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Evaluation of the Patient with Suspected Platelet Refractory State

NOTE: While evaluating the patient for suspected immune refractory state, provide ABO matched platelets if available.

<table>
<tr><td rowspan="2">1. Determine if the patient is at risk for alloimmunization:</td><td colspan="3">Alloimmunization may be directed against platelet specific antigens or, more commonly HLA antigens. Current leukoreduction methods are generally effective at preventing HLA alloimmunization. Alloimmunization may occur in multiply transfused patients.

A. Multiparous female?
B. Multiple nonleukoreduced transfusions?
C. Organ transplant?</td></tr>
<tr><td colspan="3">Assess previous platelet transfusions:
TWO previous ABO compatible/ABO matched transfusions fail to yield an increase in platelet count >10K or a CCI >5-7500

Platelet counts should be performed 10 – 60 minutes post transfusion and 24 hour post transfusion.

One hour post transfusion platelet counts that fail to demonstrate a >10K increase in platelets is suggestive of immune destruction.

Adequate one hour post transfusion platelet counts and decreased 24 hour post transfusion platelet counts are suggestive of consumption/utilization/sequestration.</td></tr>
<tr><td rowspan="7">2. Exclude non-alloimmune causes of a platelet refractory state:

These are generally disorders of platelet consumption.

Drugs are an often overlooked cause of platelet destruction.</td><td>Condition</td><td>Presentation</td><td>Laboratory Findings</td></tr>
<tr><td>Sepsis</td><td>Fever, tachycardia, tachypnea, etc.</td><td>Leukocytosis, +cultures</td></tr>
<tr><td>DIC</td><td>Bleeding, oozing</td><td>Thrombocytopenia, elevated PT, APTT, decreased fibrinogen, + fibrin degradation products</td></tr>
<tr><td>Sequestration</td><td>Splenomegaly</td><td></td></tr>
<tr><td>Drugs</td><td>▪ Antibiotics (esp Vancomycin)
▪ Heparin (Heparin induced thrombocytopenia)*
▪ Amphotericin</td><td>Thrombocytopenia, diminished response to transfusion</td></tr>
<tr><td>Auto-Immune thrombocytopenia* (aka ITP)</td><td>▪ Purpura, esp of lower extremities
▪ Easy bruising
▪ Nose bleeds
▪ Bleeding gums</td><td>Thrombocytopenia
Auto antibodies to platelet glycoprotein IIb/IIIa</td></tr>
<tr><td>Bleeding</td><td>Significant bleeding may consume platelets</td><td>Thrombocytopenia, diminished response to transfusion</td></tr>
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Evaluation of Patient (continued)

3. Evaluate for Alloimmunization

Document the presence or absence of an antibody(ies) directed against HLA Class I or platelet specific antigens. The following are the most widely available:

Assay	Method	Findings
Platelet crossmatch Donor platelets with recipient serum/plasma.	Solid phase red cell adherence (SPRCA), ELISA, Flow cytometry	At least one incompatible crossmatch
Platelet Antibody Screen Antibody screen to HLA Class I and platelet specific antibodies.	Solid phase red cell adherence (SPRCA), ELISA	Positive
Panel Reactive Antibody (PRA) Determines % of HLA antigens recipient antibodies are directed against.	Lymphocytotoxity, ELISA, Fluorescence	▪ PRA >20% is considered refractory ▪ Test is not platelet specific but focuses on anti-HLA antibodies.

4. Product Selection

At a minimum select ABO matched or compatible platelets with the longest expiration date.

If alloimmunization is present and needs will be ongoing, request HLA typing.
It typically takes a few days and one or two days after that until HLA matched platelets are available. Support with XM platelets until HLA matched are available, if possible.

Findings	Product to select	Further Actions
No risk factors for alloimmunization present?	ABO matched platelets (longest dates available)	Monitor
Risk factors for alloimmunization laboratory evaluation pending	ABO matched platelets (longest dates available)	Monitor
Incompatible platelet cross-match or + Antibody screen or PRA >20%	Crossmatch compatible platelets	Availability dependent upon inventory and assay and to number/prevalence of antigens recipient antibodies are directed against.
	HLA matched platelets	▪ Requires HLA type of recipient ▪ Specific donor called to donate if match is present ▪ 72 hour TAT typical ▪ Note that C&D grade matches are generally no better than an "off the shelf" product for these recipients.
	Antigen negative platelets (ASP method or HLA Matchmaker): selection of platelets negative for the antigens the patient antibodies are directed against.	▪ Requires identification of HLA antigens recipient antibodies are directed against. ▪ Manual process at many centers ▪ Not widely available

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Evaluation of Patient (continued)

5. Therapy Assessment	Platelet counts should be performed within 1 hour post transfusion and at 24 hour post transfusion to assess therapy. If a unit is identified that produces a desired increase in recipient platelet count take note so that the donor may be recruited for additional donations.

Platelet Transfusion Refractoriness

Platelet transfusion refractoriness is defined as a less-than-expected increase (usually less than 10,000/mm3) in a patient's platelet count on at least two occasions with assessment performed 10 minutes to 1 hour after the transfusions. This is a common occurrence in thrombocytopenic patients that have had multiple transfusions (incidence of 20 – 70% in highly transfused patient populations) especially with nonleukoreduced blood components. The causes for platelet refractoriness are diverse but frequently divided into immune and nonimmune causes.

Common Causes of Platelet Refractoriness

Non-Immune	Immune
DIC	Alloantibodies to HLA antigens
Sepsis	Alloantibodies to platelets specific antigens
Fever	Autoantibodies (ITP, etc)
Bleeding	Drugs (Heparin, etc)
Sequestration (Splenomegaly)	
Drugs (including Amphotericin B)	

Determining if a Patient is Refractory to Platelet Transfusion

Several objective methods exist to determine if a patient is refractory to platelet transfusion. The most common method used is the **corrected count increment** or **CCI**, however, other methods such as the **percent platelet recovery (PPR)** and **linear regression analysis** are occasionally used. Each method has its strengths and weaknesses. We will discuss the CCI and PPR here since they are in widespread use and easy to perform. Both methods "correct" the platelet increment for the patient's blood volume and the number of platelets transfused.

Formula for Calculation of the Corrected Count Increment

$$CCI = \frac{BSA(m^2) \times PCI \times 10^{11}}{\text{No. of platelets transfused}}$$

BSA = body surface area; PCI = platelet count increment

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Formula for Calculation

For example, a 70 kg patient has a body surface area of 1.8 m^2, a pretransfusion platelet count of 5,000/µL, and is transfused with an apheresis platelet containing 3.2 x 10^{11}. The post transfusion platelet count is 30,000/µL. The CCI would be calculated as follows.

$$CCI = \frac{1.8\ m^2 \times (30{,}000/\mu L - 5{,}000/\mu L) \times 10^{11}}{3.2 \times 10^{11}}$$

$$CCI = \frac{1.8\ m^2 \times 25{,}000/\mu L \times 10^{11}}{3.2 \times 10^{11}}$$

CCI = 14,000 platelet x m^2/µL

Alternatively, the PPR for this patient may be calculated as follows.

$$\textbf{PPR} = \frac{\textbf{TBV x PCI}}{\textbf{No of platelets transfused}}$$

TBV = total blood volume; PCI = platelet count increment. The total blood volume may be estimated for adults utilize 75 mL/kg, pediatrics 80 mL/kg and neonates 85 mL/kg.

Using the above example

$$PPR = \frac{(70\ kg \times 75\ mL/kg) \times (30{,}000/\mu L - 5{,}000/\mu L) \times 10^3}{3.2 \times 10^{11}}$$

$$PPR = \frac{5250\ mL \times 25{,}000/\mu L \times 10^3}{3.2 \times 10^{11}} \times 100$$

PPR = 41%

Both the CCI and the PPR are determined shortly after transfusion usually 10 – 60 minutes. A CCI greater than 7,500 platelets x m^2 BSA/µL or a PPR greater than 20% are considered acceptable. Platelet counts taken later in the course of therapy (12 – 24 hours later) are of less value in determining alloimmunization as they may be indicative of consumption or peripheral destruction.

Often, thrombocytopenic patients may have several reasons for a suboptimal CCI or PPR and it is helpful to look for alloimmune refractoriness by attempting to identify an antibody. The most common methods for the detection of platelet antibodies include:

- Enzyme Linked Immunosorbant Assay
- Solid Phase Red Cell Adherence Assay (SPRCA)
- Monoclonal antibody-specific Immobilization of Platelet Antigens (MAIPA)

Flow cytometry is used less commonly.

ELISA based assays are convenient because of their long shelf life but require equipment not commonly found in the blood bank (plate washer, plate reader, etc.). SPRCA assays are easy to perform and require only a plate centrifuge and inexpensive reader, however, they have a shelf life of only a few weeks and the U.S. manufacturer of FDA approved test kits occasionally runs into production problems that may prevent the kit from being available when it's needed.

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Providing Compatible Platelets for the Immune Refractory Patient

Several methods are available for attempting to provide compatible platelets for the alloimmunized patient. Generally, platelets are required immediately or very soon and the method employed is based upon what is available in the community.

Procedures used to provide compatible platelets in immune refractoriness

- Platelet Crossmatch
- HLA – Matched
- Antibody Specificity Prediction Method (ASP)

Platelet crossmatching can be performed, most commonly by SPRCA or ELISA. Just as with a red cell crossmatch, recipient plasma/serum containing the putative antibody is incubated with donor platelets in the test kit and compatibility is determined. This method is typically used to find compatible platelets for a patient with anti-HLA or anti-platelet antibodies. When HLA-matched or platelet antigen-negative donors are not available or platelets are needed emergently, some institutions use platelet crossmatching as a "first-line" test. The rationale for this practice is that if several donors are compatible then refractoriness is unlikely and if most donors are incompatible the patient is highly refractory.

HLA-matched platelets may also be used for patients with anti-HLA antibody mediated refractoriness. Platelets bear the HLA-A and HLA-B Class I antigens. Platelet donors are selected with HLA antigens that most closely approximate or match the recipient's. The HLA type of the refractory patient must be known and the blood collection agency must have a registry of HLA typed donors. Furthermore, there are several different grades for HLA matches. Match grades A and B1-B4 are acceptable. B1X may be used occasionally if donors with higher match grades are unavailable, but B2X and lower match grades are not likely to be more effective than random platelets.

Match Grades for HLA-matched Platelets

Grade	Antigen Matches
A	Donor has no mismatched antigens. All of donor's antigens are identical to patient's antigens, even if donor is homozygous at A and/or B and has fewer antigens.
B1 – B4	Donor has 1 – 4 antigens that are in the same broad antigen group as a patient antigen. All other antigens in donor are identical to a patient antigen. (This match grade should be considered almost equal to A.)
B1X – B4X	Donor has 1 – 4 antigens that are in the same cross reactive group (CREG) as a patient antigen. Other antigens are identical or in the same broad antigen group as a patient antigen.
C1 – C4	Donor has 1 – 4 antigens that are mismatched to patient's antigens. Other antigens are identical or in the same broad antigen group or in the same CREG as a patient antigen.

The **antibody specificity prediction (ASP)** method takes the opposite approach to HLA matching. In this approach, the refractory patient's serum must be tested for HLA antibodies and the specificities identified. Platelets lacking antigens the recipient has antibodies for can be selected, equivalent to crossmatch negative platelets. Antibody testing is more expensive than HLA typing the recipient, but this approach should be used if the refractory patient will need multiple platelet units and donor searches based on the patient's HLA typing are providing insufficient numbers of matched donors. This method appears to be equally efficacious to HLA-matched platelets.

Refractoriness to Platelet Specific Antigens

Refractoriness to platelet specific antigens presents a unique challenge because most blood collection agencies in the United States do not extensively type donors for platelet specific antigens. Several blood centers have donors typed for HPA-1a primarily for the treatment of neonatal alloimmune thrombocytopenia but rarely other platelet antigens. The selection of compatible platelets is similar to the ASP method for HLA induced platelet refractoriness; selection of a donor negative for the antigen to which the patient has developed the antibody.

Methods for Detection of Platelet-Specific Antibodies and Antigens

- Enzyme-linked immunosorbent assay (ELISA)
- Modified antigen capture ELISA (MACE)
- Monoclonal antibody immobilization of platelet antigens (MAIPA)
- Platelet immunofluorescence test
  - Manual
  - Flow Cytometry
- Solid-phase red cell adherence assay (SPRCA)

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- Dzik S. How I do it: platelet support for refractory patients. *Transfusion* 2007; 47:374-378.