTPs), and on platelets. Molecular variations are read as HPA-1a antigen, which evoke an antibody response. The STMPs show these antigens complexed with CD51, which potentiates the immune responses and may cause antibody-mediated platelet destruction

Several antigen systems can be seen on the surface of human platelets, including the HPAs, the ABO antigens, and the HLA class I.[33,34] So far, 29 HPA systems have been identified on six platelet membrane GPs (GPIa, GPIbα, GPIbβ, GPIIb, GPIIIa, and CD109); 12 are grouped into 6 biallelic systems (HPA-1, -2, -3, -4, -5, and -15). All but one of these HPAs represents single nucleotide polymorphisms (SNPs) that result in single amino acid substitutions.

Most HPAs are located on the GPIIb/IIIa although the distribution of various HPAs may show some ethnic/geographic variation.[35] Anti-HPA-1a alloantibodies are the major cause of immune mediated thrombocytopenia in Caucasian, whereas the HPA-4 and Naka (anti-CD36) antibodies are the predominant cause in Asian population, especially in the Japanese.

Most infants with NAIT develop mild-moderate thrombocytopenia, although these reductions can add to the morbidity and mortality if these infants become critically-ill.[36] The destruction of platelets by maternal antibodies can increase the risk of bleeding, particularly that of ICH. Alloimmunization has been best studied with the HPA-1a antigen expressed on the β3 integrin (platelet GPIIIa; CD61).[37] This integrin may be intrinsically expressed on placental STMPs or may be acquired from circulating platelets.[38] The syncytiotrophoblasts-derived microparticles show these antigens complexed with CD51, which evokes an immune response. Platelets also express various surface molecules such as the integrin β2, β3, αIIb, CD109, and the complex GPIbα that may carry various HPAs.[39] The syncytiotrophoblasts-derived microparticles can induce variable immune responses, which include fetal alloantigen tolerance or induce immune responses that cause antibody-mediated platelet destruction.[40]

Epidemiology of NAIT

The incompatibility between fetal and maternal platelet antigens evokes the synthesis of maternal IgG antibodies, which then cross the placenta to induce fetal platelet destruction and cause NAIT.[41] Similar to red cell alloimmunization such as in Rh antigen-mediated hemolysis, most cases of NAIT follow immune sensitization against platelets at the time of delivery in a previous pregnancy. However, many cases are seen in the very first pregnancy.[42]

Human platelet antigens-1a is the best-studied trigger for the production of antiplatelet antibodies and causation of NAIT.[43] In one study, the incidence of thrombocytopenia in incompatible HPA-1a positive infants was 1:1000–2000.[44] The HPA-1bb phenotype in Caucasian population was about 2.5% and out of these one-third expressed the HLA-DR antigen B3*0101.[45] One-third of infants in this subset developed antibodies against HPA-1a and with moderate-to-severe thrombocytopenia.[3]

Platelet Antigens

Important HPAs

A system of HPA nomenclature was developed by international consensus following confirmation of polymorphisms in platelet GPs. These antigens were designated as HPA1 and HPA2 in the order of discovery.[46] The suffix "a" or "b" indicated decreasing frequency of expression. The HPA-1a antigen, the first HPA implicated in NAIT, showed a leucine/proline substitution at position 33 of the integrin plexinsemaphorin.[47]

Other antigens implicated in NAIT included the platelet membrane GPs, GPIb-V-IX (von Willebrand receptor), GPIIb/IIIa, GPIa/IIa, and CD109, a glycosylphosphatidylinositol-anchored protein of uncertain function.[48] These platelet GPs and proteins interact with coagulation factors to promote hemostasis. The maternal immunization during pregnancy resulted in NAIT due to polymorphisms from 27 single amino acid substitution present in six different GPs (GPIIb, GPIIIa, GPIba,GPIbb,GP1a, and CD109).[45]

Human platelet antigens-1a contributes to NAIT in up to 85% of all cases with Caucasian and African ancestry. These figures are interesting because only 2% of women in the community are HPA-1a negative and are at risk to develop antibodies against HPA-1a.[34] Most (90%) women who express class II histocompatibility antigen DRB3*0101 produce antibodies against HPA-1a.[46,49]

Other HPAs

In the Caucasian population, nearly 95% of serologically confirmed cases of NAIT are rooted in alloimmunization against only a few antigen systems (HPA-1,- 2, -3, -5, and -15).[50] In a few cases of apparent NAIT, the maternal antibodies for these antigens were not detected and other mutations were identified. Human platelet antigens-9b has been found to be the most immunogenic, and has been detected in about 1 in 400 normal individuals and is located close to the HPA-3 antigenic site in the calf-2 domain of GPIIb.[51] Human platelet antigens-4b, HPA-6b, and HPA-21b are significantly more prevalent in Asians than in Caucasians.[51] However, the maternal alloimmunization against less frequently seen antigens contribute only a very small fraction of NAIT cases.[52]

The ABO Antigens

Platelets normally express the A and B antigens in very small concentrations.[53] One study showed that the platelets from only about 5% of normal subjects test positive for A and B blood groups. However, some mothers may express high levels of the antigens A1 and B on platelets and may be at higher risk of thrombocytopenia.

Glycoprotein IV (CD36, Nak)

Nearly 5% of infants with African and Asian ancestry seem to have lost the expression of CD36 and are at risk to undergo alloimmunization. Originally, the findings were considered to be specific for an alloantigen named Nak, but subsequent studies showed these antibodies to recognize multiple other epitopes on CD36.[43,54]

Human Leukocyte Antigens

Human leukocyte antigens antibodies account for up to a third of all cases of NAIT. Human platelets express at least 20,000 copies of class I HLA antigens, and contribute to a majority of the HLA antigens present in circulating blood.[55] Anti-HLA antibodies have been documented in nearly 31% of all pregnant women, particularly those who are multiparous.[55] However, very interestingly, the number of infants with NAIT due to these antibodies is much smaller.

Neonates born to mothers sensitized to class I HLA typically have normal platelet counts at birth. The association between the antibody concentrations and platelet concentrations has not been consistent.[55] However, some studies suggest that anti-HLA antibodies developed by the mother may cause NAIT.[56] Further studies are required to determine the impact of antibody titers, specificity, and potency of HPA and HLA antibodies.[56]

Sasaki et al.[57] reporteda neonate with NAIT caused by maternal anti-HLA A24 and B52. Treatment with platelet transfusions was ineffective because of the presence of maternal anti-B61 antibody. In another study, a high prevalence of anti-HLA antibodies was seen in mothers carrying low birth weight infants, who were thrombocytopenic.[57] The incidence of NAIT in these infants was higher than those born at term.

Antenatal Screening

Neonatal alloimmune thrombocytopenia can be associated with intracranial hemorrhages in fetuses *in utero*. About 40 in 100,000 pregnancies can present with fetal-onset NAIT, with severe bleeding episodes in about three to four of these cases.[58] Most of these bleeds seem to before 36 weeks of gestation. Hence, antenatal screening is justified in pregnancies following one with documented NAIT.

To design and implement an appropriate screening program for NAIT, resources are needed to identify women at a risk for fetal-onset NAIT.[59] We need both experienced personnel and access to cost-effective, continuously-available laboratory protocols. These antenatal screening programs need to include both HLA typing and HPA detection in at-risk pregnancies.[60]

Laboratory Diagnosis of NAIT

When thrombocytopenia is detected in a newborn, a CBC should be obtained to ascertain whether thrombocytopenia is isolated or is a part of pancytopenia syndrome. Maternal blood counts should be obtained to refute the possibility of autoimmune thrombocytopenia. These should be followed by platelet serological tests on parental blood to confirm NAIT. The diagnostic testing for NAIT has the following two objectives: (a) To determine the incompatibility between the maternal and fetal platelet antigenic profile and (b) The detection of alloantibodies in the mother's serum. Based on the results, the risk to the neonate can be projected.[24]

The assays for detecting antigen are performed on parents' blood, and if an incompatibility is detected, serum samples from the mother are tested to identify antibodies against any antigen(s) that may be detectable on the father's platelets. If there are differences in parental genotypes and there are specific antibodies in the mother's serum against the putative antigen, the diagnosis of NAIT needs consideration. The certainty of NAIT as a diagnosis is higher when an alloantibody against specific paternal antigen(s) identified on neonatal platelets is detectable in the maternal serum.[34,43] The antiplatelet antibodies in maternal serum can be detected by a variety of tests including the platelet suspension immunofluorescence test (PSIFT), monoclonal antibody immobilization of platelet antigens (MAIPA), radioimmunoprecipitation (RIA), and flow cytometry-based assays. These tests are briefly described below:

Platelet Suspension Immunofluorescence Test (PSIFT)

The intact platelets are incubated with the patient's or the control serum and allowed to bind to the antigenic epitopes. Then, fluorescence-labeled anti-human IgG/IgM are added as the secondary antibody and allowed to bind to the antibody bound to the antigenic epitope. The fluorescence-labeled platelets are then analyzed by fluorescence microscopy or by flow-cytometry.[61]

Flow-cytometry is highly sensitive to detect antibodies against most HPAs except for those against HPA-5 and HPA-15.[62] These two antigens are expressed in lower densities on the platelet surface; only about 3,000–5,000 HPA-5 antigenic sites and only 1,000 HPA-15 sites are expressed on platelets. The binding assays can be confounded due to the simultaneous presence of multiple antibodies, particularly those against the HPA and HLA. To remove reactivity against anti-HLA antibodies, platelets can be pre-treated with chloroquine or acid to destroy the platelet surface β2 microglobulin. However, it might be difficult to completely eliminate this cross-reactivity if the anti-HLA antibodies are present in high titers. To reduce the confounding effect of anti-A and anti-B antibodies, we use blood group O platelets for these assays.

Antigen Capture Assays

There are three types of antigen capture assay [antigen capture Elisa (ACE)]; the ACE, the modified ACE (MACE), and the MAIPA.[63] These methods differ in the way the GP antigens are captured. The MAIPA is widely used in Europe and other countries, whereas MACE is preferred in the USA.

(a) Antigen capture Elisa assay: Platelet lysates containing membrane GPs are placed in the wells of a microtiter plate coated with GPs-specific antibodies, which capture specific GPs. The well is then washed and incubated with the antiplatelet antibody. The antiplatelet antibody bound to the GP is detected by the addition of a peroxidase-labeled anti-human IgG, followed by an appropriate substrate.[64]

(b) Modified antigen capture Elisa assay: Platelets are incubated with antiplatelet antibodies, and then lysed. The complex consisting of GPs/antiplatelet antibody is added to the well of a microtiter plate coated with specific monoclonal antibody that capture the complex, and the captured complex is detected by the addition first of a peroxidase-labeled anti-human IgG and then an appropriate substrate.[64]

(c) Monoclonal antibody-specific immobilization of platelet antigen (MAIPA): Platelets are exposed to antibodies that can recognize *specific* target GPs, and the lysates are then placed in a microtiter plate coated with capturing antibodies. The antibody complexes can be measured using color- or fluorescence-generating laboratory methods. The antigen capture methods allow the discrimination of HPA and HLA antibodies.[63] It is important to know the strengths/weaknesses of the assays because some antibodies such as those against the Naka antigens may compete with others and may give erroneous results.

Brighton et al.[65] used MAIPA to examine the specificity of antiplatelet antibodies in patients with immune thrombocytopenia. They used direct methods in 40 patients and indirect in 23. The patients with direct positivity showed a trend, which was statistically not significant, toward more antibodies against GPIIb/IIIa. The direct-positive patients showed antibodies against anti-GPIIb/IIIa in 19 (48%), anti-GPIb/IX (21%), and to both in 16 (40%). Those with indirect positivity had anti-GPIIb/IIIa in 7 (30%), anti-GPIb/IX in 7 (30%), and against both in 9 (40%).

Radioimmunoprecipitation (RIP)

Radioimmunoprecipitation is more sensitive than MAIPA.[66] It utilizes unbound radioisotopes such as Iodine^{125} for tagging surface GPs on platelets. These immunoprecipitated GPs are captured on a solid phase such as protein agarose, where these are recognized by maternal alloantibodies. The immunoprecipitated GPs are first eluted, and then identified using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by autoradiography. These proteins are identified based on characteristics such as molecular weight. More recently, several sensitive modifications of the RIP using non-fluorescent labeling have also been developed (Fig. 3).[67]

In 2019, Vrbensky et al.[68] evaluated direct and indirect antiplatelet antibody tests for the diagnosis of immune thrombocytopenia (ITP). They concluded that the overall sensitivity of antiplatelet antibody testing was low (53%), but its specificity was high (>90%).

Newer Laboratory Tests

Bead-based Technologies

Recently, many different bead-based high-throughput techniques have been developed. Considering the relatively higher frequency of alloimmunization against HPA-1a, many of the first bead-based assays have focused on these antibodies. The bead-based technologies have been used for multiplex testing, which has lowered the cost of testing and increased efficiency.

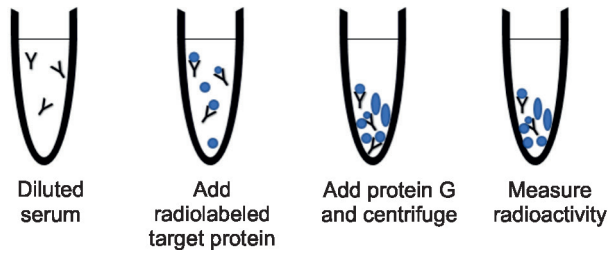

Fig. 3: Schematic representation of radioimmunoprecipitation

Immune-complex Capture Fluorescence Analysis (ICFA)

Immune-complex capture fluorescence analysis is a methodology based on antigen capture methods combined with fluorescence measurements.[35] The platelets are first exposed to the patient's serum, which might contain specific antibodies. Then, a small aliquot of the lysate is tested for detection of antibodies against HPA and HLA. The data in this article show that the assays can be used with confidence to detect antibodies against HPA-1a, -2b, -3a, -3b, -4a, -4b, -5a, -5b, -6b, and the Naka antigens. Anti-HPA-15 antibodies have not been tested extensively. These tests are based on antigen-capture methods and can be false-negative below certain diagnostic thresholds.

Fluorescent Bead-based Platelet Antibody Detection Methods

These assays have been developed using fluorescence beads for the detection of antiplatelet antibodies.[69] Currently available assays can detect antibodies against HPA-1a, -1b, -2a, -2b, -3a, -3b, -4a, -4b, -5a, -5b, and the Naka antigens, but not the anti-HPA-15a and -15b antibodies.[57] These tests show high sensitivity and are relatively easy to establish. The training of personnel is relatively simple, HPA-type platelets are not required, and only small amounts of sera are needed. Anti-HPA-15 antibodies can be clinically significant in NAIT, and therefore, specific assays are needed.[43] In addition, the tests are less-sensitive for antibodies such as anti-HPA-3a. In those cases, the methods such as the PIFT and MAIPA using appropriate monoclonal antibodies are needed.[57] In addition, low titers and low-avidity antibodies may be missed.

Assays for Platelet Genotyping

Platelet genotyping requires whole blood samples from both the mother and father. For antibody screening, maternal serum is used.[41,70] The genotypic analysis is done by PCR techniques, and antibody screening can be performed using MAIPA or RIP.[71] However, Elisa can be used for well-characterized antigens such as HPA-1a.[72] Amniocytes obtained by amniocentesis may be useful for confirming the genotype of fetal platelets.[58] When the status of the father is uncertain or the father is heterozygous, amniocentesis becomes important. Amniocytes can be grown in culture to obtain sufficient DNA needed for PCR analysis. Fetal and maternal DNA can be differentiated by using the variable number tandem repeat analysis (VNTR).[41]

In reference laboratories, several high-throughput methods are used for platelet genotyping, including sequence-specific primer-polymerase chain reaction (SSP-PCR), PCR-restriction fragment length polymorphism (PCR-RFLP), and TaqMan real-time PCR.[73]

(a) Sequence-specific primer-polymerase chain reaction: This is an allele-specific PCR that uses two reactions, using two sets of primers; one is specific for each allele and the second control primer used to monitor the efficiency of PCR.[74,75] When there is 3'-terminal nucleotide mismatch between the allele-specific primer and the target DNA, there may be some loss of efficiency of Taq polymerase in DNA amplification and this forms the basis of SSP-PCR. The HPA profile is identified by the presence or absence of DNA bands that appear after gel electrophoresis of the products obtained by PCR.[76,77] Sequence-specific primer-polymerase chain reaction is relatively simple and cost-effective for genotyping of HPA.

(b) Polymerase chain reaction-restriction fragment length polymorphism: The loss or gain of recognition sites of the restriction enzyme, which is essentially present at the polymorphic site in the target gene, constitutes the basis of PCR-RFLP. There is amplification of the gene that encodes the polymorphism followed by digestion with specific restriction enzyme. The fragments formed after the digestion are then separated according to their lengths by gel electrophoresis. After the separation according to their length, there is visualization of DNA using UV transilluminator, followed by fragment pattern interpretation. The PCR-RFLP is also simple and cost-effective, but requires an extra step of digestion which cannot be automated. One of the disadvantages of PCR-RFLP is the requirement of controlled reaction parameters for the activity of the restriction enzyme in order to avoid incomplete digestion and false results.[74]

(c) TaqMan real-time PCR: This molecular technique carries out the quantitative PCR amplification of the target gene in real time. This assay uses a sequence-specific primer (probe), that binds the SNP of interest and carries a reporting fluorophore attached to the 5′-end. The 3′-end of the probe is the quencher. The probe binds the DNA, and the extension is done by Taq polymerase. The 5′-nuclease activity of Taq polymerase displaces the fluorophore from the 5′-end of the probe, when it extends the SSP in the 5′-3′-direction, which will cause the reporter dye to cause florescence, leading to quantification of the amount of the PCR product.[77] This is an automated process and can differentiate between the homozygosity and heterozygosity in biallelic HPA systems using allele-specific probes with different reporter dyes.[74,77]

(d) High-throughput methods: The development of rapid high-throughput methods allows the amplification or multiplexing of multiple targets in a single assay, which can be used for screening of pregnant women for HPAs.[60] Because of this automation, there is a decreased risk of human error in both technical aspects and interpretation. However, these high-throughput methods require the use of expensive computer software programs and reagents.

Many bead arrays have been developed; these are useful, multiplex high-throughput methods that can be used for HPA typing. Multiple beads can be used simultaneously, each targeting a different SNP. The assays utilize allele-specific probes attached to beads tagged with florescent dyes. The target fragments of the DNA then anneal to the probes which are elongated using fluorescent labeled nucleotides. The beads are fixed to a chip or flow cytometry, where the fluorescence patterns are analyzed.[78,79]

(e) Multiplex SNP genotyping: Another high-throughput method is based on the multiplex SNP genotyping using oligonucleotide extension. This method was first used to carryout genotyping of HPA profile of platelet pheresis donors by Shehata et al.[80] In this assay, primers for multiplex PCR are designed to flank the SNP of HPA, and fragments amplified in the PCR anneal to probes with single base extensions. These probes are hybrid oligonucleotide in which one part is attached to the target that it amplifies and is in immediate proximity to the SNP of interest, and the other part, the tag portion, immobilizes the attached complex to a chip for fluorescence and laser activation.

Identification of HPA systems through high-throughput methods is valuable for blood centers in order to screen the platelet donors.[81] These methods have allowed identification of antigen-negative donors and enabled specific transfusions if needed. Human platelet antigen genotyping also has several other advantages over serological methods. First, genotyping methods do not require fresh platelets, and genomic DNA can be procured from various sources such as leukocytes, amniocytes, and buccal smears. Second, low frequency HPA can be used when serum is not available for typing. Finally, genotyping methods are mostly automated and have lower risks of error and need less time to perform the assays. However, the diagnosis of NAIT is still dependent screening of the maternal serum for antibodies, and subsequent incompatibility testing between the parents for HPA antigen likely to cause alloimmunization and platelet genotyping for HPA typing is considered to a gold standard for investigation NAIT.[76,77]

We still confront many limitations in platelet genotyping.[82] Platelet genotyping requires prior isolation of DNA of high quality and quantity, and no contamination.[75] Differences have also been reported between the genotype and the phenotype of the HPAs including HPA-1.[83,84] Primer annealing can be also be affected due to the presence of polymorphisms near SNPs in the gene of interest, which can sometimes lead to erroneous results.[62,74]

Treatment Options and Transfusion Practices for NAIT

The treatment of choices for full-term neonates with suspected NAIT, with and without bleeding includes intravenous immunoglobulins (IVIG), corticosteroids, and antigen-negative or irradiated maternal platelets as emergency supportive measures.[30,85,86]

Clinicians usually do not have continuous 24-hour access to maternal HPA-1a negative platelets.[87] Therefore, most physicians choose IVIG at a dose of 1 gm/kg weight for 2 consecutive days to the neonates who had no signs of bleeding but with platelet counts below a pre-decided threshold.[88] Small doses of corticosteroids may help by improving capillary fragility.[30] We will soon describe the current norms and preferences for treatment of NAIT in another review.

Summary

Neonatal alloimmune thrombocytopenia is an important cause of severe neonatal thrombocytopenia. The clinical presentation may range from incidental, isolated abnormalities in laboratory tests to major clinical hemorrhages with life-threatening sequalae. If the platelet counts are less than 150×10^9/L with no obvious cause to explain the thrombocytopenia, NAIT should be considered in the differential diagnosis. Although a large number of antigen systems have been clearly associated with NAIT, there are still many infants who have with suggestive clinical/laboratory profiles but unclear molecular diagnosis. These infants may have still-unidentified low-frequency and rare antigens.

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