Review: platelet alloantigens and antibodies and their clinical significance

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Antigens on human platelets are categorized according to their biochemical nature into carbohydrate antigens on glycolipids and glycoproteins (GPs) (A, B, O, P, Le antigens), protein antigens (human leukocyte antigens [HLA] Class-I A, B, and C, GPIIb/IIIa, GPIb/IX/V), and haptens (quinine, quinidine, heparin, and some antibiotics, e.g., penicillins and cephalosporins).

Many platelet antigens are shared with other blood cells, e.g., ABO and HLA class I antigens, but some of the glycoprotein antigens are expressed predominantly on platelets. These antigens are commonly referred to as platelet-specific alloantigens or human platelet alloantigens (HPAs), although some of these are also present to a lesser extent on other blood cells, e.g., HPA-5 on activated T lymphocytes.

Human Platelet Alloantigens

There are a number of well-characterized biallelic platelet alloantigen systems, and a number of rare, private, or low-frequency antigens have also been described (Table 1). Most of these antigens were first discovered during the investigation of cases of neonatal alloimmune thrombocytopenia (NAIT).

Platelet-specific alloantigens are located on platelet membrane GPs involved in hemostasis through interactions with extracellular matrix proteins in the vascular endothelium and plasma coagulation proteins. The majority of these antigens are on the GPIIb/IIIa complex, which plays a central role in platelet aggregation as a receptor for fibrinogen, fibronectin, vitronectin, and von Willebrand factor. Other important GPs are GPIb/IX/V, the main receptor for von Willebrand factor involved in platelet adhesion to damaged vascular endothelium; GPIa/IIa, which is involved in adhesion to collagen; and CD109, which also appears to be a collagen receptor. Congenital deficiency of these GPs results in bleeding disorders, e.g., lack of GPIIb/IIIa causes Glanzmanns' thrombasthenia, and absence of GPIb/IX/V results in Bernard-Soulier syndrome. The expression of platelet alloantigens located on these GPs may be altered in these disorders, and HPA typing performed by serologic assays (phenotyping) may give discrepant results when compared with results obtained by molecular typing (genotyping).

Inheritance and nomenclature

Most of the platelet-specific alloantigen systems reported to date have been shown to be biallelic, with each allele being codominant. Historically, systems were named by the authors first reporting the system, usually using an abbreviation of the name of the patient in whom the antibody was detected. Some systems were published simultaneously by different laboratories and with different names, e.g., Zw and Pl^A, or Zav/Br/Hc, and only later were they found to be the same polymorphism. In 1990, a working party for platelet immunology of the ISBT agreed on a new nomenclature for platelet polymorphisms, the Human Platelet Antigen (HPA) nomenclature. Any new platelet GP alloantigens are now accepted and named according to guidelines established by the recently founded International Platelet Nomenclature Committee.[1]

In the HPA nomenclature, each system is numbered consecutively (HPA-1, -2, -3, and so on) according to its date of discovery. The high-frequency allele in each system is designated *"a"* and the low-frequency allele *"b."* Newly discovered systems are only officially included when confirmed by a second party and approved by the nomenclature committee. If an antibody against only one allele has been reported, a "w" (for workshop) is added after the antigen name, e.g., HPA-10bw. One possible reason why antibodies

Table 1. Platelet-specific alloantigen systems

System	Antigen	Alternative names	Phenotype frequency* (%)	Platelet membrane glycoprotein	Nucleotide change	Amino acid change
HPA-1	HPA-1a	Zw^a, Pl^{A1}	97.9	GPIIIa	T^{196}	$Leucine^{33}$
	HPA-1b	Zw^b, Pl^{A2}	28.8		C^{196}	$Proline^{33}$
HPA-2	HPA-2a	Ko^b	> 99.9	GPIbα	C^{524}	$Threonine^{145}$
	HPA-2b	Ko^a, Sib^a	13.2		T^{524}	$Methionine^{145}$
HPA-3	HPA-3a	Bak^a, Lek^a	80.95	GPIIb	T^{2622}	$Isoleucine^{843}$
	HPA-3b	Bak^b	69.8		G^{2622}	$Serine^{843}$
HPA-4	HPA-4a	Yuk^b, Pen^a	> 99.9	GPIIIa	G^{526}	$Arginine^{143}$
	HPA-4b	Yuk^a, Pen^b	< 0.1		A^{526}	$Glutamine^{143}$
HPA-5	HPA-5a	Br^b, Zav^b	99.0	GPIa	G^{1648}	$Glutamic\ acid^{505}$
	HPA-5b	Br^a, Zav^a, Hc^a	19.7		A^{1648}	$Lysine^{505}$
HPA-6				GPIIIa	A^{1564}	$Arginine^{489}$
	HPA-6bw	Ca^a, Tu^a	0.7		G^{1564}	$Glutamine^{489}$
HPA-7				GPIIIa	C^{1267}	$Proline^{407}$
	HPA-7bw	Mo	0.2		G^{1267}	$Alanine^{407}$
HPA-8				GPIIIa	T^{2004}	$Arginine^{636}$
	HPA-8bw	Sr^a	< 0.01		C^{2004}	$Cysteine^{636}$
HPA-9				GPIIb	G^{2603}	$Valine^{837}$
	HPA-9bw	Max^a	0.6		A^{2603}	$Methionine^{837}$
HPA-10				GPIIIa	G^{281}	$Arginine^{62}$
	HPA-10bw	La^a	< 1.6		A^{281}	$Glutamine^{62}$
HPA-11				GPIIIa	G^{1996}	$Arginine^{633}$
	HPA-11bw	Gro^a	< 0.25		A^{1996}	$Histidine^{633}$
HPA-12				GPIbβ	G^{141}	$Glycine^{15}$
	HPA-12bw	Iy^a	0.4		A^{141}	$Glutamic\ acid^{15}$
HPA-13				GPIa	C^{2531}	$Threonine^{799}$
	HPA-13bw	Sit^a	0.25		T^{2531}	$Methionine^{799}$
HPA-14	HPA-14bw	Oe^a	< 0.17	GPIIIa	$\Delta\ AAG^{1929-1931}$	$\Delta\ Lysine^{611}$
HPA-15	HPA-15a	Gov^b	74	CD109	C^{2108}	$Serine^{703}$
	HPA-15b	Gov^a	81		A^{2108}	$Tyrosine^{703}$
HPA-16					C^{517}	$Threonine^{140}$
	HPA-16bw	Duv^a	< 1	GPIIIa	T^{517}	$Isoleucine^{140}$
		Va^a	< 0.4	GPIIb/IIIa		
		Pl^T	> 99.9	GPV		
		Vis		GPIV		
		Pe^a		GPIbα		
		Dy^a		38 kD GP		
		Mou^a	26	unknown		

* Frequencies based on studies in Caucasians

against the "a" antigen have not yet been reported for many of the recently discovered systems is that the *"b"* allele is of such low frequency that *"bb"* homozygous individuals either do not exist or are extremely rare.

In Caucasian populations, the allele frequency for the majority of HPA systems is skewed toward the *"a"* allele and homozygosity for the *"b"* allele is below 3 percent. This places significant pressures on the blood services in the management of alloimmunized patients, as compatible RBCs and platelets are difficult to obtain for patients with antibodies against the "a" alloantigen. Allele frequencies vary between populations, e.g., HPA-1b is extremely rare or absent in the Far East, while HPA-4b does not occur in Caucasians (Table 2). These differences are important when investigating cases of suspected platelet alloimmunity in different ethnic groups.

Until the early 1990s, platelet typing was performed by serologic assays. These assays required the use of monospecific antisera, which were relatively uncommon, as the majority of immunized individuals produced HLA Class-I antibodies in addition to the platelet-specific antibodies. Therefore, typing that could be performed was limited, and many laboratories were only able to phenotype for HPA-1a. The publication of more advanced assays, such as monoclonal antibody-specific immobilization of platelet antigens (MAIPA) (Fig. 1), permitted more extensive phenotyping, but some antisera were simply not available.[2]

With the advent of techniques such as immunoprecipitation of radioactive labeled platelet-membrane proteins, and the PCR, the molecular basis of the majority of clinically relevant platelet-specific alloantigen systems was elucidated. The molecular basis for all of the HPA alloantigen systems has been determined, and in all but one (Oe[a]) the difference between the two alleles is based on a single nucleotide difference that results in a single amino acid substitution. Based on this molecular knowledge, a plethora of molecular typing techniques has been developed over the last decade, and these have largely overcome problems in platelet typing. One such assay is the PCR using sequence-specific primers (PCR-SSP). This is a fast and reliable molecular typing technique with minimal post-PCR handling. It has become one of the cornerstone techniques in HLA typing and is widely used for HPA genotyping (Fig. 2).[3,4] Novel, high-throughput, DNA-based typing techniques with automated readout are under development and will be used in platelet immunology reference laboratories in the near future.

Knowledge of the genetic basis of platelet-specific antigens makes it possible to carry out molecular genotyping on whatever DNA-containing material is available, e.g., platelet typing using fetal DNA from amniocytes or from chorion villous biopsy samples. However, in the setting of first trimester fetal HPA typing, extreme caution is required to exclude possible contamination with maternal cells and consequent erroneous typing.

Platelet Antibodies

Platelet antigens can be targeted by different types of antibodies: autoantibodies, alloantibodies, isoantibodies, and drug-dependent antibodies.

Platelet Autoantibodies

Platelet autoantibodies cause the persistent thrombocytopenia (peripheral platelet count < 150 × 10^9/L) seen in idiopathic thrombocytopenic purpura (ITP). Autoantibodies bind to platelet antigens and cause the premature destruction of platelets in the reticuloendothelial system.[5] The main targets appear to be the platelet membrane GP GPIIb/IIIa and GPIb/IX/V. Such autoantibodies bind to the platelets of all individuals, regardless of their HPA types.

ITP may be seen both in children and in adults, where it can follow an acute or chronic course. In adults, the condition typically has an insidious onset and runs a chronic course. Symptoms and signs are variable and include bruising, mucocutaneous bleeding, and frank hemorrhage. In general, serious bleeding symptoms are rare unless the ITP is severe (platelet count < 30 × 10^9/L).[6] Adult chronic ITP has an

Table 2. Platelet antigen frequencies in different populations

	HPA-										
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	Nak[a]
Caucasians	97.5	30.8	99.8	11.8	86.1	62.9	100	0	98.8	20.7	100
African blacks	100	16	97	33	85	60	100	0	96	38	97.6
Japanese	99.9	3.7	?	25.4	78.9	70.7	99.9	1.7	99.8	8.7	97
Chinese	99.9	0.15	?	9	78.5	71.9	99.9	0.17	99.9	17.7	85.7

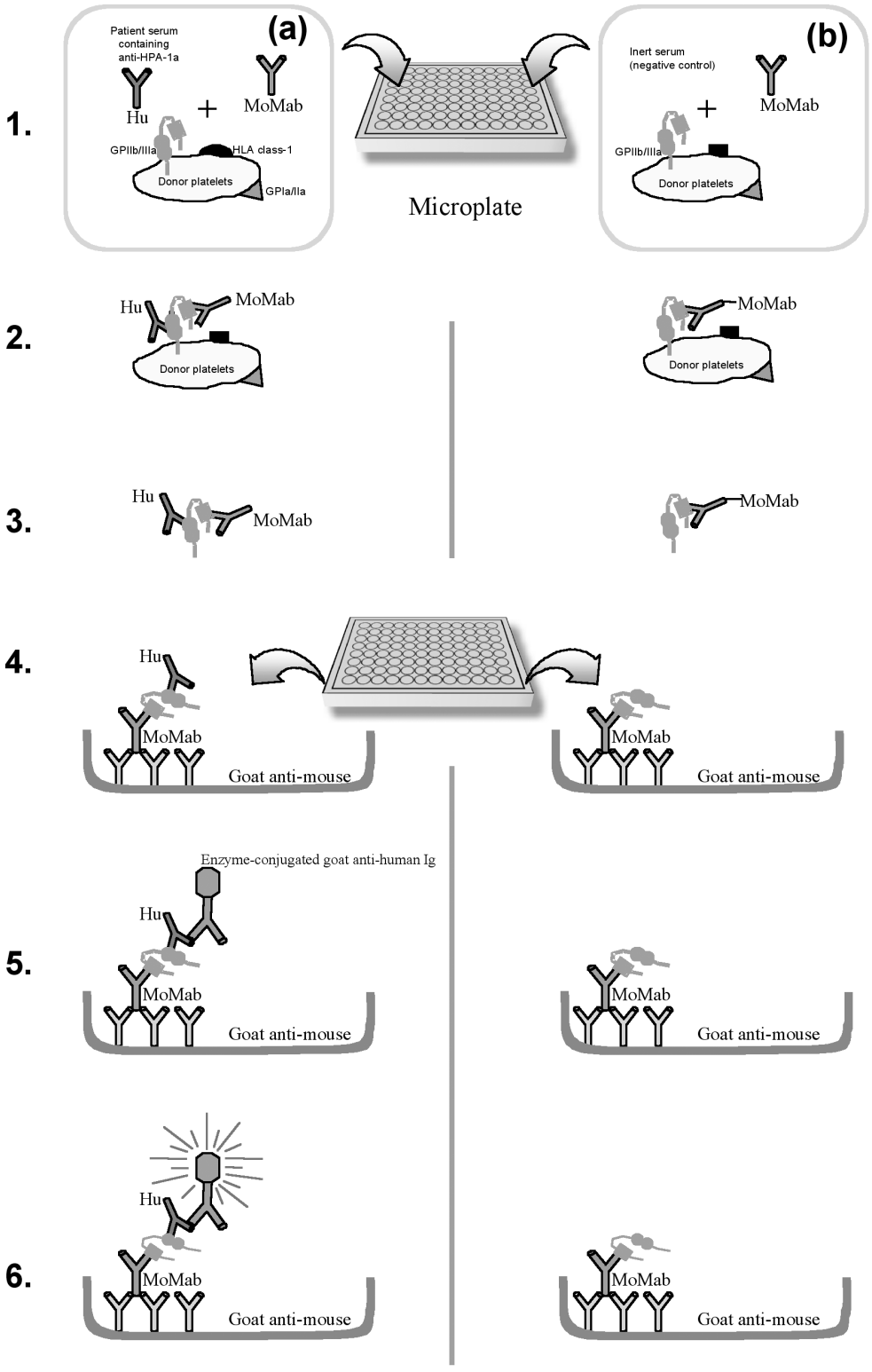

Fig. 1. Monoclonal antibody-specific immobilization of platelet antigens. (1) A cocktail of target platelets; murine monoclonal antibody (MoMab) directed against the glycoprotein being studied, e.g., GPIIb/IIIa; and human serum is prepared in (a) the test serum containing anti-HPA-1a and (b) no anti-platelet antibodies. (2) After incubation, a trimeric (a) or dimeric (b) complex is formed. Excess serum and MoMab is removed by washing. (3) The platelet membrane is solubilized in a non-ionic detergent, which releases the complexes into the fluid phase, and particulate matter is removed by centrifugation. (4) The lysates containing the glycoprotein/antibody complexes are added to the wells of a microtiter plate previously coated with goat anti-mouse antibody. (5) Unbound lysate is removed by washing and an enzyme-conjugated goat anti-human antibody added. (6) Excess conjugate is removed by washing and a substrate solution is added. Cleavage of the substrate, i.e., a color reaction indicates binding of human antibody to the target platelets.

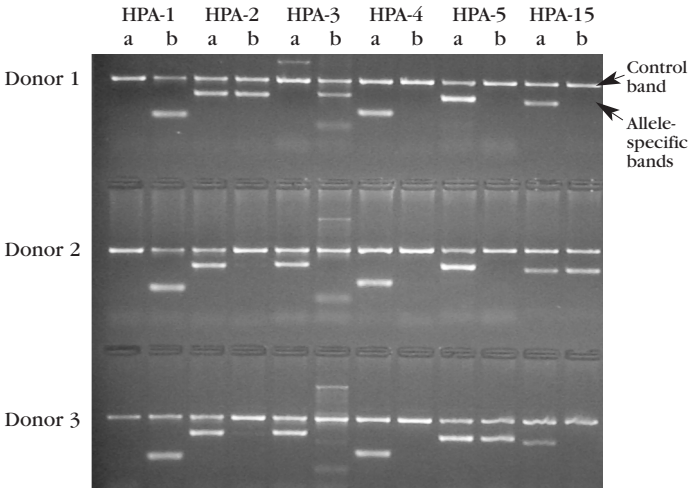

Fig. 2. Simultaneous genotyping for HPA-1, -2, -3, -4, -5 and -15 by PCR-SSP. The upper band present in all lanes is the 429-bp product of human growth hormone. The lower bands are the products of sequence-specific primers. Results are read from left to right, i.e., lane 1 is HPA-1a, lane 2 is HPA-1b, etc. The genotypes of the 3 donors shown are:

Donor 1: *HPA-1b1b; 2a2b; 3b3b; 4a4a; 5a5a; 15a15a*

Donor 2: *HPA-1b1b; 2a2a; 3a3a; 4a4a; 5a5a; 15a15b*

Donor 3: *HPA-1b1b; 2a2a; 3a3a; 4a4a; 5a5b; 15a15a*

Courtesy of Dr Paul Metcalfe.

incidence of 58 to 66 new cases per million population per year in the United States[7] and affects predominantly women of childbearing age.[8] In children, the incidence of ITP appears to be lower than in adults, with published figures of between 4.0 and 5.3 per 100,000.[9,10] The disorder also tends to run a different clinical course, with an acute and abrupt onset, often following a viral illness or immunization. The majority of children with the disorder require no treatment, and it resolves within 6 months. However, 15 percent of affected children develop a chronic form of ITP similar to that seen in adults.

The diagnosis of ITP usually relies on a typical history, blood count, and blood film. Although there are assays available for the detection of platelet autoantibodies, these are not robust enough to establish the diagnosis of ITP alone. The direct platelet immunofluorescence test (PIFT) detects platelet-associated immunoglobulin (PAIg) levels, which are found to be increased in most patients with ITP. Unfortunately, the assay is not specific, since positive results can also be seen in nonimmune thrombocytopenias, for example secondary to septicemia.[11] Assays for antibodies to specific platelet membrane GPs IIb/IIIa and Ib/IX are specific (90%) in ITP but less sensitive (50–65%), and currently their routine use in the diagnosis of ITP is not considered to be justified.[12,13]

Testing for platelet autoantibodies may be of value when there is a combination of bone marrow failure associated with immune-mediated thrombocytopenia, in ITP patients refractory to first and second line treatments, in drug-dependent immune thrombocytopenias, and in rare disorders such as acquired antibody-mediated thrombasthenia.[13]

Secondary immune thrombocytopenias can occur in association with underlying autoimmune disorders, e.g., systemic lupus erythematosus. Platelet autoimmunity is also frequently associated with B-cell malignancies, and autoantibody formation is not infrequent in the posthematopoietic stem cell transplantation period, during immune cell re-engraftment. In these situations, the presence of autoantibodies might contribute to refractoriness to donor platelets.

Platelet Alloantibodies

Detection of platelet alloantibodies

Tests for the detection of platelet-specific antibodies have evolved over the last 4 decades. Currently the most widely used assay is the indirect PIFT, but it is unable to distinguish between platelet-specific and HLA Class I antibodies. The MAIPA assay has become the gold standard for the identification of platelet-specific antibodies. This capture assay uses murine monoclonal antibodies specific for platelet GPs and can analyze complex mixtures of antibodies in patient sera. Third generation antibody detection assays, using purified or recombinant platelet GP, have also been developed; however, the sensitivity of these assays is not satisfactory for all alloantigen systems and some systems (e.g., HPA-15) are not included.[14]

Clinical significance of HPA alloantibodies

HPA alloantibodies are responsible for the following clinical conditions:

- Neonatal alloimmune thrombocytopenia (NAIT), sometimes called fetomaternal alloimmune thrombocytopenia (FMAIT)
- Posttransfusion purpura (PTP)
- Refractoriness to platelet transfusions

NAIT

Definition

NAIT is defined as thrombocytopenia in the neonate (platelet count < 150×10^9/L) due to transplacental transfer of maternal platelet alloantibodies. However, thrombocytopenia can also occur in utero,

resulting in intrauterine death or intracranial hemorrhage (ICH).

Incidence

The estimated incidence of severe thrombocytopenia due to maternal alloantibodies is 1 per 1200 pregnancies in a Caucasian population, of which the majority are due to fetomaternal incompatibility for HPA-1a.[15] NAIT is probably underrecognized in clinical practice.[16]

Pathophysiology

NAIT is the platelet equivalent of the anemia seen in HDN. Maternal IgG alloantibodies against a fetal platelet alloantigen cross the placenta and bind to fetal platelets, resulting in their increased destruction in the reticuloendothelial system. Unlike HDN, up to 50 percent of cases of NAIT occur in the first pregnancy. In Caucasian women, the antibody most commonly implicated in NAIT (78–89% of cases) is anti-HPA-1a.[17] Anti-HPA-5b occurs more frequently in pregnancy, but it tends to cause mild thrombocytopenia,[18] and clinically significant NAIT is less common (6–15% of cases). The HPA-15 system, described a decade ago, has recently been found to have clinical significance, with HPA-15 alloantibodies being the third most frequently encountered antibodies. Rarely, NAIT can occur due to incompatibility for other HPA, HLA, or blood group ABO antigens. In non-Caucasian populations, other HPA alloantigens are more commonly implicated, for example HPA-4b in Orientals.

Approximately 2.5 percent of pregnant Caucasian women are HPA-1a negative and most of these women will carry HPA-1a positive fetuses, but only about 12 percent of these women will become HPA-1a alloimmunized.[15] The ability to develop anti-HPA-1a is HLA class II restricted, with a strong association seen with HLA-DRB3*0101 positivity.[19] As a negative predictive factor, the absence of HLA-DRB3*0101 for HPA-1a alloimmunization in HPA-1a negative women is > 90 percent, but its positive predictive value as a single marker is only 35 percent.[15]

The pathogenicity of antibodies in an unselected population is highly variable. In a study including 26 cases of NAIT due to anti-HPA-1a, 9 of the 26 (34%) had severe thrombocytopenia; 35 percent (7 of 26 cases) had a normal cord platelet count, with a further 38 percent (10/26) having a "safe" platelet count of > 50 × 10^9/L.[15]

Clinical features

The typical infant with NAIT is full term and otherwise healthy, with a normal coagulation screen and isolated thrombocytopenia. The thrombocytopenia may be severe and unexpected, and it may occur in the firstborn child. Clinically, the neonate may present with petechiae, purpura, and/or ecchymoses.[17] The most feared complication is ICH, which occurs in 14 to 26 percent of cases,[17,20,21] resulting in death in 7 percent of affected cases[20,21] and long-term morbidity with neurologic sequelae in 21 percent.[20] Eighty percent of ICHs occur in utero, with 14 percent occurring before 20 weeks' and a further 28 percent before 30 weeks' gestation.[21] This is consistent with the expression of platelet antigens from 16 weeks' gestation and placental transfer of IgG antibodies that can occur from 14 weeks. There are also less common presentations of NAIT, including fetal hydrocephalus, hydrops fetalis, or recurrent miscarriages.[22]

The differential diagnoses of thrombocytopenia in the newborn include infection, prematurity, intrauterine growth retardation, maternal platelet autoimmunity, and inadequate megakaryocytopoiesis (such as in the thrombocytopenia absent radii [TAR] syndrome).

Laboratory diagnosis

The diagnosis of NAIT requires the demonstration of maternal platelet alloantibodies that react against platelet-specific antigens present in the father and infant but not in the mother. Detection of maternal platelet-specific antibodies is usually carried out by two techniques, the indirect PIFT and the MAIPA assay using a panel of HPA-typed platelets. The serum of the mother is also tested against paternal platelets by both tests so that alloantibodies against low-frequency alloantigens and private antigens are not missed. The parents are also genotyped for the HPA-1,-2,-3, -5, and -15 alloantigens (the frequency of other alloantigens being comparatively low in the Caucasian population). It should also be noted that in up to 30 percent of cases of NAIT associated with fetomaternal incompatibility for the HPA-1a antigen, maternal HPA-1a antibodies may not be detectable.[20] However, some authors have shown that the choice of MoAb used to capture GPIIb/IIIa in the MAIPA assay is critical and falsely negative results may be obtained if the epitope recognized by the MoAb is blocked by the human antibody.[23] Others have reported that an increased serum:cell ratio is required to detect low levels of

antibody. Sometimes the antibodies that were not detectable at the time of delivery become detectable a few weeks or months after delivery.

Postnatal management

The postnatal management of NAIT is influenced by the degree of thrombocytopenia and clinical symptoms present. Any neonate with suspected NAIT who is bleeding or has a platelet count of $< 30 \times 10^9$/L should be transfused with compatible platelets to minimize the risk of ICH, without waiting for confirmatory laboratory tests.

Anti-HPA-1a and anti-HPA-5b are responsible for approximately 75 percent and 20 percent of cases of NAIT, respectively.[17,24] Approximately 2 percent of blood donors are negative for both these antigens and can be easily identified using established platelet typing methods.[25,26] It is possible to establish in blood centers a stock of HPA-1a-negative and 5b-negative platelet concentrates from donors who donate regularly, and these can be issued without delay for the treatment of suspected cases of NAIT before laboratory confir-mation of the diagnosis.[27] An alternative for platelets is random donor platelets, which, while more readily available, have been shown to have poorer responses, with one study of 36 cases of NAIT reporting a median increase in platelet count 24 hours posttransfusion of only 3.5×10^9/L.[21] Washed maternal platelets have been found to be successful in the treatment of NAIT[28] and may also be used. However, maternal platelets need to be collected by apheresis machines, washed to remove the maternal HPA antibodies, which may prolong the thrombocytopenia, and gamma-irradiated to prevent transfusion-associated graft-versus-host disease; all of which is time consuming and requires specialist equipment and facilities.

In the stable neonate, an alternative approach is to use IVIG at a dose of 1g/kg/day for 2 days. In a study of 12 cases of NAIT, a 75 percent response rate was reported. However, the increase in platelet count was delayed by 24 to 48 hours.[29] In urgent situations, where antigen-negative platelets are not available and the neonate is bleeding, a combination of random platelets and IVIG should be given until compatible donor platelets become available.

Monitoring of neonates with NAIT is important in the postnatal period, irrespective of initial platelet level and clinical symptoms. The platelet count may continue to fall after birth, particularly in the first 48 hours, and thrombocytopenia may persist for up to 6 weeks postnatally (although 1–2 weeks is more usual). All neonates with NAIT should also undergo some form of cerebral imaging to exclude ICH.

Antenatal management

The realization that spontaneous ICH may occur in utero has led many to a search for methods of preventing serious antenatal bleeding. Unfortunately, 30 percent of cases of NAIT occur in the first pregnancy, and at present there are no reliable tests available to predict which women will become alloimmunized and which of those will have severely affected babies. What is well recognized is that mothers with HPA-1a alloimmunization and previously affected infants (particularly those with ICH) have a high risk of recurrence and poor outcome in subsequent pregnancies.[30] These are the women targeted for antenatal treatment.

In women with a known history of alloimmunization and a previously affected pregnancy, HPA typing of the partner is important. This allows the risk of the fetus being HPA-1a positive to be assessed (i.e., all infants are affected when the father is homozygous for the pathogenic platelet antigen as opposed to 50 percent of infants affected in heterozygous cases). Where the father is heterozygous it may be useful to HPA type the fetus by chorionic villous or amniotic fluid sampling.

There is considerable experience in the antenatal management of FMAIT where there has been a previously affected pregnancy, but which is the optimal management remains controversial.[31] The therapeutic options that have been explored are maternal administration of IVIG and/or steroids and fetal platelet transfusions. Early cesarean section alone is not considered to be effective in preventing antenatal or perinatal hemorrhage. For both approaches to antenatal management, fetal blood sampling (FBS) has been used for initial assessment of the fetal platelet count, usually at 20 to 22 weeks' gestation, and for the monitoring of the effectiveness of treatment in the early studies.

Bussel et al.[30] found maternal administration of IVIG to be successful, with no instances of ICH and most, but not all, infants, achieving a platelet count of greater than 30×10^9/L at the end of pregnancy. The addition of steroids did not add to the effect of IVIG. However, ICH has been found during maternal treatment with IVIG,[32] and a group of European centers

treating 37 pregnancies only found success with the use of maternal IVIG in 7 of 27 cases (26%), and steroids in 1 of 10 cases (10%).[33] It is difficult to understand why there was such a difference in the success of maternal administration of IVIG between these two studies. Relevant factors may include the methods used for assessing the success of treatment and the dose, timing, and type of IVIG used. The selection of cases may also be important.

A number of studies have shown the value of platelet transfusions given by cordocentesis in raising the platelet count, but the platelet count is raised for only a few days. A single predelivery transfusion may protect against bleeding at the time of delivery, but the fetus remains at risk of spontaneous ICH earlier in pregnancy. Weekly in utero platelet transfusions have been shown to be effective in preventing ICH in severe cases of FMAIT,[34] but this approach is invasive.

In a recent study from the European Study Group for NAIT, the outcome of 56 fetuses receiving antenatal treatment for NAIT due to HPA-1a alloimmunization compared favorably with the outcome in previous pregnancies.[35] Cases with a sibling history of antenatal ICH or severe thrombocytopenia (platelet counts of $< 20 \times 10^9$/L) had significantly lower pretreatment platelet counts than cases whose siblings had less severe thrombocytopenia or postnatal ICH. Maternal therapy resulted in a platelet count exceeding 50×10^9/L in 67 percent of cases. None of the fetuses managed by serial platelet intrauterine transfusions (IUTs) suffered ICH after treatment started. However, the most serious complications encountered by the study cases were associated with fetal blood sampling (FBS). The results of this study support the use of maternal therapy as first line treatment for the antenatal management of NAIT. The observations of this study suggest that the commencement of maternal therapy can be stratified on the basis of the sibling history of NAIT. In two recent studies, concern regarding the safety of FBS led to the use of a less invasive treatment strategy involving maternal administration of IVIG without FBS for monitoring of the fetal platelet count, without an increased incidence of ICH.[36,37]

Antenatal treatment appears to have the potential to improve the outcome of severely affected cases of NAIT, but there is little information on the long-term development of children who have been treated in utero.

Routine antenatal screening

Advances in laboratory diagnosis and antenatal management have drawn attention to the fact that the first affected fetus/neonate in a family is only recognized after bleeding has occurred, and this has raised the question of whether routine screening for NAIT would be advantageous.

An important initial question is whether to carry out screening antenatally or postnatally. The advantages of antenatal screening are that alloimmunized women can be identified during pregnancy, allowing time for antenatal intervention if it is agreed that this is appropriate. Even if no antenatal intervention is undertaken, the mode and timing of delivery can be planned to ensure minimal trauma to the baby's head and that compatible platelets are available, if needed. Postnatal screening can be achieved by simply carrying out a platelet count on a cord blood sample, but the major drawback of this approach is that the key objective of screening, to prevent morbidity and mortality from ICH, is unlikely to be achieved in the index pregnancy.

Although it is recognized that antenatal hemorrhage due to NAIT can produce devastating clinical effects, significant shortcomings exist in the knowledge about NAIT necessary for the introduction of an antenatal screening program. Further research is required on a number of issues, including the range of clinical outcomes in affected cases, the identification of factors useful for predicting severe disease, and the preferred option for antenatal management in women with anti-HPA-1a but no previous history of affected pregnancies.[38]

Posttransfusion Purpura

A clinical case of thrombocytopenia developing 7 days after elective surgery, then spontaneously resolving 3 weeks later, was first described in 1959 by van Loghem and colleagues.[39] The 51-year-old woman involved was found to have a strong platelet alloantibody, subsequently described as the first human platelet alloantibody, Zw. Two years later, a similar case was described by Shulman and colleagues, who coined the term posttransfusion purpura (PTP).[40] In each case the same human platelet alloantibody was implicated (named Zw in the first case and anti-PI^{A1} in the case by Shulman, now known as HPA-1a).

Definition

PTP describes an acute episode of severe thrombocytopenia occurring 5 to 12 days after a blood

transfusion. It is usually seen in HPA-1a negative women previously alloimmunized by pregnancy or transfusion. The implicated transfusion is thought to induce a secondary immune response, boosting the production of HPA-1a antibodies and destruction of transfused donor platelets. At the same time, the patient's own platelets are also destroyed.

Incidence

PTP is considered to be a rare complication of transfusion, although the true incidence is unknown. A UK-based voluntary and confidential scheme for reporting serious hazards of transfusion (SHOT), set up in 1996, reported 43 cases in the first 6 years of the scheme, during which approximately 20 million blood components were transfused, giving an approximate incidence of 1 case in 465,000 transfusions. However, since the introduction of universal leukoreduction of blood components in 1999, there has been a reduction in the annual number of cases reported to SHOT.[41]

As in NAIT, the susceptibility of HPA-1a negative individuals to PTP appears to reflect their ability to make HPA-1a antibodies, which is strongly associated with HLA Class II –DRB3*0101.

Clinical features

The typical patient is a middle-aged or elderly woman who has had a previous exposure to platelet antigens through pregnancy, transfusion, or both. The occurrence of PTP has also been reported in a small number of male patients. The time interval between the initial sensitizing event and the subsequent transfusion stimulating PTP is variable, with previous reports ranging from 3 to 52 years.

Clinically, the patient presents 5 to 12 days after transfusion with an acute severe thrombocytopenia (platelet count < 10×10^9/L) that has fallen from normal within 12 to 24 hours. Associated hemorrhage is very common and sometimes severe, with widespread purpura, and bleeding from mucous membranes and the gastrointestinal and urinary tracts. If untreated, the condition usually lasts between 7 and 28 days, although occasionally it may persist longer.

Implicated blood products include whole blood, packed RBCs, and RBC concentrates. PTP has also been reported following transfusion of plasma.

Laboratory investigations

A clinical diagnosis of PTP needs to be confirmed by the finding of platelet-specific alloantibodies in antigen-negative individuals. The majority of cases of PTP (80 to 90%) are associated with the development of HPA-1a antibodies, but other HPA antibodies have also been implicated, including HPA-1b, HPA-3a, HPA-3b, HPA-4a, HPA-5a, HPA-5b, HPA-15b, and Nak^a, and occasionally multiple antibodies are present.

Platelet-specific antibodies are detected using the MAIPA assay, which is able to resolve mixtures of antibodies, including HLA antibodies, that are often present in patients with PTP but do not appear to have a pathological role.

Other causes of a rapid onset of severe thrombocytopenia should also be excluded, such as disseminated intravascular coagulation (the coagulation screen is normal in uncomplicated cases of PTP), autoimmune thrombocytopenia, and drug-induced thrombocytopenia, e.g., heparin-induced thrombocytopenia (HIT). Rarely, thrombocytopenia may occur within 48 hours of a transfusion secondary to passively transfused platelet-specific alloantibodies from an immunized blood donor.

Pathophysiology

A rapid secondary antibody response (usually against HPA-1a) is stimulated by the RBC transfusion, with the result of acute thrombocytopenia about 1 week later. This time course of events is well established. However, it is still unclear why the patient's own antigen-negative platelets are destroyed. A number of hypotheses have been suggested:

- Transfused HPA-1a-positive platelets release HPA-1a antigen, which is adsorbed onto the patient's HPA-1a-negative platelets, making them a target for anti-HPA-1a. Support for this hypothesis comes from observations such as the elution of anti-HPA-1a from HPA-1a-negative platelets in some cases of PTP and the demonstration of the adsorption of HPA-1a antigen onto HPA-1a-negative platelets after incubation with plasma from HPA-1a-positive stored blood.
- The released HPA-1a antigen forms immune complexes with anti-HPA-1a in the plasma and the immune complexes become bound to the patient's platelets, causing their destruction.
- The transfusion stimulates the production of platelet autoantibodies as well as anti-HPA-1a. Evidence in favor of this mechanism is the detection of positive reactions of some PTP patients' sera from the acute thrombocytopenic phase with autologous platelets and the isolation

of autoantibodies that recognize calcium-dependent epitopes on GPIIb/IIIa during the acute phase.[42]
- In the early phase of the secondary antibody response, anti-HPA-1a may be produced, which has the ability to crossreact with autologous as well as allogeneic platelets.

Management

The main aim of treatment is to prevent morbidity and mortality associated with severe thrombocytopenia by shortening the duration of thrombocytopenia. A particular risk is ICH, which may cause early fatalities. Prompt treatment is therefore essential, to prevent this.

There have been no randomized controlled trials of treatment for PTP, and comparison of various therapeutic measures is complicated because of the occurrence of spontaneous remissions. Currently the treatment of choice is high-dose intravenous immunoglobulin (IVIG) (2g/kg given over 2 or 5 days), with rapid responses seen in about 85 percent of cases.[43] Steroids and plasma exchange were the preferred treatments before the availability of IVIG, and plasma exchange, in particular, appeared to be effective in some, but not all, cases.

Platelet transfusions are usually ineffective in raising the platelet count, but they may be needed in large doses to control severe bleeding in the acute phase, particularly in patients who have recently undergone surgery before their response to high-dose IVIG. There is no evidence that platelet concentrates from HPA-1a-negative platelets are more effective than those from random donors; the dose probably is more important.

Prevention

PTP may recur, although this is unpredictable and occurs many years later. Patients with a previous episode should be issued a card to indicate that they need special blood products, and ideally future blood or platelet transfusions should be either autologous or from HPA-compatible donors.

The reduction in cases of PTP reported to the SHOT scheme since the introduction of universal leukoreduction in the UK suggests that leukoreduced blood products may be safe.

Refractoriness to Platelet Transfusions

Platelet transfusions are effective in decreasing hemorrhagic complications of severe thrombocytopenia. They may be given therapeutically to patients with active bleeding or prophylactically to patients with thrombocytopenia secondary to bone marrow failure. Serious spontaneous hemorrhage is unlikely to occur at platelet counts above 10×10^9 /L,[44] and this level has been widely adopted as a threshold above which prophylactic platelet transfusions are not required in an otherwise stable, nonbleeding patient.[45] Although prophylactic platelet transfusions are standard practice for patients with bone marrow failure, no recent randomized controlled trials have compared therapeutic versus prophylactic transfusions in terms of incidence of hemorrhage and associated morbidity. Indeed, concerns about platelet refractoriness secondary to alloimmunization have led some to suggest lowering the platelet transfusion threshold to 5×10^9 /L.[45]

Definition

Platelet refractoriness is defined as the repeated failure to achieve satisfactory responses to platelet transfusions. This can be assessed clinically in a bleeding patient. However, where platelet transfusions have been given prophylactically, response is assessed by measuring the posttransfusion platelet count increment.

Various formulae have been devised to assess the platelet increment, including the percent platelet recovery and CCI. In practice, a 24-hour increment of $< 5 \times 10^9$/L on two or more occasions is a good indicator of refractoriness to random donor platelets.

Causes of platelet refractoriness

Refractoriness is due to the shortened survival of the transfused platelets in the recipient's circulation, historically described in 20 to 60 percent of patients receiving multiple transfusions.[46] The causes may be classified as immune or nonimmune, but in any individual patient there may be a multifactorial etiology.

Nonimmune clinical factors include disseminated intravascular coagulation (DIC), splenomegaly, and intravenous antibiotics (especially antifungal drugs such as amphotericin B).[47,48] Fever has also been implicated in causing poor responses to platelet transfusions, although whether this is a reflection of sepsis, associated DIC, or antibiotic therapy rather than the temperature itself is unclear.[49] Another unknown factor is whether platelet refractoriness may be due in part to inadequate dosage of platelets. Further studies

are required to assess the optimal dosage of platelets, which may need to be adjusted according to a patient's blood volume.

The most common cause of alloimmune platelet refractoriness is HLA alloimmunization. Other immune causes include HPA alloimmunization, high titer ABO antibodies in the recipient, platelet autoantibodies, and drug-related platelet antibodies.

HLA antibodies predominantly occur in women with a history of pregnancies and/or a history of multiple transfusions. If they are suspected as a cause for refractoriness, a combination of screening tests for both cytotoxic and noncytotoxic HLA antibodies should include the lymphocytotoxicity test (LCT) with either the lymphocyte or PIFT, or an ELISA-based method.

The role of platelet-specific antigens in platelet refractoriness is unclear. HPA antibodies occur at a frequency of 8 percent[50] to 20–25 percent[51] in various studies and are usually found in combination with HLA antibodies,[52] although rarely they may occur in isolation. Most commonly, HPA alloimmunization is directed toward antigens with phenotypic frequencies below 30 percent. Some studies have suggested that there is no clear correlation between HPA antibodies and poor responses to platelet transfusions,[53,54] but others have found that matching for platelet-specific antigens in those refractory to HLA-matched platelets may be beneficial.[55]

Management

The management of platelet refractoriness first requires an assessment of possible nonimmune clinical causes. These should be corrected if possible and prophylactic platelet transfusions from random donors continued in the usual way. If poor responses to platelet transfusions persist, HLA antibodies should be sought in the patient's serum and, if they are present, platelet transfusions matched for the HLA-A and -B antigens of the patient should be used.

If poor responses continue, consideration should be given to ABO and/or HPA incompatibility. Testing for platelet-specific antibodies may identify HPA antibodies, and future platelet transfusions lacking the relevant antigen may be indicated. Platelet crossmatching may be helpful in cases where the platelet-specific antibodies have no obvious HPA specificity and the only way of identifying compatible donors is to find those who are crossmatch negative.[45] ABO incompatibility is an unusual cause of platelet refractoriness, seen usually when there are high-titer ABO antibodies in the recipient. ABO-identical platelet concentrates should be given to exclude this possibility.

Prevention

Leukoreduction of blood components has been shown to reduce the incidence of HLA alloimmunization and platelet refractoriness.[50]

Drug-Dependent Antibodies

Drug-dependent antibodies are an important cause of shortened platelet survival that can often be overlooked, especially in hemato-oncology patients who may have thrombocytopenia for a variety of reasons. In some cases, drugs too small to elicit an immune response by themselves may bind as a hapten to platelet GPs in vivo. This haptenized platelet GP can trigger the formation of antibodies that only bind to the GP in the presence of the hapten. A classic example is quinine and its optical stereo-isomer, quinidine. Typically, quinine-dependent antibodies are against GPIIb/IIIa, GPIb/IX/V, or a combination of both. Vancomycin has also been shown to induce the formation of IgG antibodies that bind specifically to GP IIb, IIIa, or both in the presence of the antibiotic.[56] Similarly, the interaction of heparin with platelet factor 4 can cause antibody formation and lead to a drug-dependent thrombocytopenia.[57,58] Evidence is also emerging implicating teicoplanin as a cause of immune-mediated thrombocytopenia.[59]

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