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# Neonatal alloimmune thrombocytopenia: pathogenesis, diagnosis and management

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## Summary

Neonatal alloimmune thrombocytopenia, (NAIT) is caused by maternal antibodies raised against alloantigens carried on fetal platelets. Although many cases are mild, NAIT is a significant cause of morbidity and mortality in newborns and is the most common cause of intracranial haemorrhage in full-term infants. In this report, we review the pathogenesis, clinical presentation, laboratory diagnosis and prenatal and post-natal management of NAIT and highlight areas of controversy that deserve the attention of clinical and laboratory investigators.

### Keywords

neonatal thrombocytopenia; platelet antigens; alloimmune thrombocytopenia

In 1953, two infants born with severe thrombocytopenia to mothers with normal platelet counts were described (Harrington *et al*, 1953). Despite severe bleeding and other complications, recovery occurred after 2 and 8 weeks, respectively. Although definitive serological studies were not feasible at the time, this appears to be the first formal report describing the condition now designated neonatal alloimmune thrombocytopenia (NAIT). Shulman *et al* (1962) first implicated a maternal antibody raised against a defined platelet alloantigen as the cause of platelet destruction in an infant with this condition. In two of Shulman's cases, placental transfer of maternal antibodies specific for a platelet alloantigen designated $Pl^{A1}$ was the cause of platelet destruction in the newborn. $Pl^{A1}$ was later found to be identical to an antigen designated $Zw^{a}$ by Dutch workers (Van Loghem *et al*, 1959) and is now known as 'HPA-1a' (human platelet antigen 1a). In subsequent years, numerous other platelet-specific antigens were shown to be capable of inducing maternal immunization during pregnancy and causing fetal platelet destruction. NAIT is now recognized as an important complication of pregnancy that can present difficult diagnostic



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**Authorship contributions**

J Peterson, J McFarland, BR Curtis and R Aster have each for many years been engaged in the study of NAIT. Dr. McFarland was the primary author of sections dealing with incidence, clinical presentation and management. Dr Curtis (Director of the Platelet and Neutrophil Immunology Laboratory of BloodCenter of Wisconsin) edited the section dealing with laboratory evaluation of NAIT. Dr Peterson wrote sections concerning HPA antigen systems and edited all sections of the paper. Dr Aster provided oversight and edits to all sections of the paper.

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The authors have no disclaimers to make or conflicts to disclose.

and therapeutic challenges. In this review, we will consider NAIT pathogenesis, diagnosis and management and will highlight areas of controversy that deserve the attention of clinical and laboratory investigators.

## Incidence of NAIT

Several large prospective studies of women negative for HPA-1a, the most common trigger for antibodies causing NAIT, showed that between one in 1000 and one in 2000 HPA-1a-positive infants had neonatal thrombocytopenia caused by maternal antibodies (Blanchette *et al*, 1990; Williamson, 1998; Kjeldsen-Kragh *et al*, 2007). The incidence of the HPA-1a-negative (HPA-1bb) phenotype in Caucasian populations is about 2.5%. One third of these individuals are positive for the HLA-DR antigen B3*0101 and are at high risk to become immunized against HPA-1a when they carry an HPA-1a positive fetus (L'Abbe *et al*, 1992; Williamson, 1998); this occurs in about 35% of cases. Of these, about one in three will deliver an HPA-1a-positive child with significant thrombocytopenia (less than $50 \times 10^9$ platelets/l). In contrast to maternal immunization against fetal red cell antigens, it is common for immunization against platelet alloantigens to occur during a first pregnancy and for a firstborn infant to be affected by NAIT. However, most instances of maternal immunization may be triggered by exposure to fetal blood at the time of delivery, setting the stage for an infant to be born subsequently with thrombocytopenia (Stuge *et al*, 2011).

## Clinical manifestations

NAIT is the leading cause of severe thrombocytopenia in the fetus and neonate (Sainio *et al*, 2000), can produce serious bleeding, intracranial haemorrhage and death (Pearson *et al*, 1964; Mueller-Eckhardt *et al*, 1989b; Bonacossa & Jocelyn, 1996), and is the leading cause of intracranial haemorrhage in full term infants (Bussel, 2009). A severely affected infant will present with florid petechial haemorrhages and purpura and a profoundly low platelet count. Typically, no other explanation for thrombocytopenia is discovered after evaluation for bacterial and viral infection, disseminated intravascular coagulation and other congenital conditions associated with thrombocytopenia. A similar, but usually less serious condition can be caused by passive transfer of platelet autoantibodies; in such cases the mother will usually have a low platelet count and/or a history of autoimmune thrombocytopenia.

Prospective studies (Blanchette *et al*, 1990; Williamson, 1998; Kjeldsen-Kragh *et al*, 2007) have shown that the degree of thrombocytopenia in neonates at risk for NAIT (mother immunized against HPA-1a) can be quite variable. A recent report that 'severe' thrombocytopenia is more than twice as common in infants born to a group A mother than in those born to a mother whose blood group is O (Ahlen *et al*, 2012) requires confirmation. An infant born to a mother who previously gave birth to an infant with NAIT tends to have more severe disease than its older sibling (Fenichel *et al*, 1984; Bussel *et al*, 1997a; Bussel & Sola-Visner, 2009).

The most serious complication of NAIT is intracranial haemorrhage, which occurs in 10–20% of symptomatic infants (Mueller-Eckhardt *et al*, 1989b; Bussel *et al*, 1991, 2005; Kaplan *et al*, 1991). Up to 80% of these bleeds occur prenatally (Spencer & Burrows, 2001). After delivery, the greatest risk of bleeding is in the first 96 h of life (Mueller-Eckhardt *et al*, 1989b; Bussel *et al*, 1991, 2005; Kaplan *et al*, 1991; Spencer & Burrows, 2001). Untreated, the thrombocytopenia in the neonate typically resolves within the first two weeks of life. For reasons not well understood, a low count sometimes persists for longer periods of time, occasionally for several months.

## Antigens implicated in NAIT

### Platelet-specific (HPA) antigens

Antigens capable of triggering NAIT are carried on platelet membrane glycoproteins (GPs) GPIb-V-IX (von Willebrand receptor), GPIIb/IIIa (αIIb/β3 integrin, fibrinogen receptor) GPIa/IIa (a collagen receptor) and CD109, a glycosylphosphatidylinositol (GPI)-anchored protein of uncertain function. Together, these platelet GPs interact with proteins of the extracellular matrix and coagulation factors to facilitate haemostasis (Clemetson, 2012). Genetic polymorphisms resulting from at least 27 single amino acid substitutions located in six different glycoproteins (GPIIb, GPIIIa, GPIbα, GPIbβ, GPIa, CD109) have been shown to cause maternal immunization during pregnancy resulting in NAIT (Fig 1). When it became apparent that platelet GPs are remarkably polymorphic, a system of Human Platelet Antigen (HPA) nomenclature was developed by international consensus under which antigen systems are designated HPA-1, HPA-2, etc., more or less in the order of their discovery (von dem Borne *et al*, 1995; Metcalfe *et al*, 2003). In each system, the more common and less common alleles are designated ‘a’ and ‘b’ respectively. An HPA database is maintained by several groups in the United Kingdom and can be reached at http://www.ebi.ac.uk/ipd/hpa/index.html.

### The HPA-1 antigen system

The first human platelet antigen (HPA) implicated in NAIT, HPA-1a (originally called $Pl^{A1}$) (Shulman *et al*, 1962), results from a leucine/proline substitution at position 33 of the PSI (plextrin-semaphorin-integrin) homology domain of the GPIIIa subunit of the GPIIb/IIIa complex (αIIb/β3 integrin) (Newman *et al*, 1989). Maternal-fetal incompatibility for HPA-1a is by far the most common cause of NAIT in families of Caucasian and African ancestry, accounting for about 85% of cases in which an HPA-specific antibody is identified (Davoren *et al*, 2004; McQuilten *et al*, 2011) despite the fact that only 2% of women are HPA-1a negative and at risk to make antibodies with this specificity. More than 90% of HPA-1a antibodies are made by women positive for the Class II histocompatibility antigen DRB3*0101 (DR52a), present in about one-third of the general population (L’Abbe *et al*, 1992; Braud *et al*, 1994; Williamson *et al*, 1998; Kjeldsen-Kragh *et al*, 2007). This correlation appears to be related to high affinity of a GPIIIa peptide containing Leu33 for the peptide binding groove of DRB3*0101 (Maslanka *et al*, 1996; Rayment *et al*, 2009). The history of HPA-1a, from its first discovery to characterization of its molecular basis and elucidation of its role in disease was recently reviewed (Aster & Newman, 2007).

### HPA-1a sensitization in surrogate pregnancies

Four instances have been described in which HPA-1a-positive fertilized eggs were implanted into HPA-1a-negative woman, two of whom had previously given birth to children with NAIT (Curtis *et al*, 2005). Among a total of seven infants carried by these women, four had profound thrombocytopenia at birth, two had antenatal intracranial haemorrhage and one died *in utero*. The severity of NAIT in these cases could in part be related to fact that the infants were homozygous for HPA-1a, a condition that is normally impossible when the mother is HPA-1a-negative. It was recommended that women being considered as surrogate mothers be typed routinely for HPA-1a (Curtis *et al*, 2005).

### Other HPA antigens

More than 95% of serologically confirmed NAIT cases in Caucasian families are caused by maternal immunization against antigens belonging to five antigen systems (HPA-1, -2, -3, -5, and -15) consisting of two alleles, each of which is relatively common in most populations (Table I). Studies performed in cases of apparent NAIT in which maternal

antibodies specific for one of these 'common' HPA antigens are not detected have led to the identification of mutations encoding a series of less common HPA antigens. At this writing, a total of 23 such mutations have been identified, each encoding a low frequency antigen. Some of these have been described only in a single case report. Low frequency antigens shown to be capable of triggering NAIT are listed in Table II. The most immunogenic of these appears to be HPA-9b, found in about one of 400 normal individuals and located close to the HPA-3 antigen site in the calf-2 domain of GPIIb (Fig 1). HPA-4b (Kupatawintu *et al*, 2005), HPA-6b (Tanaka *et al*, 1996) and HPA-21b (Peterson *et al*, 2012b) are significantly more common in Asian than in Caucasian populations. The number of identified low frequency HPA antigens is likely to increase in the future. On average, however, maternal sensitization against this group of antigens probably accounts for only a small fraction of all NAIT cases (Ghevaert *et al*, 2009).

### ABO antigens

It has been known for many years that platelets normally express small quantities of A and B antigens on their surface (Moreaux & Andre, 1954). Ogasawara *et al* (1993) first showed that about 5% of normal subjects positive for blood groups A or B possess platelets that carry unusually large numbers of A and B antigen sites and showed that such platelets survive poorly when transfused to an ABO incompatible recipient. Curtis *et al* (2000) confirmed these findings and showed that in a subset of individuals ('Type 2 high-expressers'), platelet A1 and B antigen levels are extremely high, ranging up to 20 000 antigen sites per platelet. These findings raised the possibility that some infants possessing the Type 2 high expresser trait could be at risk for thrombocytopenia if born to an ABO incompatible mother. This prediction was borne out in a study of a family in which two infants who were Type 2 high expressers of blood group B were born with thrombocytopenia (one requiring platelet transfusions) and low grade ABO haemolytic disease to a group O mother whose serum contained high titre IgG anti-B (Curtis *et al*, 2008). The platelet-reactive antibody in maternal serum was completely absorbed with group B but not group O red cells. A third infant whose blood type was $A_2$ was borne to the same mother with a normal platelet count. These findings indicate that NAIT can be caused by maternal IgG anti-B (and presumably anti-A) antibodies under some circumstances. Typing of a father's platelets for the high expresser trait can be helpful in some cases of apparent NAIT not resolved on the basis of HPA incompatibility.

### Glycoprotein IV (CD36, Nak)

Glycoprotein IV (CD36) is a member of the class B scavenger receptor family of proteins expressed on platelets, red cells, endothelial cells and other tissues (Silverstein & Febbraio, 2009). About 5% of persons of African or Asian ancestry have inherited mutations that lead to failure of CD36 expression (Type 2 CD36 deficiency) and are at risk to become immunized if exposed to the protein by transfusion or pregnancy (Curtis *et al*, 2002). The resulting antibodies were originally considered to be specific for an alloantigen designated 'Nak' (Ikeda *et al*, 1989). With the recognition that the antibodies probably recognize multiple epitopes on the target protein, it is more appropriate to designate them as isoantibodies having CD36 specificity. The clinical picture of NAIT associated with maternal immunization against CD36 is similar to that seen in infants affected by HPA-specific antibodies (Curtis *et al*, 2002). It is of particular interest that affected infants can have severe thrombocytopenia without evidence of other tissue damage despite the fact that CD36 is expressed on endothelium and other blood cells (Curtis *et al*, 2002). NAIT caused by anti-CD36 antibodies has not been reported in persons of Caucasian ancestry, presumably because of the rarity of the CD36-negative phenotype in that group.

### Human leucocyte antigen (HLA) antigens

Human platelets carry at least 20 000 copies of class I HLA antigens (Janson *et al*, 1986; Pereira *et al*, 1988) and account for the majority of HLA antigens present in circulating blood (Pereira *et al*, 1988). Given that about one-third of multiparous women are sensitized to Class I HLA (King *et al*, 1996), the possibility exists that class I HLA antibodies could cause NAIT in some newborns. However, women sensitized to Class I HLA antigens routinely give birth to infants with normal platelet counts and at least one formal study failed to show any correlation between neonatal platelet counts and the presence or absence of such antibodies in the mother (King *et al*, 1996). Nonetheless, the literature contains many anecdotal reports of infants born with apparent NAIT possibly caused by maternal anti-HLA (Saito *et al*, 2003; Moncharmont *et al*, 2004; Thude *et al*, 2006). Accordingly, whether Class I HLA antibodies cause some cases of NAIT not accounted for by maternal-fetal HPA incompatibility is presently unresolved. Maternal HLA antibodies vary greatly in potency; the possibility that high titre maternal antibodies or those specific for HLA antigens that are strongly expressed on platelets (Szatkowski *et al*, 1978; Szatkowski & Aster, 1980) can cause neonatal thrombocytopenia or contribute to its severity deserves investigation.

## Laboratory diagnosis of NAIT

As noted, sensitization to fetal platelet antigens can occur during a first pregnancy. Norwegian investigators typed women pregnant for the first time for HPA-1a and screened those found to be negative for HPA-1a antibody at various times during gestation in an attempt to identify infants at risk for NAIT (Kjeldsen-Kragh *et al*, 2007; Killie *et al*, 2008; Skogen *et al*, 2010). Some have argued that it may be cost-effective to perform such screening routinely and offer special case management to the 10% of HPA-1a-negative women who produce antibody (Husebekk *et al*, 2009) but at the present time this is not practiced in the absence of a family history of NAIT, e.g., in a sister. Therefore, the first suspicion of NAIT usually arises when a newborn exhibits petechial haemorrhages, echymoses or other bleeding symptoms. As time is required for serological studies to be performed, the initial diagnosis is made and treatment is started on the basis of the platelet count and other clinical findings. Even in mildly affected infants, however, serological investigation is indicated because results can be critical for effective management of future pregnancies. Proper laboratory diagnosis of NAIT requires sophisticated testing, a thorough understanding of platelet serology and, often, personal communication between the testing laboratory and the attending physician and should be undertaken only by laboratories that possess these capabilities. For the most informative evaluation, it is important to study blood samples from both mother and father.

Here, we will describe the approach used by the Platelet and Neutrophil Immunology Laboratory of BloodCenter of Wisconsin to investigate newly referred cases of suspected NAIT. Approaches used by other laboratories may differ in some details but are likely to be generally similar. Readers interested in a more detailed description of antibody detection and platelet typing methods can refer to a recently published review (Curtis & McFarland, 2009).

### Serological studies

Flow cytometry using secondary probes specific for IgG and IgM immunoglobulin isotypes (Curtis & McFarland, 2009) provides a rapid and sensitive means of detecting platelet-reactive antibodies and is used to test maternal serum against washed paternal and maternal platelets and a small panel of platelets from normal group O donors typed for selected common HPA antigens. A screen for class I HLA antibodies is also performed and paternal and maternal red cells are typed for ABO. The glycoprotein for which maternal antibody is

specific can often be identified by performing one or more solid phase assays. In one approach, designated MACE (modified antigen capture enzyme-linked immunosorbent assay [ELISA]), target platelets are incubated with maternal serum, washed and lysed with a detergent, such as triton X-100. The glycoprotein of interest is then captured on a solid surface to which a monoclonal antibody specific for it has been fixed. After washing, maternal antibody bound to the captured GP is detected by ELISA (Curtis & McFarland, 2009). MACE is used routinely to detect maternal antibodies reactive with HPA antigens carried on GPIIb/IIIa and GPIa/IIa using paternal platelets and a small platelet panel as targets. In addition, an antigen capture ELISA (ACE) assay is used to screen maternal serum for antibodies against GPIb/IX, the carrier for HPA-2a/b, CD36 (GPIV) and class I HLA. In selected cases, MACE is used to detect HPA antibodies reactive with HPA-2a/b. A slightly different approach, designated monoclonal antibody immobilization of platelet antigens (MAIPA) is widely used in Europe (Kaplan *et al*, 2007). MACE and MAIPA are probably equivalent in sensitivity and specificity. Other solid phase assays suitable for HPA antibody detection are reviewed by Curtis and McFarland (2009). Detection of maternal antibodies specific for HPA-15a and -15b requires MAIPA using fresh target platelets because the carrier protein, CD109, is only weakly expressed (about 1000 copies per platelet) and is relatively labile (Ertel *et al*, 2005; Maslanka *et al*, 2012).

## Platelet typing

Development of DNA-based methods has made serological typing for HPA antigens obsolete. The nucleotide changes that encode individual antigens can be rapidly and accurately identified with allelic discrimination assays using 5′ fluorescently-labelled hydrolysis probes that anneal to specific alleles (Ruan *et al*, 2007). A quencher that inhibits fluorescence is attached to the 3′ end of the probe. When specific probe annealing and extension occurs, the quencher is removed and the resulting fluorescence reports the presence of the targeted allele (Arinsburg *et al*, 2012). Other polymerase chain reaction (PCR)-based techniques, such as those that use PCR-sequence-specific primer amplification (Skogen *et al*, 1994; Curtis, 2008) followed by electrophoresis and visualization of DNA bands, are used by some laboratories. Our practice is to type paternal and maternal DNA routinely for antigens of the HPA-1 through 6, -9 and -15 systems.

## Interpretation of test results

A reaction of maternal IgG with paternal but not maternal platelets and a negative screening result for class I HLA antibodies suggests that an HPA antibody may be present. Reaction of maternal serum with paternal platelets but not with any member of the normal panel suggests that the antibody could be specific for a low frequency HPA antigen. However, if the father is incompatible with mother's serum for blood group A1 or B, this reaction can be due to a blood group antibody. If the reaction is strong, paternal platelets are tested with monoclonal antibodies specific for blood groups A or B and the strength of this reaction is compared with that of 'normal' group A1 or B platelets to identify fathers who are Type 2 high expressers of A1 or B (Curtis *et al*, 2008). If a high expresser state is identified, maternal serum is absorbed with washed group A1 or B red cells. If the reaction with paternal platelets is abolished, it is assumed to be a result of ABO incompatibility.

In 20–35% of cases, an antibody specific for an HPA antigen present in the father but not the mother is found in maternal serum (Mueller-Eckhardt *et al*, 1989b; Davoren *et al*, 2004; Ghevaert *et al*, 2007; McQuilten *et al*, 2011). HPA-1a is targeted in 75–90% of these 'resolved' cases, HPA-5b in 8–15%, HPA-1b in 1–4% HPA-3a in 1–2% and HPA-5a in about 1% (McQuilten *et al*, 2011). In a recent study, antibodies specific for HPA-15b were found in 8 of 200 cases (4%) (Ghevaert *et al*, 2007). Occasionally, two HPA antibodies are present in the same mother (Davoren *et al*, 2004; McQuilten *et al*, 2011). HPA-4b, -6b and

-21b in mothers of Asian descent and GPIV (CD36) in African American women are targeted much more often than in Caucasian women.

As noted, reaction of a maternal antibody only with paternal platelets in flow cytometry that is not accounted for by ABO incompatibility suggests maternal immunization against a low frequency HPA antigen (Table II). In such cases, identification of the carrier protein in a solid phase assay using paternal platelets as targets can be helpful. Given that panel cells carrying low frequency HPA antigens are not readily available, sequencing of relevant exons encoding known low frequency HPA antigens in paternal DNA may be required to identify the probable sensitizing antigen. Transfected cell lines expressing low frequency antigens (Kroll *et al*, 2003) or recombinant glycoprotein fragments engineered to carry relevant epitopes (Stafford *et al*, 2008a,b) that can be used to identify such antibodies may become available in the future. Although many low frequency antigens have been described (Table II), sensitization to these markers probably accounts for no more than a few percent of all cases of NAIT (Ghevaert *et al*, 2009).

## Unresolved cases: possible explanations

As noted, about two-thirds of suspected NAIT cases referred for study are not resolved on the basis of maternal-fetal incompatibility for one of the antigens listed in Tables I and II. In some of these, neonatal thrombocytopenia is undoubtedly a consequence of one of the many non-immune conditions that can lower the platelet count in a newborn (Arnold *et al*, 2008; Chakravorty & Roberts, 2012). If the mother is thrombocytopenic or has a history of autoimmune thrombocytopenia (ITP), maternal autoantibodies not readily detected in laboratory assays may be responsible.

In the remaining cases, a cause of thrombocytopenia other than NAIT is usually not identified. In many such instances, class I HLA antibodies, sometimes very high titre, are present in the mother. As already noted, anecdotal reports claim that HLA antibodies can cause NAIT. In fact, the very first report of NAIT caused by HPA-1a immunization described two additional cases associated with maternal immunization against a newly identified antigen designated '$Pl^{B1}$', which was later found to be the class I HLA antigen HLA-A2 (Shulman *et al*, 1962). Although there is no consensus as to whether class I HLA antibodies can cause NAIT, further studies to define whether such antibodies cause the condition or contribute to its severity are needed.

Antibodies specific for HPA-3a and HPA-3b can be extremely difficult to detect in standard serological assays for reasons not yet fully understood (Lin *et al*, 1995; Harrison *et al*, 2003; Socher *et al*, 2008). Zhu *et al* (2008) recently found that the region of the GPIIb calf-2 domain where these antigens are located is not resolved in the crystal structure of the GPIIb/IIIa ectodomain, suggesting that this region is not rigidly constrained. The resulting lability of the antigen structure could explain difficulties encountered in serological assays. The low frequency antigens HPA-9b and HPA-27b are located in calf-2 very close to HPA-3a/b and antibodies specific for these markers can also be difficult to detect (Kaplan *et al*, 2005; Peterson *et al*, 2005; Jallu *et al*, 2012), perhaps for the same reason. Maternal-fetal incompatibility for HPA-3a and -3b is common; perhaps improved assays for HPA-3 antibody detection will show that HPA-3 incompatibility causes NAIT more often than has been suspected.

Recent studies suggest yet another explanation for the failure of laboratory studies to resolve apparent cases of NAIT – that thrombocytopenia is caused by low avidity HPA antibodies not detected in standard assays that require washing of the target antigen (Socher *et al*, 2009; Bakchoul *et al*, 2011; Peterson *et al*, 2012c). Antibodies of this type can be detected using surface plasmon resonance (SPR) analysis in which a signal translated into 'resonance units'

is generated in real time as antibody (ligand) binds to immobilized antigen (GPIIb/IIIa). A typical tracing obtained with IgG from an HPA-1a negative, 'antibody-negative' mother who gave birth to an infant with NAIT is shown in Fig 2 where it can be seen that perfused antibody accumulated on the HPA-1a-positive but not the HPA-1a-negative target and then dissociated rapidly when perfusion with buffer was begun. In contrast, a 'conventional' HPA-1a antibody dissociated very slowly. Potential pathogenicity of low avidity HPA-1a antibodies can be demonstrated by showing that they cause destruction of circulating human platelets in a non-obese diabetic severe combined immunodeficiency (NOD/SCID) mouse model (Bakchoul *et al*, 2011; Peterson *et al*, 2012c). SPR analysis is technically demanding and is presently unsuitable for routine antibody detection. However, it is important that further studies be done to define the extent to which low avidity HPA antibodies cause 'antibody-negative' NAIT.

Serological confirmation of an NAIT diagnosis is particularly important in guiding the management of subsequent pregnancies, where fetal genotyping for the implicated incompatible antigen can be done if indicated. However, there are cases of 'true' NAIT for which serological confirmation is not available due to one of the scenarios mentioned above, or to the lack of an appropriately timed maternal serum sample, i.e., one obtained before antibody became detectable or after it disappeared. Such cases need to be assessed on clinical grounds alone, particularly if multiple pregnancies have been affected in a family. Although the absence of a serological diagnosis adds more uncertainty to management of a subsequent pregnancy, the clinician may well offer empiric antenatal therapy as discussed below if the clinical suspicion of NAIT is particularly high.

## Treatment

A first affected neonate with NAIT in a family is normally identified when clinical signs of bleeding are evident at or shortly after birth and a platelet count confirms isolated thrombocytopenia. The immediate treatment for very severe thrombocytopenia ($<30 \times 10^9$/l), especially if serious bleeding signs are evident (petechiae, ecchymoses, gastrointestinal, gentio-urinary or intracranial haemorrhage), is platelet transfusion (Sola-Visner *et al*, 2008). Random donor platelets appropriate for neonates (ABO compatible; volume reduced, if indicated; cytomegalovirus negative; and irradiated) will usually elevate the platelet count at least transiently and reduce the likelihood of bleeding even when they are incompatible with the maternal antibody. In addition, intravenous immunoglobulin (IVIG) at 0.4–1.0 g/kg/d for 2-5 d can be given to potentially prolong the survival of the incompatible platelets and lessen the overall period of thrombocytopenia (Mueller-Eckhardt *et al*, 1989a; Bussel, 2009; McQuilten *et al*, 2011). Compatible, HPA-1b/b platelets may be available from some blood providers who have HPA-typed donors available (Verran *et al*, 2000).

HPA-compatible platelets can also be obtained by performing platelet pheresis on the mother of the affected infant and can be helpful in unusual cases requiring transfusion support over an extended period of time. If maternal platelets are used, it is absolutely essential that they be washed to remove antibody-containing maternal plasma. Methods of plasma removal include simple volume reduction by centrifugation with resuspension in normal saline, or more exhaustive washing of platelets in saline or normal AB plasma. Maternal platelets should always undergo gamma irradiation prior to transfusion to prevent transfusion-induced graft-versus-host disease in the infant (McFarland, 2008). While it may seem obvious that maternal antibody should be removed before maternal platelets are transfused to an affected infant, we are aware of several instances in which failure to observe this precaution led to prolonged thrombocytopenia in the infant, in one case for more than two months.

Moderately severe thrombocytopenia (e.g. between 50 and 30 × $10^9$/l (Williamson *et al*, 1998) without obvious haemorrhage can be managed with IVIG treatment alone. Typically, total doses of 2 g/kg are given over 2–5 d (Mueller-Eckhardt *et al*, 1989a; Bussel, 2009).

## Management of subsequent pregnancies

As noted, NAIT tends to be more severe in infants born subsequently to a mother who previously gave birth to an infant with this condition. Accordingly, later pregnancies should be managed in consultation with physicians experienced in NAIT diagnosis and management. Several steps should be considered in such cases. One is to determine whether the infant being carried is incompatible with the maternal alloantibody previously demonstrated, whether or not it is still detectable. A second (if the infant is incompatible) is to estimate the degree of fetal thrombocytopenia so as to gauge the risk of antenatal intracranial haemorrhage. A third is to offer risk-stratified antenatal therapy to the mother to ameliorate fetal thrombocytopenia and reduce the likelihood of prenatal and postnatal bleeding. The HPA genotype of the father can be used to determine whether the infant has a 100% (father homozygous) or 50% (father heterozygous) chance of possessing the implicated antigen. If the father is homozygous all subsequent fetuses will be obligate heterozygotes and will be incompatible with the maternal antibody. If the father is heterozygous the fetus will have a 50% chance of inheriting the marker. In the latter case, fetal genotyping can be performed on amniotic fluid or chorionic villus material to determine whether the fetus is at risk (McFarland *et al*, 1991; Skogen *et al*, 1994). The former material can be typed at 18–20 weeks gestation and the latter as early as 8–10 weeks.

When a fetus has been determined to be at risk for NAIT, an estimate should be made of its likely severity. The most direct way to accomplish this is to perform a platelet count on a fetal blood sample. However, this procedure carries significant risk, particularly if the fetus happens to have a severely depressed platelet count (Paidas *et al*, 1995; Bussel, 2009). Non-invasive methods for estimating the severity of NAIT during pregnancy include testing of the mother's serum for the strength of the anti-HPA antibody and considering the severity of disease in previously affected siblings. Several investigators found that there is a roughly inverse relationship between the strength of maternal antibody measured serologically and the platelet count in the newborn (Jaegtvik *et al*, 2000; Killie *et al*, 2007, 2008; Bessos *et al*, 2009). While this approach may hold some promise, there are many examples of an infant incompatible with a very strong maternal antibody having only mild thrombocytopenia at birth and, conversely, an infant being born with severe thrombocytopenia to a mother whose antibody is quite weak. The severity of NAIT in previously affected siblings can be a helpful predictor, especially if an older sibling experienced intracranial haemorrhage (Christiaens *et al*, 1997; Bussel, 2009).

From earlier antenatal treatment trials in which serial fetal blood sampling was done prior to and after administering treatment to mothers, it is clear that, without intervention, fetuses at risk for NAIT who had previously affected older siblings usually experience a progressive drop in platelet count as pregnancy progresses (Bussel *et al*, 1997a). Beginning in 1997, trials of antenatal maternal treatment using high dose IVIG with or without corticosteroids have shown that both the degree of thrombocytopenia in the fetus and the risk of intracranial haemorrhage can be reduced by such therapy (Bussel *et al*, 1988, 1991, 1997b,b; Bussel, 2009). It is now recognized that prenatal treatment can be stratified. More intense and earlier therapy should be given if a previous untreated fetus experienced an early (i.e. prior to 28 weeks gestation) *in utero* haemorrhage (Bussel *et al*, 2010).

A treatment algorithm for stratifying risk (Table III) and customizing antenatal therapy on the basis of prior clinical trials and expert opinion has been proposed (Pacheco *et al*, 2011).

The authors recommend monitoring of women in Stratum 1 with serial testing to detect anti-HPA antibodies, including serological crossmatches with paternal platelets to detect rare specificities at 12 weeks, 24 weeks and 30 weeks gestation, and withholding antenatal therapy unless an HPA antibody is detected. (However, as noted above, if the clinical suspicion for NAIT is particularly high, empiric therapy could be considered in this group without serological confirmation.) Stratum 2 pregnancies, known to have an at-risk fetus either from paternal zygosity or fetal genotyping, are offered antenatal therapy at approximately 20 weeks gestation with IVIG (1 g/kg/week and prednisone (0.5 mg/kg/d) or IVIG at 2 g/kg/week, and therapy is increased to IVIG 2 g/kg/week plus prednisone at 32 weeks gestation without fetal blood sampling. Caesarean delivery is done electively at 37–38 weeks. Stratum 3 mothers with at-risk fetuses are offered IVIG at 1 g/kg/week at 12 weeks gestation and therapy is increased (doubling the IVIG dose or adding prednisone) at 20 weeks and again at 28 weeks (all mothers receiving IVIG 2 g/kg/week plus prednisone) with elective delivery as per Group 2. Stratum 4 mothers are given IVIG at 2 g/kg/week beginning at week 12 with prednisone added at week 20 and further acceleration of treatment as per Group 3 at week 28 with elective delivery as per Groups 2 and 3.

Based on previous clinical trials in which at risk pregnancies were classified on the basis of severity of a previously affected sibling, (Berkowitz *et al*, 2006, 2007; Bussel *et al*, 2010), this approach seeks to minimize the risk of antenatal intracranial haemorrhage while avoiding fetal blood sampling, which has been shown to have a high complication rate, especially for at- risk fetuses with extremely low platelet counts (Paidas *et al*, 1995).

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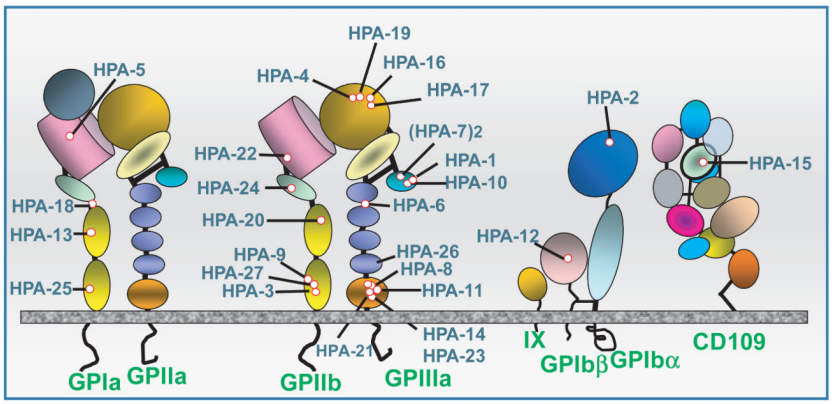


**Fig 1.**

Antigens known to trigger maternal sensitization leading to neonatal alloimmune thrombocytopenia are carried on four different platelet membrane glycoproteins (GP) and glycoprotein complexes. Structural domains identified by crystallographic studies are shown schematically. Adapted from (Peterson *et al*, 2012a).

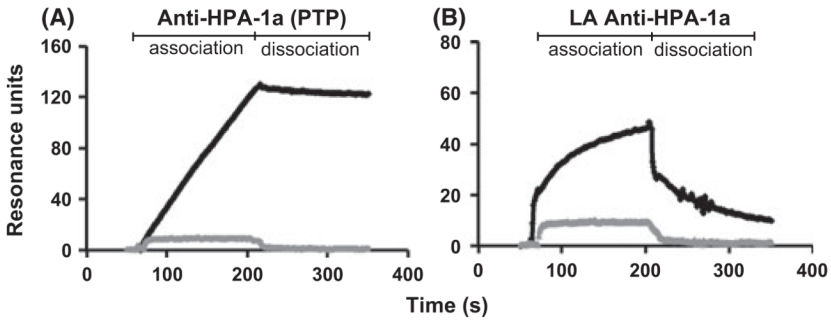


**Fig 2.**

Detection of HPA-1a antibodies by surface plasmon resonance analysis (SPR). Equal quantities of GPIIb/IIIa purified from HPA-1a-positive and HPA-1a-negative platelets were fixed to CM5 Biacore chips using standard linkage chemistry and were perfused with IgG containing HPA-1a antibodies from a patient with post-transfusion purpura (PTP) (A) and from an HPA-1a-negative woman who delivered an infant with apparent neonatal alloimmune thrombocytopenia (NAIT) but had no HPA-1a antibody detectable by conventional serological methods (B). The progressive increase in SPR signal during the first 200 s of perfusion reflects binding of IgG to HPA-1a-positive GPIIb/IIIa (dark line). IgG from a healthy individual is depicted by the grey line. During subsequent perfusion with buffer, the HPA-1a antibody from the PTP case remained associated with its target but the low avidity (LA) antibody from the NAIT case dissociated almost completely. HPA-1a antibodies from NAIT cases that could be detected by standard serology produce intermediate tracings (not shown). Adapted from (Peterson *et al*, 2012c).

**Table I**

Common human platelet antigens.

| Antigen | Alias | GP | AA | Reference |
|---|---|---|---|---|
| HPA-1a/b | $Pl^{a/b}$ | GPIIIa | L33P | Newman *et al* (1989) |
| HPA-2a/b | $Ko^{b/a}$ | GPIba | T145M | Kuijpers *et al* (1992) |
| HPA-3a/b | $Bak^{a/b}$ | GPIIb | I843S | Lyman *et al* (1990) |
| HPA-5a/b | $Br^{b/a}$ | GPIa | E505K | Santoso *et al* (1993) |
| HPA-15a/b | Zav, $Gov^{a/b}$ | CD109 | S703Y | Schuh *et al* (2002) |

GP, glycoprotein; AA, amino acid.

## Table II

Low frequency human platelet antigens.

| Antigen | Alias | GP | AA | Validated cases (*n*) | Index reference |
|---|---|---|---|---|---|
| *HPA-4a/b | (Yuk$^{a/b}$) Pen$^{a/b}$ | GPIIIa | R143Q | a:5; b:9 | Wang *et al* (1992) |
| *HPA-6b | Ca/Tu$^{a}$ | GPIIIa | R489Q | 5 | Wang *et al* (1993) |
| HPA-7b | Mo$^{a}$ | GPIIIa | P407A | 1 | Kuijpers *et al* (1993) |
| HPA-7c | Hit | GPIIIa | P407S | 1 | Koh *et al* (2010) |
| HPA-8b | Sr$^{a}$ | GPIIIa | R636C | 3 | Santoso *et al* (1994) |
| HPA-9b | Max$^{a}$ | GPIIb | V837M | 14 | Noris *et al* (1995) |
| HPA-10b | La$^{a}$ | GPIIIa | R62Q | 2 | Peyruchaud *et al* (1997) |
| HPA-11b | Gro$^{a}$ | GPIIIa | R633H | 3 | Simsek *et al* (1997) |
| HPA-12b | Iy$^{a}$ | GPIbb | G15E | 2 | Sachs *et al* (2000) |
| HPA-13b | Sit$^{a}$ | GPIa | T799M | 2 | Santoso *et al* (1999) |
| HPA-14b | Oe$^{a}$ | GPIIIa | K611del | 1 | Santoso *et al* (2002) |
| HPA-16b | Duv$^{a}$ | GPIIIa | T140I | 1 | Jallu *et al* (2002) |
| HPA-17b | Va$^{a}$ | GPIIIa | T195M | 1 | Stafford *et al* (2008b) |
| HPA-18b | Cab$^{a}$ | GPIa | Q716H | 1 | Bertrand *et al* (2009) |
| HPA-19b | Sta | GPIIIa | K137Q | 1 | Peterson *et al* (2010) |
| HPA-20b | Kno | GPIIb | T619M | 1 | Peterson *et al* (2010) |
| *HPA-21b | Nos | GPIIIa | E628K | 3 | Peterson *et al* (2010) |
| HPA-22b | She, Sey | GPIIb | K164T | 1 | Peterson *et al* (2012a) |
| HPA-23b | Hug2 | GPIIIa | R622W | 1 | Peterson *et al* (2012a) |
| HPA-24b | Cab2, In | GPIIb | S472N | 1 | Jallu *et al* (2011) |
| HPA-25b | Swi$^{a}$ | GPIa | T1087M | 1 | Kroll *et al* (2011) |
| HPA-26b | Sec$^{a}$ | GPIIIa | K580N | 1 | Sachs *et al* (2012) |
| HPA-27b | Cab3, Ak | GPIIb | L841M | 3 | Jallu *et al* (2012) |

GP, glycoprotein; AA, amino acid.

*HPA-4b, HPA-6b and HPA-21b are more common in Asian populations.

## Table III

Stratification of NAIT cases according to risk of intracranial haemorrhage *.

| Stratum | Definition | Risk |
|---|---|---|
| 1 | History of previous fetus or newborn with thrombocytopenia or intracranial haemorrhage of unknown aetiology; no HPA antibody detected. | Unknown |
| 2 | History of previous fetus or newborn with serologically confirmed fetal or neonatal alloimmune thrombocytopenia having only thrombocytopenia and no evidence of an intracranial haemorrhage. | Standard |
| 3 | History of serologically confirmed fetal or neonatal alloimmune thrombocytopenia and previous fetus or newborn with intracranial haemorrhage at 28 weeks of gestation or more (includes peripartum and neonatal intracranial haemorrhage). | High |
| 4 | History of serologically confirmed fetal or neonatal alloimmune thrombocytopenia and previous fetus with intracranial haemorrhage at less than 28 weeks. | Very High |

* Adapted from Pacheco et al, 2011.