In many ways platelet and red blood cell (RBC) immunology are similar, but there are important differences. At least 272 RBC antigens have been described. These have been placed into 22 systems (194 antigens), 7 collections (20 antigens), and 2 series (high-frequency antigens [12] and low-frequency antigens [46]). Platelets express human leukocyte antigens (HLA) (class 1 only), ABH, P (not P1), Lewis, I, and platelet-specific antigens on their membranes. Platelet-specific antigens can be classified into five biallelic systems, originally called PlA1 (Zw), Ko(Sib), Bak, Yuk (Pen), and Br. Each system includes a high-frequency antigen occurring in 96 percent to >99 percent of the population, and homozygosity for a lower-frequency antigen, occurring in 4 percent to <1 percent of the population. In addition, a number of additional low-frequency (private) antigens (e.g., Moa, Caa, Sra, Vaa, Iya, Pea, Groa, Maxa) have been described.2–4 Like RBC antigens and antibodies, platelet-specific antigens and antibodies were first named using letters from the name of the proposer (e.g., Bak, Br, Yuk).

In 1990 an international working party on platelet serology suggested a numerical terminology.5 The term HPA was introduced to indicate human platelet antigen. The antigens were numbered in order of their discovery. Allelic antigens were named alphabetically, with the high-incidence allele first (= a) and the lower-incidence allele second (= b). Table 1 shows equivalent platelet-specific alloantigen terms. In 1995 four more systems were added and designated HPA-6W, -7W, -8W, and -9W. The “W” stands for “workshop,” or “not yet worked out,” as both alleles have not been defined.6 Just as with RBC numerical terminology, universal acceptance of the HPA terminology has been slow. In addition, the nomenclature does not consider the underlying genetic polymorphism. Because platelet-specific antigens differ by a single amino acid substitution at the protein level, it has been suggested that the nomenclature be based on the findings at the molecular level, but this too is controversial.6–7

The term “platelet specific” antigen is now known to be a misnomer. As with many RBC antigens (e.g., ABH, Lewis, P, Cr, Lu, and LW), some of the so-called “platelet specific” antigens have recently been detected on other cells.8 HPA-1 (PlA1) and HPA-4 (Yuk/Pen) have been detected on endothelial cells, fibroblasts, and smooth muscle cells. HPA-5 (Br) is expressed on long-term activated T lymphocytes and on endothelial cells.8 So far, HPA-3 (Bak) and HPA-2 (Ko) have been found only on platelets.8

### Investigation of Immune Thrombocytopenia

Most technologists, pathologists, and even hematologists are more knowledgeable about RBC immunology than platelet immunology. Our reference laboratory often receives requests for “platelet antibodies,” and it is obvious that the individuals ordering the test do not know the optimal tests to request for a particular investigation. These same individuals would never send in a
request for “RBC antibodies.” They would usually ask for a direct antiglobulin test (DAT), serum antibody screen, hemolytic disease of the newborn (HDN) or autoimmune hemolytic anemia (AIHA) workup, cross-match, etc., whichever was appropriate for the patient’s condition.

The rationale for the appropriate tests to investigate immune thrombocytopenia is very similar to the rationale for investigating immune hemolytic anemia. The classification of the immune thrombocytopenias parallels that of the immune hemolytic anemias (see Table 2). The rationale behind the diagnostic tests is quite similar, but there are some major differences in the predictive value (PV) of the tests.

Antibody-sensitized RBCs and platelets probably are destroyed in a very similar way, and the factors affecting the pathogenicity of antibodies are quite similar.\(^9\) RBCs or platelets, sensitized with IgG (IgG1 or IgG3), can interact with Fc receptors on macrophages and be destroyed by cytotoxicity or phagocytosis within the spleen or liver. Complement may also be activated by IgM or IgG antibodies, leading to intravascular destruction of the RBCs or platelets, but it is thought that platelets are more often destroyed extravascularly.\(^8\)–\(^10\)

Therapy for immune thrombocytopenia and immune hemolytic anemia are also quite similar (e.g., steroids, splenectomy, transfusion). A major difference is the more extensive use of intravenous immunoglobulin (IVIg) for thrombocytopenia. IVIg is commonly used to treat adults with autoimmune thrombocytopenic purpura (AITP),\(^8\)–\(^10\),\(^12\) and posttransfusion purpura (PTP),\(^10\) but its use for treating neonatal immune thrombocytopenic\(^13\),\(^14\) and refractory patients is controversial.\(^15\)–\(^19\) IVIg is not used routinely for the treatment of AIHA, although some investigators have suggested it may be useful.\(^12\) It is unknown how IVIg works.\(^20\) The earliest, and still perhaps the most popular, hypothesis is that the large amount of IgG causes a reticuloendothelial system (RES) blockade, by blocking macrophage Fc receptors. Others have argued that there are many reasons to think that this hypothesis is not valid and have suggested other hypotheses. Mueller-Eckhardt et al. (see recent review by Salama and Mueller-Eckhardt\(^21\)) suggested that IVIg is contaminated with RBC alloantibodies (e.g., anti-A and -B) and that these sensitize the patient’s RBCs, and it is the IgG-sensitized RBCs that compete with the IgG-sensitized platelets for Fc receptors. They went on to support their hypothesis by showing that intravenous Rh immune globulin (IVRhIg) given to Rh+ patients with AITP was as efficient and much cheaper than IVIg. An intravenous RhIg (WinRho SD, Univax Biologicals, Inc., Rockville, MD) has been licensed recently by the FDA for use in the United States. Another popular theory for the IVIg mechanism is that the beneficial effects have nothing to do with an RES-blockade, but rather are due to the presence of anti-idiotypic in the IVIg.\(^20\)

From a laboratory viewpoint it is important to know whether the patient has received the above products. IVIg can cause falsely positive platelet antibody assays,\(^22\) and IVRhIg can cause anomalous results because anti-D may be detected in a patient unexpectedly.\(^23\)

**Table 2. Classification of the immune thrombocytopenias and immune hemolytic anemias**

<table>
<thead>
<tr>
<th>Hemolytic anemia</th>
<th>Thrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>autoimmune</td>
<td>autoimmune</td>
</tr>
<tr>
<td>acute</td>
<td>chronic</td>
</tr>
<tr>
<td>chronic</td>
<td>neuronal</td>
</tr>
<tr>
<td>hemolytic disease of newborn</td>
<td>hemolytic transfusion reaction</td>
</tr>
<tr>
<td>delayed</td>
<td>poor survival of transfused red cells</td>
</tr>
<tr>
<td>drug-induced</td>
<td>drug-induced</td>
</tr>
<tr>
<td>immune hemolytic anemia</td>
<td>immunohematologic anemia</td>
</tr>
</tbody>
</table>

**Platelet antibodies**

When a report states that “platelet antibodies” are detected, it indicates that an antibody(ies) was (were) detected that reacted with platelets by one of many in vitro assays.\(^24\) A platelet-reactive antibody may be reacting with any antigens that are on platelets. These include HLA (Class I only), platelet-specific antigens, ABH, P (not P\(_1\)), Lewis, and I antigens. The most common reactants are HLA or platelet-specific antigens. One must also remember that if the ABO type of the target platelets is unknown, or if they are ABO incompatible, then IgG anti-A or -B may be detected as a “platelet” antibody. If “platelet” antibodies are detected, it is important that specificity tests be performed; at the very least, HLA antibodies should be differentiated from platelet-specific antibodies. HLA and platelet-specific antibodies can be differentiated relatively easily. A patient’s serum can
be tested with untreated and acid- or chloroquine-treated platelets. HLA antigens, but not platelet-specific antigens, are depressed by acid or chloroquine treatment.25,26 A better test is to test the patient’s serum against specific platelet glycoproteins (GPs).27 Specific GPs are usually attached to a solid phase (e.g., microplate), and after adding patient’s serum and washing, a labeled (e.g., with an enzyme or radioisotope) anti-IgG is added. Freedman and Hornstein28 described a novel approach using flow cytometry to differentiate HLA and platelet-specific antibodies.

Autoimmune Thrombocytopenic Purpura (AITP)

Thrombocytopenic purpura is often autoimmune in nature and equivalent to AIHA, but AITP is much more common than AIHA.2 There are three distinct syndromes of AITP: (1) idiopathic thrombocytopenic purpura (ITP): a chronic thrombocytopenia of insidious onset, without any identifiable antecedent or associated illness, that typically affects young and middle-aged adults, predominantly women; (2) secondary AITP: this resembles ITP clinically, but is associated with other autoimmune diseases, malignancy, or a variety of other disorders that have in common a disorder of the immune system; and (3) acute postviral thrombocytopenia: an acute self-limiting thrombocytopenic purpura that typically affects young children within about 3 weeks of an acute viral infection and occasionally after immunization (also described as acute or childhood ITP).8,10,29 The term “ITP” is often used generically for all these conditions.

The most useful diagnostic tests for AITP are similar in principle to those used for AIHA (Table 3). The most valuable test is that used for platelet-associated IgG (PAIgG), the equivalent of a DAT on RBCs.

Detection of PAIgG is more complicated than the detection of IgG on RBCs, as simple agglutination tests (e.g., DAT) cannot be used. PAIgG can be detected by using anti-IgG combined with a measurable marker (e.g., a radioisotope, enzyme, fluorochrome, or RBCs). Another complication is that when patients (especially children) are severely thrombocytopenic, it is difficult to obtain enough platelets to perform some of these assays.

Unlike RBCs, the majority of PAIgG is not on the surface of the cell, but is associated with the α-granules inside the platelet.30 Some investigators have argued that it is more valuable to measure total IgG (e.g., in a platelet lysate) than to measure only the IgG on the surface of the platelet.8,10,30 Others have argued that measuring the IgG on the membrane is more important because that is the only IgG available for interaction with the Fc receptors on macrophages. Most investigators use assays that measure only the PAIgG on the platelet membrane.

As in AIHA, proteins other than IgG (e.g., IgA, IgM, and complement) can be detected on the platelets of patients with AITP. As in the AIHA literature, different authors disagree on the merits of detecting such proteins in AITP.

One difference between RBCs and platelets is that platelets tend to accumulate proteins, nonimmunologically, on their surface.31,32 Several groups have shown that PAalbumin increases in proportion to PAIgG in AITP.32 It has been suggested that this PAIgG might be due to trapping of plasma proteins in platelet interstices or microvesicles, and that this trapping might be enhanced by antibody-induced damage to platelets. An alternative explanation is that plasma proteins may be incorporated preferentially into young (newly formed) platelets (e.g., in AITP).10 Because of the presence of PAlgG that is not autoantibody, the predictive value (PV) of measuring PAIgG is low. Some investigators claim that their assays detect IgG autoantibody preferentially to nonimmune IgG.33,34 Approximately 80 percent of AITP patients have increased PAlgG; unfortunately, PAlgG can also be increased in many other conditions (e.g., systemic lupus erythematosus and septicemia).31,32 Kelton et al.32 calculated that the positive PV of a positive PAIgG assay in a thrombocytopenic patient was only 46 percent, but the PV of a negative assay was 82 percent. In contrast, in a patient with hemolytic anemia, the PV of a positive DAT is 83 percent and the PV of a negative DAT is 99 percent.35 If too few platelets are available for testing for PAIgG, then tests for platelet antibodies in the serum can be performed. It should be emphasized that, as in AIHA, most of the circulating antibody may be adsorbed in vivo onto the patient’s platelets, and the serum may contain very little or no antibody.

Table 3. Most useful tests for diagnosing autoimmune hemolytic anemia (AIHA) and autoimmune thrombocytopenic purpura (AITP)

<table>
<thead>
<tr>
<th>AIHA</th>
<th>AITP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is autoantibody on cell?</td>
<td>Direct antiglobulin test</td>
</tr>
<tr>
<td>2. Does serum contain autoantibody?</td>
<td>Indirect antiglobulin test</td>
</tr>
<tr>
<td>3. Specificity of serum antibody?</td>
<td>autoimmune, allo (both)</td>
</tr>
</tbody>
</table>
The common Rh specificity of warm autoantibodies associated with AIHA became obvious when broadly reactive sera were found to be nonreactive with RBCs of the rare Rhnull and -D– phenotypes. Similarly, the specificity of autoantibodies associated with AITP became obvious when platelets from patients with the rare Glanzmann’s thrombasthenia known to be severely deficient in GPllb/IIa failed to react with the autoantibodies. Autoantibodies associated with AITP in adults or children are usually directed against GPllb/IIa glycoproteins. Table 1 shows the various platelet-specific antigens and the GPs with which they are associated.

Because of the low PV of diagnostic serologic tests, the diagnosis of AITP is often one of exclusion.

**Neonatal Alloimmune Thrombocytopenia (NAIT)**

NAIT is the equivalent of HDN (i.e., maternal IgG platelet antibody crosses the placenta and attacks fetal platelets). It is thought that 1:2,000 to 1:5,000 random neonates suffer from NAIT. The diagnostic approach is similar to the investigation of HDN (Table 4). One major difference from HDN is that 60 percent of NAIT occur during the first pregnancy. Another difference is that it is not routine practice to screen all mothers, so the problem often presents as an emergency following the birth of an affected newborn. When a mother has a history of thrombocytopenic newborns, or has just delivered a thrombocytopenic newborn, the maternal serum should be tested for platelet antibodies. In Caucasians, about 50 percent of NAIT is associated with maternal anti-HPA-1a (PlA1). Anti-HPA-1a will react with 97 percent to 98 percent of the Caucasian population. Thus, the mother and newborn are usually typed for HPA-1a. The newborn’s platelets can also be tested for PAIgG. If the antibody is not anti-HPA-1a and/or the mother is HPA-1a positive, other specificities should be considered (examples of NAIT caused by anti-HPA-1b, -2, -3, -4, -5, and -6 have been published). Of 424 mothers of children with NAIT, studied in a 10-year period in Germany, 40 percent were found to be homozygous for HPA-1b (HPA-1a-negative); in 75 percent of these, anti-HPA-1a was detected in their serum. Sixty percent were HPA-1a-positive; 26 percent of these had platelet-reactive antibodies that were identified as anti-HLA; only 6 percent had platelet-specific antibodies. Although other platelet-specific specificities were confirmed, it was not proven that specificities other than anti-HPA-1a were responsible for NAIT. In contrast, in Japan the HPA-1b phenotype is hardly ever found, so anti-HPA-1a is not detected; anti-HPA-4b is the most common cause of NAIT in Japan.

The role of HLA antibodies in NAIT is highly controversial. IgG HLA antibodies certainly cross the placenta and the fetal platelets have HLA antigens, but HLA antigens are widely distributed on cells other than platelets, so there is competition for the HLA antibody that crosses the placenta. On rare occasions, sufficient uninhibited antibody may remain, and could react with fetal platelets. This situation would be similar to ABO RBC antibodies and the low incidence of clinically significant HDN due to ABO antibodies. There are some data in the literature that support the possibility of NAIT being caused by HLA antibodies. As with HDN, on rare occasions antibodies to low-frequency antigens are present, leading to a negative serum screen. If this situation is suspected, the maternal serum should be tested against the father’s platelets (one should remember that ABO incompatibility may lead to falsely positive results).

McFarland et al. found that the detection of platelet-specific antibodies in maternal sera has a good PV for moderate to severe NAIT. However, they found, as have other authors, that failure to detect such antibodies does not always predict a normal neonatal platelet count. Thus, as with AITP, the diagnosis of NAIT is often one of exclusion. It has been argued that the theoretical risk is that 1 in 42 pregnant women could have a baby with NAIT. Nevertheless, the highest reported incidence in a published series is 1 in 678; most series report that only 1 in 2,000–5,000 neonates have NAIT. Obviously, many factors, such as those described as affecting RBC
destruction, play a role. One important factor that does not pertain to RBC alloimmunization is the effect of Class II HLA genes. There is a strong association in women who make anti-HPA-1a with DRW52a.42

Recently, especially in Europe, it has been recommended that all pregnant women should be typed for HPA-1a, and that the HPA-1a-negative women be tested for DRW52a, and for the presence of anti-HPA-1a in their serum.35-45 If anti-HPA-1a is detected or there is a history of NAIT, then some investigators suggest percutaneous umbilical blood sampling (PUBS) at 20 weeks gestation.44 They recommend that if the platelet count of the fetus is <100 × 10^9/L, then 1g/Kg IVIg should be given weekly and PUBS performed again 4–6 weeks later. If the platelet count is 300 × 10^9/L, IVIg treatment should be continued; if <30 × 10^9/L, the fetus should receive platelet transfusions once a week until delivery.31 These approaches are obviously controversial and open to cost-benefit arguments.24,25 A recent publication by Panzer et al.46 suggests that there is little benefit in the above approach. In a prospective study of 933 mother-child pairs, in which the mother typed negative for HPA-1, -2, -3, or -5, antibody screening was performed. Anti-HPA-1a was not detected in 11 HPA-1b mothers. One anti-HPA-3a and 17 anti-HPA-5b were detected; all of the babies, in the presence of these antibodies, had normal platelet counts. Only nine neonates had platelet counts <150 × 10^9/L; these low platelet counts were associated with, but not proven to be due to, anti-HLA(5) and anti-A(4).46

Transfusion of newborns with neonatal thrombocytopenia due to anti-HPA-1a

As in HDN, the newborn can be transfused in utero or ex utero; ideally the cells should lack the putative antigen. As only 2 percent to 3 percent of the United States (US) population are HPA-1a-negative, platelets lacking HPA-1a are available only if the blood center (e.g., our own blood center) actively screens apheresis donors with anti-HPA-1a to detect HPA-1a-negative donors. Unfortunately, these donors are not always available quickly enough, and transfusion with the mother’s HPA-1a-negative platelets should be considered for the first transfusion. If possible, these platelets should be washed to remove maternal anti-HPA-1a. If washing is not possible, then as much antibody-containing plasma as possible should be removed from the maternal platelets before transfusion to the affected child.

Posttransfusion Purpura (PTP)

Delayed hemolytic transfusion reactions (DHTRs) have been reported to occur in 1 in 854 (0.12%) patients.47 These are usually noticed because the patient becomes jaundiced or has an inappropriate response to the transfusion, or antibodies are detected 7–14 days following the transfusion. As platelets have a shorter life span than RBCs, and the clinical signs of increased platelet destruction are less obvious, there is no direct parallel with DHTRs. Nevertheless, there is a platelet syndrome that is a delayed transfusion reaction. This syndrome is known as PTP, and occurs much less frequently than DHTRs.

PTP is an uncommon but serious disorder characterized by the development of acute, potentially life-threatening thrombocytopenia about 1 week following transfusion of RBCs, plasma, or platelets.8 About 90 percent of affected patients are women who formed anti-HPA-1a during pregnancy. Although the patient’s own platelets are destroyed, her platelets usually type as HPA-1a-negative. Thus, transfusing HPA-1a-negative platelets during the acute phase has no more value than transfusing HPA-1a-positive platelets. Nevertheless, it is recommended that during the recovery phase or for future transfusions, “antigen-negative” (e.g., HPA-1a-negative) blood products be used.8

The pathophysiology of PTP is unknown. Duration of symptomatic thrombocytopenia in patients given only supportive therapy with RBC or platelet transfusions has been from 1 to 35 days, the average being approximately 14 days. The total period of thrombocytopenia until platelet counts return to normal has varied from 6 to over 100 days, the average being approximately 24 days. Patients with thrombocytopenia lasting over 40 days usually had underlying serious illnesses that complicated interpretation of recovery. Duration of PTP or responsiveness to treatment cannot be predicted by the initial platelet level, hemorrhagic symptomatology, or antibody titer.8

PTP may have more in common with DHTRs than has been previously thought. Recent studies on DHTRs have shown that the alloantibody causing the DHTR can be recovered (i.e., eluted) from the patient’s RBCs that lack the putative antigen many months following the reaction.47,48 It is still unclear whether that antibody would sensitize other “antigen negative” RBCs transfused at that time. There are also many interesting reports of autologous RBCs being hemolyzed by an “innocent bystander” lysis process (e.g., in transfused patients with sickle cell
Similarities—platelet and red cell immunology

serum against platelets in the presence of the drug, using the same technique used for platelet antibody detection. This approach has not been very productive for detecting heparin antibodies, which are probably the most common cause of DIIT. It has been reported that 3 percent to 30 percent of patients receiving heparin develop thrombocytopenia. Most of these patients have a mild to moderate thrombocytopenia, but about 25 percent of patients with heparin-induced thrombocytopenia (HIT) develop a life-threatening thrombocytopenia. It is unclear what percentage of these cases have an immune etiology. Recent studies indicate that the new low-molecular-weight heparins may not induce HIT as commonly as the higher-molecular-weight products.

The most reliable laboratory tests for HIT have not been serologic tests but functional assays such as platelet aggregation and serotonin-release assays. Recently, the mechanism for HIT has been clarified. Antibodies causing HIT are specific for a complex of heparin and a heparin-binding protein, factor-4 (PF-4), found in the alpha granules of platelets. Thus, the antibody will only be detected in vitro by using an assay where conditions are optimal for PF-4-heparin complex formation. This explains why serologic assays using whole platelets were unreliable. Assays using PF-4 (e.g., recombinant PF-4) would seem to be the most accurate approach.

Drug-Induced Immune Thrombocytopenia (DIIT)

DIIT is detected more often than drug-induced immune hemolytic anemia. Over 100 drugs have been reported to cause immune thrombocytopenia. Most of these drugs do not bind to proteins (i.e., on platelet membranes) very well, and it is not known how they act as immunogens. As drugs are low-molecular-weight substances, it is assumed that they must act as haptenss, and use protein carriers in order to induce antibodies. If this assumption is true, it does not involve the classic haptenic mechanism involving covalent bonding of hapten and protein.

It is controversial how most drug antibodies cause thrombocytopenia. It was first suggested (in the 1940s and 1950s) that the antibody reacted with an antigen formed by the drug + platelet. In the 1960s, Shulman suggested that it was more likely that drug antibody combined with the drug, resulting in drug-anti-drug immune complexes, and that these immune complexes attached “nonspecifically” to platelets, often activating complement, thus causing the platelets’ destruction. This theory reigned supreme for about 25 years, but recently has been criticized, and a return to the earlier hypothesis involving formation of a target “neoantigen” created by the drug and the platelet membrane has been favored. Shulman and Reid have suggested a modification of Shulman’s earlier immune complex hypothesis, incorporating an explanation for the data criticizing his earlier hypothesis.

The most common drugs to cause DIIT are heparin and quinine/quinidine. Antibodies to quinine/quinidine are usually demonstrable by testing the patient’s serum against platelets in the presence of the drug, using the same technique used for platelet antibody detection.

This approach has not been very productive for detecting heparin antibodies, which are probably the most common cause of DIIT. It has been reported that 3 percent to 30 percent of patients receiving heparin develop thrombocytopenia. Most of these patients have a mild to moderate thrombocytopenia, but about 25 percent of patients with heparin-induced thrombocytopenia (HIT) develop a life-threatening thrombocytopenia. It is unclear what percentage of these cases have an immune etiology. Recent studies indicate that the new low-molecular-weight heparins may not induce HIT as commonly as the higher-molecular-weight products.

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Refractoriness to Platelet Transfusions

It is common practice to use prophylactic platelet transfusions for thrombocytopenic patients undergoing chemotherapy or bone marrow transplantation. Approximately half of these patients (20%-50% of leukemic patients, and 80% of patients with aplastic anemia) become refractory to further platelet transfusions. Refractoriness is defined as a lack of expected response following two platelet transfusions. There are two parameters that characterize a platelet response: the initial posttransfusion platelet recovery (PPR) or corrected count increment (CCI) and the subsequent survival of transfused platelets.

The PPR is calculated as follows:

$$\text{PPR} = \text{platelet increment} \times \frac{\text{patient's weight (kg)}}{\text{blood volume}} \times \frac{\text{platelet count of infused platelets}}{\text{volume of platelets (mL)}} \times \text{volume of platelets (mL)}$$

where

- *platelet increment* = posttransfusion count (at 10–60 minutes) – pretransfusion count (normal PPR = 66 ± 8%)
The CCI is calculated as follows:

\[
CCI = \frac{\text{platelet increment} \times (\text{body surface area in } \text{M}^2)}{\text{number of platelets transfused} \times 10^{11}}
\]

Guidelines

- <30% recovery at 1 hour or survival <2 days = abnormal response
- CCI of <7.5 \times 10^9 or <10 \times 10^9/L (depending on the investigator) within 1 hour of transfusion, and/or <4.5 or <7.5 \times 10^9/L within 18–24 hours = unsuccessful transfusion
- CCI of 30 \times 10^9/L = 100 percent recovery
- CCI of 18 \times 10^9/L = 60 percent recovery
- CCI of 7.5–10 \times 10^9/L = 25 percent to 30 percent recovery
- CCI of 4.5–7.5 \times 10^9/L = 15 percent to 25 percent recovery
- If platelet recovery or CCI is not calculated, then an increment of <5 \times 10^9/L after two sequential transfusions of six units of platelet concentrates (or 1 apheresis unit) can be used as an indication to further evaluate the refractoriness.

Causes of refractoriness

Refactoriness may be due to immune or nonimmune causes. Immune causes of refractoriness include HLA antibodies, platelet-specific autoantibodies and alloantibodies, ABO antibodies, and immune complexes. Bishop et al.59 identified splenectomy, splenomegaly, bone marrow transplantation, disseminated intravascular coagulation, and use of intravenous amphotericin B as the major nonimmune causes affecting the CCI. A number of other factors including antibiotics, bleeding, and temperature also played a role. A poor response to a single transfusion (or even several transfusions) may also be due to the age of platelets transfused; this factor is often not taken into account. Doughty et al.60 found that 44 percent of a series of patients with hematologic malignancies were refractory to platelet transfusions. In 88 percent of these patients, the poor response was in the presence of nonimmune factors. Nonimmune factors were present alone in 67 percent, and in combination with immune factors (“platelet” antibodies) in a further 21 percent. “Platelet” antibodies were present in 25 percent of unsuccessful transfusions, and were the only factor present in 13 percent of the patients. Although there was a poor response to 60 percent of the platelet transfusions given to six patients with HLA antibodies, nonimmune factors could have played a role in 92 percent of these transfusions.60 Friedberg et al.61 found that no one single factor consistently explained the CCI variation in refractory patients.

The role of HLA antibodies in refractoriness

The most common immune cause of refractoriness is thought to be IgG HLA antibodies in the recipient’s plasma reacting with HLA antigens on the transfused platelets.

Patients appear to form HLA antibodies more commonly than they form RBC antibodies. Approximately 40 percent of patients with malignant disorders receiving chemotherapy and 1 percent to 5 percent of pregnant women (30%–70% of multiparous women) make HLA antibodies.58 Only 0.3 percent to 2 percent of random patients and pregnant women make RBC alloantibodies; in selected groups of multitransfused patients (e.g., those with sickle cell anemia), the incidence of RBC antibodies can be 20 percent to 30 percent. More recent reports show an incidence of 0 percent to 22 percent HLA immunization in patients transfused with modified blood products (e.g., leukodepleted). Some patients never become alloimmunized to HLA, despite repeated transfusions. Dutcher et al.62 found that 54 percent of 114 patients undergoing chemotherapy never made HLA antibodies. Other workers have reported that in two thirds of the patients with HLA antibodies, the antibodies disappear and do not reappear despite subsequent transfusions.63

HLA antibodies can be detected by lymphocyte cytotoxicity tests (LCTs) or platelet antibody tests. There are many more HLA antigens on lymphocytes than on platelets, so one would expect that weaker HLA antibodies may be detected by LCT and not by a test utilizing platelets as a target. However, LCT is a complement-dependent test and will not detect IgG noncomplement-activating antibodies. It is not clear if this is the reason why HLA antibodies are sometimes detectable by platelet antibody tests and not by LCT. Some investigators argue that platelets, not lymphocytes, are being transfused, therefore, the target in our test system for HLA antibodies should be platelets, not lymphocytes. Another advantage of the platelet test is that it should detect platelet-specific in addition to HLA antibodies.

The role of platelet-specific antibodies in refractoriness

On rare occasions, platelet-specific autoantibodies or alloantibodies are thought to cause decreased platelet survival. If platelet-specific autoantibodies are causing
AITP, then as with AIHA, they can cause shortened survival of transfused platelets. Because the autoantibodies are being continually adsorbed by the patient’s own platelets, there usually is a limited amount of autoantibody left in the patient’s plasma to react with transfused platelets. Most refractory patients are those undergoing chemotherapy or bone marrow transplant and do not have AITP. Platelet-specific alloantibodies are rarer than HLA antibodies, but some examples have been shown to cause decreased survival of transfused platelets. The only platelet-specific antigen system that, on theoretical grounds, would be expected to play a significant role in refractoriness is the HPA-3 (Bak) system. This situation is possible because 22 percent of Caucasians will be homozygous for HPA-3b, and upon HPA-3a immunization will react with 88 percent of transfused platelet donations; 36 percent of recipients who are homozygous for HPA-3a will react with 64 percent of platelet donations. Interestingly, anti-HPA-3 does not appear to be more commonly reported as a cause of refractoriness than does HPA-1b, -2b, and Nak. Some authors have suggested that 20 percent to 30 percent of refractory patients may have platelet-specific antibodies. The platelet-specific antibodies are not always obvious because they are often masked by HLA antibodies and, if present, may not be the cause of the refractoriness. Platelet-specific antibodies should be detectable by platelet crossmatching methods, and compatible units can be selected for transfusion, or preferably the specificity can be identified and platelets lacking the putative antigen selected and crossmatched.

The role of ABO and other antibodies in refractoriness

Anti-A and anti-B are the most clinically significant antibodies as far as RBCs are concerned. This is not the case for platelets. The major reason for this fact is that the number of A and B antigen sites on platelets is far less than on RBCs, and there is a much larger variation from one individual to another. Although ABO is often ignored as a matter of convenience when transfusing platelets to refractory patients, it is well established that anti-A and anti-B can shorten platelet survival and sometimes may be the cause of the refractoriness. When a recipient (usually group O) has a high titer anti-A or anti-B, and the donor platelets have a high expression of A or B antigens, then platelet destruction may be severe enough to cause refractoriness. Ogasawara et al. found that 7 percent of Japanese group A, B, and AB donors had a high expression of A or B antigens. Heal et al. have suggested that it is not always good enough to have a major ABO match—ABO-identical platelets are optimal. In a prospective study, patients given ABO-identical platelets required only half as many transfusions in the first 30 days as patients in the ABO mismatch group. A smaller percentage of patients in the ABO-identical group became refractory (36% vs. 75%).

It has also been suggested that circulating immune complexes (e.g., CICs) can cause shortened platelet survival. Heal et al. have suggested that CICs formed by anti-A or anti-B with A and B substances in plasma can cause shortened platelet survival. The same group and other investigators have also pointed to CICs formed by antibodies to plasma proteins (e.g., IgG, C2, C3, albumin, and fibrinogen). The antibodies can be in the recipient’s or the donor’s plasma. Heal et al. showed that after 20 transfusions, >90 percent of recipients made antibodies to foreign plasma proteins.

Selecting Platelets for the Refractory Patient

**HLA-based selection**

The major emphasis for selecting platelets has always been based on the belief that HLA antibodies were the main cause of the refractoriness. For many years (and perhaps even now) the major approach was based on typing the recipient for HLA (A and B) and trying to match that type as closely as possible. Platelet donors are HLA-typed and entered in a local registry. A computer program is used to select the best “match.” A popular form of the “match” definition is shown in Table 5.

<table>
<thead>
<tr>
<th>“Match”</th>
<th>Definition</th>
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<tbody>
<tr>
<td>A</td>
<td>All 4 antigens identical</td>
</tr>
<tr>
<td>BU*</td>
<td>2 or 3 antigens identical with blanks in balance of haplotype</td>
</tr>
<tr>
<td>BX*</td>
<td>2 or 3 antigens identical with balance being cross-reactive antigens</td>
</tr>
<tr>
<td>C</td>
<td>3 antigens identical, but 4th is nonidentical</td>
</tr>
<tr>
<td>D</td>
<td>2 or more antigens are nonidentical</td>
</tr>
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*Sometimes designated as BU1 or BUX, indicating that 3 antigens are identical, or BU2 or BU2X, indicating that 2 antigens are identical.

The problem with the above list is that A and BU are the only real matches, BX and C are the best mismatches, and D is probably no better than random selections. Thus, a preferred term to “HLA matched” would be “HLA-based” selection.

Without a very large HLA-typed donor base, it is very difficult to provide A matches. Schiffer et al. reported that 2,470 donors yielded 1.3 perfectly matched (i.e., A match) donors; 9 with no mismatches (i.e., BU match-
es) and 40 with one or more crossreactive antigens (BX matches). Sixty-two percent of their refractory patients had no A-matched donors, and 38 percent had no BU-matched donors available. In contrast, in Japan, where the population is much more homogenous, Takahasi et al. reported that with 5,000 donors they could get more than five perfectly matched donors for 80 percent of their refractory patients. In the United States, a donor base of 18,000–25,000 would be needed for comparable results.

Another approach is one that parallels the way RBC incompatibility is approached. The HLA antibody is detected and the specificity defined using LCT. When a specificity is determined, then platelets are selected from a donor that lacks the putative HLA antigen. The lack of popularity of this method probably relates to the fact that cytotoxic antibodies are broadly reactive and difficult to identify. Nevertheless, some institutions (e.g., Stanford University) use this approach routinely.

**Crossmatching**

Compatibility testing is performed before all RBC transfusions. Such testing is used to detect ABO incompatibility and the presence of any alloantibodies of potential clinical significance. Considering that a lower percentage of patients have RBC antibodies than HLA antibodies, which are known to shorten platelet survival, it is perhaps surprising that platelet crossmatching is not performed more often (e.g., for every platelet transfusion, or at least for every refractory patient).

Some RBC antibody specificities have clearly been shown to shorten RBC survival, and sometimes lead to acute hemolytic transfusion reactions (HTRs). The clinical sequelae of HTRs can sometimes be life-threatening. Most platelet transfusions are given prophylactically to thrombocytopenic patients who are thought to be at risk of bleeding. Many (if not most) of these transfusions are probably not necessary, and the results are monitored poorly. At levels between 75,000 and 140,000/μL, patients are generally asymptomatic. Below 75,000/μL, patients experience easy bruising, enhanced menstrual blood loss, and occasional epistaxis. At levels between 20,000/μL and 75,000/μL, these bleeding tendencies may be further exacerbated but are still generally not clinically significant. At levels between 10,000/μL and 20,000/μL, petechiae, spontaneous purpura, dental bleeding, and spontaneous bleeding from the kidneys may occur. Nevertheless, it is still quite unusual that such patients would experience any mas-sive bleeding or intracranial hemorrhage unless there are aggravating factors such as infection with fever, mucositis, gastritis, gastroenteritis, colitis, or proctitis. At levels below 10,000/μL, spontaneous cerebral hemorrhage in the absence of other aggravating factors may occur, as well as fatal or life-threatening hemorrhage from the gastrointestinal tract if the aggravating factors named previously are also present. Platelet data equivalent to the RBC data concerning antibody specificity, cell survival, and clinical response to transfusions are poor, especially in nonrefractory patients (i.e., the majority of recipients). Therefore, there has been no pressure to use antibody screening or crossmatching for routine platelet transfusions, and the value for refractory patients is still controversial.

There are many publications suggesting that crossmatching has a high PV for successful transfusion of refractory patients, but many of these studies can be criticized. Factors to look for when analyzing these studies are as follows: (1) Is the study prospective or retrospective? (2) Were patients with nonimmune factors known to cause refractoriness excluded from the study? (3) Were the patients preselected to include only those who had detectable platelet antibodies? (in which case there would be a much better PV than in random refractory patients)? (4) Were there any associations with producers of commercial products? (5) Were ABO-compatible or ABO-identical platelets used?

Freedman reviewed the best crossmatching studies published up until 1989. Since then, several other studies have been published. It should be remembered that crossmatching is of value only for patients with detectable platelet-reactive antibodies. Among refractory patients, the incidence of those with platelet-reactive antibodies has been reported to be as low as 25 percent to 50 percent.

As with crossmatching RBCs, there are many methods of crossmatching platelets. All of them depend on incubating the patient’s serum with platelets, and then detecting uptake of platelet antibody (usually IgG only) onto the platelets by adding a labeled anti-human globulin (AHG). The most popular methods have utilized AHG (e.g., anti-IgG) labeled with I125 (radioimmune assay), alkaline phosphatase (enzyme-linked immunosorbent assay [ELISA]), fluorescein (immunofluorescence [IF]), or a solid-phase red cell adherence (SPRCA) assay utilizing RBCs coated with anti-IgG. As with methods for detecting RBC antibody, sensitivity and speci-
ficity vary between methods, and each method has its advocates. The SPRCA assay is perhaps the most utilized, because it is the only test that is commercially available (Immucor, Inc., Norcross, GA).

The American Red Cross organized a five-center national prospective study comparing the use of platelets selected by HLA type or by crossmatching of random platelets for refractory patients. Patients were excluded if they had any nonimmune factors that were thought to influence refractoriness. Sera were tested for platelet antibodies by three different methods (IF, ELISA, and RIA) and for HLA antibodies by LCT. The results were as follows:

1. The overall rate of successful transfusion was similar when an HLA-based method of donor selection that included all grades of matching and mismatching was compared to a crossmatch-based method of donor selection.
2. HLA-based selection that restricts recipients to grade A and BU matches was superior to a selection method based upon crossmatching alone.
3. Selection of donors based on HLA cross-reactive groups (defined by in vitro serologic cross-reactivity) was no more successful than grade C and D mismatches, and was no more successful than selection by crossmatching alone.
4. Lymphocytotoxic and “platelet” antibodies were not detected in the sera of approximately half of the enrolled patients, even though patients demonstrating nonimmune factors were eliminated from the study (a more recent, unpublished study by our laboratory showed that approximately 75% of random refractory patients had platelet antibodies detectable by the Immucor SPRCA assay).

The three methods used in the above study and the Immucor SPRCA method were also compared in a blinded study using antibodies associated with known clinical significance (i.e., associated with a known CCI). The PV of a negative crossmatch in sera containing HLA antibodies (LCT-positive) were as follows: RIA 65 percent; ELISA (2 centers) 57 percent/65 percent; IF (2 centers) 61 percent/74 percent; and SPRCA 68 percent. In sera that were LCT-negative, the PV was 42 percent to 46 percent. The efficiency (i.e., taking into account sensitivity and specificity) of the various assays was as follows: RIA 73 percent; ELISA 60 percent/69 percent; IF 66 percent/69 percent; and SPRCA 75 percent. Based on the results of the above study, we use the protocol shown in Table 6 for refractory patients.

**Conclusions**

Treatment of refractory patients is usually a trial-and-error approach. Refractoriness is often due to nonimmune factors, or these factors add to the antibody-mediated problems. Approximately 60 percent to 80 percent of refractory patients respond well to HLA-matched platelets, but it is important to remember that A matches (all HLA-A and -B antigens identical) and BU matches (2 or 3 antigens identical with blanks in the balance of the phenotype) are really the only matched products. Some patients (i.e., those with antibodies detectable by platelet antibody assay) may do better with crossmatched platelets. Finally, some patients, even those with no antibodies detectable by platelet antibody assays, may do well with platelets lacking antigens to the HLA cytotoxic antibody. An optimal product would also be ABO identical, or at least ABO compatible.

**References**


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**Table 6.** Protocol used by American Red Cross Blood Services, Southern California Region, for transfusing refractory patients

1. Try HLA-matched (A or BU match) platelets.*
2. If A or BU matches are not available, then—
   a. If patient has a detectable platelet (HLA) antibody, crossmatch BX-matched platelets* (or C matched platelets* if BX is not available).
   b. If patient has no detectable platelet (HLA) antibodies, then try BX- or C-matched platelets.*
3. If above does not work, then try identifying HLA cytotoxic antibody and finding a donor that lacks putative antigen(s).

*It is always preferable to use ABO-identical platelets; if these are not available, then use ABO-compatible platelets. The HLA match always supersedes the ABO type (e.g., an ABO-incompatible A match is preferable to an ABO-compatible C “match”).
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