ALLOIMMUNE THROMBOCYTOPAENIC DISORDERS: A REVIEW

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ABSTRACT

Alloimmune thrombocytopaenia (AIT) is caused by alloantibodies against specific platelet glycoproteins. Alloimmune thrombocytopaenic disorders include alloimmune neonatal thrombocytopaenia, post-transfusion purpura, refractoriness to platelet transfusions, passive AIT, and transplantation-associated AIT. In this review we have summarised five thrombocytopaenic syndromes caused by platelet-reactive alloantibodies. Increased awareness of these syndromes, together with the greater availability of highly specialised laboratory methods to detect and to characterise platelet-reactive alloantibodies, will lead to their more frequent diagnosis.

Keywords: Thrombocytopaenia, alloimmune, alloantigens.

INTRODUCTION

Thrombocytopaenia defined as a platelet count below 150,000/mm^3 is a common cause of abnormal bleeding. A low platelet count can result from decreased production or increased destruction of platelets. Decreased platelet production can result from suppression or failure of the bone marrow. Thrombocytopaenia is also caused by shortened platelet survival and this is much more common than thrombocytopaenia caused by inadequate production. Platelet destruction is most commonly immune-mediated. Platelets perform innate and adaptive immunity functions through ligand receptor interactions involving the many glycoproteins expressed on their surface membranes. It is known that 33 human platelet alloantigens (HPAs) are expressed on six different platelet glycoproteins: GPIIb, GPIIIa, GPIa, GPIb, GPIa, and CD109. Twelve antigens are clustered into six biallelic groups (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-16). These are numbered in order of their discovery. This review specifically discusses the diagnosis and management of benign alloimmune disorders of platelets.

HUMAN PLATELET ALLOANTIGENS (HPAs)

Antibody formation against alloantigens[1] of the human platelet membrane is responsible for clinical syndromes and transfusion-related conditions such as neonatal alloimmune thrombocytopaenia (NAIT), post-transfusion purpura (PTP), platelet transfusion refractoriness, and passive alloimmune thrombocytopaenia (PAIT).

PLATELET-SPECIFIC ALLOANTIGENS

Platelet-specific alloantigens (PSAs[2,3]) are antigens which are unique to the platelet membrane. These antigens cause the two well-characterised thrombocytopaenic disorders PTP and NAIT. Although not yet established as a frequent cause of refractoriness to platelet transfusions in multi-transfused thrombocytopaenic patients, the platelet-specific antigens may potentially be an important factor in refractoriness. The study of the immunochemistry of the platelet-specific antigens has been important because of their locations on functionally important platelet surface glycoproteins. They play an important role in

platelet function: they serve as receptors for the physiological stimulators thrombin, adenosine diphosphate, and collagen. Furthermore, the receptors for von Willebrand factor and fibrinogen are glycoproteins. Associated with these glycoproteins are the platelet-specific antigens (Table 1).

HPAs[4] are located in receptors in the platelet membrane and are frequently involved in alloimmunisation. The Class I human leukocyte antigens (HLAs) HPA-1, -2, -3, -4, -5, and -15 are present in the GPIIIa, GPIba, GPIIb, GPIIIa, GPIa, and CD109 glycoproteins, respectively. According to Ghevaert et al.[5] 95% of the antiplatelet antibodies are specific for HPA-1a or 5b; 5% of the cases involve allele antibodies for HPA-2, -3, and -15. To date 33 HPAs have been described and their molecular basis has been defined. 24 PSAs have been defined by immune sera, of which 12 have been grouped into 6 biallelic systems (HPA- 1, -2, -3, -4, -5, and -16). DNA-typing methods[6] based on polymerase chain reaction (PCR) restriction fragment length polymorphism or the use of allele-specific oligonucleotide hybridisation and single specific primer PCR enables rapid typing for HPA systems, which makes these techniques feasible in most clinical settings where urgent HPA typing is required.

NAIT

NAIT refers to a disorder[7] in which foetal platelets contain an antigen that the mother lacks, and is inherited from the father. These antibodies cross the placenta and bind to the foetal platelets. Clearance of the antibody coated platelets results in foetal/neonatal thrombocytopaenia, a condition that is responsible for severe life-threatening bleeding of the newborn.[8,9] Early diagnosis or suspicion of NAIT is essential for effective therapy even if the identity of the offending platelet antibody is unknown. The passively transmitted maternal antibodies can affect either a foetus or neonate, and failure to recognise this condition results in low platelet counts that can cause intracranial haemorrhage of an otherwise healthy infant either *in utero* or at birth. Major platelet antigens are fully expressed as early as the 19th week of gestation.[10] The severity of clinical symptoms of the disorder can vary from no observable indication of disease (whereby the thrombocytopaenia is discovered incidentally) to severe intracranial bleeding. Intracranial haemorrhage in NAIT has been estimated to cause neurologic impairment in 20% of affected infants and death in 10%.[11] HPA-1a is the most common platelet antigen implicated in NAIT, causing ~78% of proven cases. The other specific platelet antibodies implicated are anti-HPA-5b (~19%), and anti-HPA-2, -3, and -4 (~3%). Less common platelet antigens have also been reported to cause infrequent cases of NAIT.[12]

Although platelet-specific antigens and antibodies are known to cause NAIT, it is important to differentiate between HPA and HLA antigen-antibody reactions. HLA Class II determinants may be associated with HPA-1a alloimmunisation. Because many HPA-1a-negative women who have become sensitised to the HPA-1a antigen have HLA-B8, HLA-DR3, and DR52a antigens, it is speculated that these markers may increase the risk of alloimmunisation.[12] Routine typing to determine an HLA phenotype is not feasible because immunisation may not occur even if the markers are present, or severe alloimmunisation may occur when they are absent.[10]

Table 1: Alloimmune thrombocytopaenic syndromes (ATSs) caused by platelet-specific alloantibodies.

Classic ATSs	Platelet antigen system	Protein antigen	Antigen frequency
Neonatal alloimmune thrombocytopaenia	HPA-1a	GPIIIa	>80%
Post-transfusion purpura	HPA-4	GPIIa	>99%
Passive alloimmune thrombocytopaenia	HPA-1a	GPIIIa	>80%
Platelet transfusion refractoriness	HPA-5b HPA-1b	GPIa GPIIIa	20-90%
Transplatation-associated thrombocytopaenia	HPA-1a and HPA-5b	GPIIIa GPIa	20-99%

HPA: human platelet alloantigen; GP: glycoprotein.

NAIT should be considered when a thrombocytopaenic neonate does not respond to transfusion of random platelets or intravenous immunoglobulin (IVIg), but demonstrates and sustains an adequate platelet count shortly after transfusion (i.e. 1 h post transfusion of maternal platelets). The incidence[5,11-13] of NAIT has been estimated to be 1 in 1,500-5,000 live births, and as 60% of identified cases occur in first pregnancies that are otherwise uneventful, it is difficult to predict who may be at risk. The first indication of NAIT may be the presence of unexplained petechiae and/or purpura in a newborn with a platelet count of <100,000/mm^3. A diagnosis of NAIT should only be made after exclusion of maternal history of an autoimmune disorder, thrombocytopaenia, or drug abuse. Cordocentesis to determine the foetal platelet count has been used in managing pregnancies complicated by NAIT, but this approach is being minimised or avoided due to the significant procedure-related risks. The earlier the intracranial haemorrhage occurred in the previous sibling, the greater the risk for intracranial haemorrhage in the currently affected foetus.

We perform[5,11,14,15] maternal and paternal platelet antigen typing, as well as maternal human anti-platelet antibody evaluation when the woman or her sister has an obstetrical history suggestive of this diagnosis (e.g. foetal death due to intracranial haemorrhage, neonatal thrombocytopaenia of undetermined aetiology). We perform paternal platelet antigen genotyping if the foetus is at risk of NAIT. If the father is heterozygous HPA-1a/1b, the foetal HPA status should be determined by typing foetal DNA for platelet antigens by PCR. The most widely accepted prevention strategy in the USA is weekly maternal antenatal administration of intravenous gamma globulin, ranging between 1-2 g/kg/week and/or prednisone 0.5-1 mg/kg/day. Therapy has been initiated as early as at 12 weeks of gestation in pregnancies in which a previous intracranial haemorrhage occurred, with good perinatal outcomes. Although use of glucocorticoids in pregnant women has been associated with an increased risk of pre-term premature rupture of membranes, this has not been described in the literature of pregnancies complicated by NAIT. Experts suggest cesarean delivery with consideration of vaginal birth only if the foetal platelet count is greater than 100,000/mm^3 prior to delivery.

POST-TRANSFUSION PURPURA (PTP)

PTP[16] is a rare bleeding disorder caused by alloantibodies specific to platelet antigens. The antibody against HPA-1a is responsible for most cases. Patients[17] with PTP can present with severe thrombocytopaenia (e.g. platelet count ≤20,000/mm^3) that develops approximately 5-10 days following transfusion. The thrombocytopaenia often lasts from days to weeks. This condition appears in patients pre-exposed to foreign platelet-specific antigens by pregnancy or blood transfusion, and develops following a booster of incompatible platelets by producing high titre anti-HPA antibodies. These antibodies paradoxically destroy recipient platelets.[17] Several mechanisms have been proposed to explain the destruction of the patients' own platelets along with transfused platelets: adsorption of antigen-antibody complexes, cross-reactive antibodies, or autoantibody production. The majority of PTP[17] cases occur in patients with HPA-1b/b genotype producing anti-HPA-1a antibodies after transfusion of HPA-1a antigen; occasionally exposure to other platelet antigens induces the disease. More than one species of platelet-specific antibody may be implicated in rare cases of PTP. However, specific tests to determine the platelet antigenic composition and/or the presence of anti-platelet antibodies may not be readily available.

PTP[17-19] is an immunologically mediated thrombocytopaenia and may be confused with drug-induced or immune thrombocytopaenia (ITP), since the blood and bone marrow smears are consistent with immune platelet destruction in all of these disorders (i.e. thrombocytopaenia, occasional large platelets on the blood smear, increased megakaryocytes in the bone marrow). Since drug-induced thrombocytopaenia is relatively rare, and *de novo* ITP developing in someone who has recently been transfused rarer still, the possibility of a provisional diagnosis of PTP in someone with a history of a recent transfusion is reasonable. The preferred therapy for PTP is IVIg in high doses (400-500 mg/kg per day, usually for 5 days); alternatively, 1 g/kg per day for 2 days can be given for severe thrombocytopaenia. It usually takes about 4 days for the platelet count to exceed 100,000/mm^3.

REFRACTORINESS TO PLATELET TRANSFUSIONS

Platelet refractoriness[20,21] is a complication of platelet transfusion that affects variable proportions of patients, mostly depending on their diagnosis, previous immunologic stimuli, and type of blood products used for transfusion. Refractoriness to platelet transfusion can be separated into immune and non-immune causes. Immune causes include alloimmunisation to HLA and/or platelet-specific antigens due to prior exposure from pregnancy, transfusions, and/or transplantation. Non-immune causes, based on studies in patients with acute myeloid leukaemia or haematopoietic progenitor cell transplants, include fever, sepsis, splenomegaly, disseminated intravascular coagulation, bleeding, veno-occlusive disease, graft-versus-host disease, and medications. A large recent[20,22] study showed that platelet refractoriness develops in 13% of patients with acute leukaemia transfused with traditional blood products and in 3-4% of recipients of white-cell-reduced blood components. Alloimmunisation should be suspected when patients fail to have adequate platelet count increments following transfusion.

In general, poor increments following at least two ABO compatible transfusions stored for less than 72 hours should be documented prior to searching for histocompatible transfusions because, for reasons that are sometimes elusive, patients can have poor increments to a single transfusion with excellent responses to subsequent transfusions. Immune causes of platelet consumption include HLA Class I or HPA antibodies, major and minor ABO incompatibility, drug-induced antibodies, and antibodies to plasma proteins. Different[21,23] serological tests were developed to distinguish immune from non-immune causes of platelet refractoriness. In each assay, patient serum is incubated with a source of donor target antigen to demonstrate the presence of alloantibodies. There is no consensus regarding which test (lymphocytotoxicity test, platelet immunofluorescence test, lymphocyte immunofluorescence test, enzyme-linked immunosorbent assay [ELISA], antigen capture ELISA, monoclonal antibody-specific immobilisation of platelet antigens, solid-phase red cell agglutination test) yields optimum results. Multiplex flow cytometric bead assays are ideal for diagnosing refractoriness. Although studies have compared different testing strategies, there is no clear gold standard.

Once it is determined that a patient is alloimmunised to HLA antigens, compatible platelets are required for transfusion.[24] The large number of polymorphisms in the HLA system complicates the provision of HLA-matched platelets. With approximately 70 antigens to consider, the probability of finding matched donors for recipients with fewer common HLA phenotypes is low, even if a large number of HLA-typed plateletpheresis donors are available. Because of this, different strategies of donor selection are used, such as platelet cross-matching. Alternative methods for treating patients who are refractory to platelets and have thrombocytopaenic bleeding include the use of IVIg or anti-D immunoglobulin in patients who are Rh-positive.

PAIT

PAIT[23,25,26,27] is characterised by abrupt onset of thrombocytopaenia within a few hours of transfusing a blood product (usually plasma) that contains high-titre platelet-specific antibodies. In this syndrome, in contrast with PTP, the thrombocytopaenia immediately follows the transfusion and the duration is shorter, from several hours to a few days. Although the PSA can be detected in the donor's plasma and on the recipients platelets, it is not detectable in the recipient's plasma, suggesting that virtually 100% of the transfused alloantibodies bind soon after transfusion.[22,28] It is very important to investigate these cases because of the potential for multiple recipients to develop this syndrome, the responsible blood donor must be excluded from future blood donation. In comparison with PAIT caused by anti-HPA-1a, the severity of the thrombocytopaenia is consistent with other alloimmune syndromes (NAIT, PTP), and is consistent with the concept that the alloimmune thrombocytopaenic syndromes differ in severity largely on the basis of the number of antigen sites per platelet.

TRANSPLANTATION-ASSOCIATED ALLOIMMUNE THROMBOCYTOPAENIA (TAIT)

This syndrome[25,29,30] can occur as a severe complication either with a solid organ

transplantation, or an allogeneic bone marrow transplant. Both anti-HPA-1a and anti-HPA-5b alloantibodies can cause thrombocytopaenia that may develop immediately after or a long time after transplantation. An immune mechanism[27,28,31] was suggested by the repeated platelet count increase after treatment with high dose γ-globulin. Thrombocytopaenia by an alloimmune mechanism has been reported in patients after autologous peripheral blood stem cell transplantation. Solid organ transplants can rarely lead to AIT.[32] Reduced platelet levels are commonly seen after liver transplantation. In one reported series of 76 such procedures, a minimal mean platelet count of 86,000/mm^3 was measured on the third postoperative day and sequestration of platelets in the liver was demonstrated by the use of radio-labelled platelets. In another recently reported series of 43 liver transplantations, however, the nadir platelet count occurred about 1 week after transplantation and averaged 65,000/mm^3.

CONCLUSION

Alloimmune thrombocytopaenia is not the commonest cause of thrombocytopaenia, which has a wide variety of underlying causes. It is important to consider the clinical context of the thrombocytopaenia to guide rational investigation. The best practice guidelines for treatment aim to reduce the risk of severe haemorrhage in thrombocytopaenic patients. The outcome of ongoing and future studies will be crucial for determining the precise role of alloantibodies in the pathophysiology of disease. In the clinical setting it is also important to consider unusual alloimmune thrombocytopaenic disorders in which alloantigens that could be limited to just one family could cause important disease (i.e. NAIT caused by a private alloantigen; theoretically, PAIT or TAIT related to directed donations of blood or bone marrow). These considerations underscore the need for serological investigation to involve the family members rather than to rely on standard platelet-typing donor pools.

REFERENCES

1. Santoso S, Kiefel V. Human platelet alloantigens. Wiener Klinische Wochenschrift. 2001;113(20-21):806-13.

2. Rozman P. Platelet antigens. The role of human platelet alloantigens (HPA) in blood transfusion and transplantation. Transplant immunology. 2002;10(2-3):165-81.

3. Metcalfe P et al. Nomenclature of human platelet antigens. Vox Sang. 2003;85(3):240-5.

4. Jones DC et al. Human platelet alloantigens (HPAs): PCR-SSP genotyping of a UK population for 15 HPA alleles. Eur J Immunogenet. 2003;30(6):415-9.

5. Ghevaert C et al. Alloantibodies against low-frequency human platelet antigens do not account for a significant proportion of cases of fetomaternal alloimmune thrombocytopenia: evidence from 1054 cases. Transfusion. 2009;49(10):2084-9.

6. Klüter H et al. Rapid typing for human platelet antigen systems-1, -2, -3 and -5 by PCR amplification with sequence-specific primers. Vox Sang. 1996;71(2):121-5.

7. Pacheco LD et al. Fetal and neonatal alloimmune thrombocytopenia: a management algorithm based on risk stratification. Obstet Gynecol. 2011;118(5):1157-63.

8. Kaplan C. Neonatal alloimmune thrombocytopenia. Haematologica. 2008;93:805-7.

9. Peterson JA et al. New platelet glycoprotein polymorphisms causing maternal immunization and neonatal alloimmune thrombocytopenia. Transfusion. 2012;52(5):1117-24.

10. Rothenberger S. Neonatal alloimmune thrombocytopenia. Ther Apher. 2002;6(1):32-5.

11. Kamphuis MM et al. Incidence and consequences of neonatal alloimmune thrombocytopenia: a systematic review. Pediatrics. 2014;133(4):715-21.

12. Harkness M. Neonatal alloimmune thrombocytopenia. Br J Midwifery. 2002;10(2):99-103.

13. Bussel JB et al. Clinical and diagnostic comparison of neonatal alloimmune thrombocytopenia to non-immune cases of thrombocytopenia. Pediatr Blood Cancer. 2005;45(2):176-83.

14. Ouwehand WH et al. Management of severe alloimmune thrombocytopenia in the newborn. Arch Dis Child Fetal Neonatal Ed. 2000;82(3):F173-5.

15. Arnold DM et al. Diagnosis and management of neonatal alloimmune thrombocytopenia. Transfus Med Rev. 2008;22(4):255-67.

16. Gonzalez CE, Pengetze YM. Post-transfusion purpura. Curr Hematol Rep. 2005;4(2):154-9.

17. Shtalrid M et al. Post-transfusion purpura: a challenging diagnosis. Isr Med Assoc J. 2006;8(10):672.

18. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. Anesth Analg. 2009;108(3):759-69.

19. Brand A. Immunological aspects of blood transfusions. Transpl immunol 2002;10(2):183-90.

20. Leitner GC et al. Post-transfusion purpura without detectable antibodies: their adsorption from the plasma by multiple incompatible platelet transfusions. Br J Haematol. 2002;117(4):994-5.

21. Rebulla P. Refractoriness to platelet transfusion. Curr Opin Hematol. 2002;9(6):516-20.

22. Dan ME, Schiffer CA. Strategies for managing refractoriness to platelet transfusions. Curr Hematol Rep. 2003;2(2):158-64.

23. Hod E, Schwartz J. Platelet transfusion refractoriness. Brit J Haematol. 2008;142(3):348-60.

24. Schiffer CA. Diagnosis and management of refractoriness to platelet transfusion. Blood Rev. 2001;15(4):175-80.

25. Toor AA et al. Bleeding risk and platelet transfusion refractoriness in patients with acute myelogenous leukemia who undergo autologous stem cell transplantation. Bone Marrow Transplant. 2000;26(3):315-20.

26. Reik RA et al. Unique donor-suitability issues. Vox Sang. 2006;90(4):255-64.

27. Saner F et al. Small-for-size syndrome after living-donor liver transplantation treated by 'portal vein wrapping' and single plasmapheresis. Transplantation. 2005;79(5):625.

28. Diaz GC et al. Transplantation-mediated alloimmune thrombocytopenia: Guidelines for utilization of thrombocytopenic donors. Liver Transpl. 2008;14(12):1803-9.

29. Psaila B, Bussel JB. Fc receptors in immune thrombocytopenias: a target for immunomodulation? J Clin Invest. 2008;118(8):2677-81.

30. Warkentin TE et al. Thrombocytopenia caused by passive transfusion of anti-glycoprotein Ia/IIa alloantibody (anti-HPA-5b). Blood. 1992;79(9):2480-4.

31. Friend PJ et al. Transmission of idiopathic (autoimmune) thrombocytopenic purpura by liver transplantation. N Engl J Med. 1990;323(12):807-11.

32. McFarland JG. Detection and identification of platelet antibodies in clinical disorders. Transfus Apher Sci. 2003;28(3):297-305.