EDUCATIONAL COMMENTARY – PLATELET REFRACTORINESS

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Learning Outcomes

On completion of this exercise, the participant should be able to

- define platelet refractoriness;
- list nonimmune mechanisms for refractoriness;
- discuss the etiology of immune platelet refractoriness;
- describe test methods for human leukocyte (HLA) antigen and antibody determinations; and
- discuss strategies for platelet transfusion in the patient with platelet refractoriness.

Introduction

Platelets are a critical component for maintaining hemostasis in patients who are actively bleeding and those with low number of platelets or with non-functional platelets. When a blood vessel is injured, platelets adhere to the exposed collagen, forming the primary platelet plug. Platelets then aggregate at the injured site, secreting various substances that promote vasoconstriction, clot formation, and fibrinolysis. Once the primary platelet plug has been stabilized by fibrin, forming a clot, platelet thrombasthenin is responsible for clot retraction.

Platelets are transfused to patients who are actively bleeding, as a prophylactic measure for those with non-functional or low numbers of platelets, and to those with bone marrow aplasia, whose bone marrow is not producing a sufficient quantity of platelets to maintain hemostasis. In 2015, 1,983,000 platelet units were transfused in the United States, of which 91% were apheresis platelet units.¹

Platelet Refractoriness: Definition and Determination

When platelets are transfused, the 1-hour post-transfusion platelet count is expected to increase by 5 × 10³ to 10×10³ /µL for each whole blood–derived platelet concentrate or by 20 × 10³ to 60 × 10³ /µL for each apheresis platelet unit. When after two consecutive platelet transfusions the expected increase in platelet count is not attained, the patient’s condition is considered refractory to platelets.

The effectiveness of a platelet transfusion may be evaluated using the post-transfusion platelet increment (PPI), which is also known as the count increment (CI) or the corrected count increment (CCI) (Box 1).
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The PPI is a straightforward calculation in which the pre-transfusion platelet count is subtracted from the post-transfusion count to determine the increase (increment) achieved. The CCI is determined using the PPI, the patient’s body surface area, and the number of platelets transfused. Refractoriness is indicated when the CCI is less than $5 \times 10^3$ to $7.5 \times 10^3/\mu$L following 1-hour CCI values on two consecutive days. CCIs should be performed twice at a minimum and should be measured 10 to 60 minutes after a platelet transfusion.

Box 1. Calculation of PPI and CCI.

| PPI = Post-transfusion platelet count minus pre-transfusion platelet count |
| CCI = PPI (/µL) × body surface area (m²) / Number of platelets transfused (10¹¹) |

There are a variety of reasons a patient may become refractory to platelet transfusions. These can result from immune and nonimmune mechanisms.

Nonimmune Mechanisms

The most common reason a platelet transfusion fails to achieve the expected PPI is a nonimmune mechanism. Nonimmune reasons for platelet refractoriness can be broadly grouped into those that increase platelet consumption and those that stimulate platelet activation. Treating the underlying condition generally restores the platelet count. The most common nonimmune mechanisms include the following:

- **Fever**: One of the most common causes for platelet refractoriness, especially in hematology/oncology patients, fever can greatly reduce the post-transfusion platelet count. This is particularly true when the fever is greater than 38.4°C (101°F). Accompanying sepsis and antibiotic use appear to contribute to the problem.
- **Splenomegaly**: The spleen normally stores approximately one-third of the body’s platelets. When the spleen is enlarged, the proportion of stored platelets increases.
- **Bleeding**: Platelets are lost during the bleeding episode and are consumed as they form the primary platelet plug in damaged blood vessels.
- **Disseminated intravascular coagulopathy (DIC)**: DIC is a condition in which there is systemic clotting and simultaneous fibrinolysis. Platelets are consumed in the clotting process.
- **Sepsis**: Sepsis appears to increase platelet activation. Severe sepsis can trigger DIC. Fever and antibiotic use can contribute to the platelet dysfunction seen in sepsis.
- **Thrombotic thrombocytopenic purpura (TTP)**: TTP is a hematologic disorder caused by a deficiency of ADAMTS13 or an acquired antibody to ADAMTS13 (a disintegrin and
metalloproteinase with a thrombospondin type 1 motif, member 13), an enzyme that cleaves von Willebrand factor. TTP is characterized by systemic thrombosis, which consumes platelets.

- Medications: Heparin, Vancomycin, Amphotericin, and many more. For example, platelets exposed to the antifungal agent amphotericin B demonstrate increased membrane expression of P-selectin, a marker of platelet activation. In addition, these platelets show increased adhesion to granulocytes, which leads to clearance of platelets from circulation.3

Immune Mechanisms

Once nonimmune causes have been eliminated as the source of refractoriness, an investigation into immune causes should be initiated. In most immune-mediated cases, antibody-coated platelets are removed from circulation by the mononuclear phagocytic system.

**Antibodies to Human Leukocyte Antigens**

Most cases of immune-mediated platelet refractoriness are caused by alloantibodies directed against human leukocyte (HLA) antigens. HLA antigens, also known as major histocompatibility complex (MHC) antigens, are glycoproteins found on the surface of nucleated cells throughout the body. Platelets express HLA-A and HLA-B antigens, and small amounts of HLA-C antigens.

Patients who have repeatedly received transfusions of non-leukoreduced blood components, which are those containing WBCs, and women who have had multiple pregnancies are most likely to produce HLA antibodies due to repeated exposure to foreign HLA antigens on leukocytes. Organ transplant recipients are also at risk. Transfusion of leukoreduced blood components has successfully decreased the incidence of refractoriness in these patients. Exposing pooled platelet concentrates to UVB radiation is equally effective for preventing refractoriness.4

**Antibodies to Human Platelet Antigens**

Although they are much less common than HLA antibodies, antibodies to human platelet antigens (HPAs) should be suspected as the source of refractoriness when there is no improvement in the CCI after transfusing leukoreduced components, when testing has failed to demonstrate the presence of HLA antibodies, or when transfusion of HLA-matched platelets does not achieve the expected PPI increment.

Currently 35 platelet antigens are recognized, found on six platelet-membrane glycoproteins. These antigens appear to be the result of single-nucleotide polymorphisms in the genes that control their expression.
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Anti-HPA-5b and anti-HPA-1b are most frequently detected in patients when the platelet refractoriness is caused by HPA. Platelet antibodies are more often found in conjunction with HLA antibodies than as a single antibody.

ABO Antibodies

A, B, and H antigens are found naturally as platelet membrane glycolipids and on platelet-surface glycoproteins. Soluble antigens can also be adsorbed from the plasma onto the platelet membrane. Major ABO incompatibility (donor platelets possess antigens for which the recipient has antibodies) and minor incompatibility (donor plasma contains antibodies directed against recipient antigens) have both been implicated in refractoriness. ABO-incompatible platelets also appear to inhibit platelet aggregation and may stimulate an increase in ABO antibody titers.

Other immune sources of refractoriness include the following:
- Immune thrombocytopenia: Autoantibodies to platelet antigens, most frequently GPIIb/IIIa
- Heparin-induced immune thrombocytopenia (HIT): Antibodies directed against heparin/platelet factor 4 complexes cross-link platelet Fcy IIA receptors, inducing platelet activation
- Post-transfusion purpura: Seen most often in multiparous women with a platelet antibody formed during pregnancy, post-transfusion purpura manifests after an RBC or platelet transfusion. The platelet antibody in these cases destroys both autologous and transfused platelets. Anti-HPA-1a is most frequently implicated.
- Cell-mediated clearance: Recent studies in mice suggest that CD8+ T lymphocytes can remove platelets independent of antiplatelet antibodies.

Management of Platelet Refractoriness

Management of refractoriness will depend on the etiology and the patient’s risk for bleeding. Nonimmune causes are best managed by treating the underlying disorder. Platelets should be transfused if there is ongoing bleeding or the patient is scheduled to undergo an invasive procedure with a risk for bleeding.

Treatment of the patient with immune-mediated refractoriness will depend on the causative antibody. Because most immune-mediated refractoriness is caused by HLA antibodies, the standard method for preventing refractoriness is to determine the recipient’s HLA antigen type and provide platelets that are antigen matched. For platelet-matching purposes, only the HLA-A and HLA-B antigens are determined.

HLA antigen typing for platelet matching is generally performed using molecular techniques such as reverse sequence-specific oligonucleotide probes (rSSO). In rSSO, oligonucleotide probes specific for a
single HLA allele are attached to a solid-phase matrix, such as a microbead, which has been labelled with a unique fluorescent tag. The recipient’s DNA from the HLA-A and HLA-B loci are individually amplified by polymerase chain reaction. Each of the recipient’s DNA samples is allowed to mix with the probes, and if the recipient’s DNA is complementary to the probe, a fluorescent signal is produced, which is measured by flow cytometry.

Once the recipient’s HLA antigen type is determined, the search begins for donor platelets with similar antigen content, with the ultimate goal of an identical four-antigen match. Turnaround time to obtain an HLA-matched platelet may be prolonged while searching for a suitable donor. A perfect four-antigen match may be difficult to find when the frequency of the desired haplotype(s) is low. An extensive search can be quite costly. When an identical match is not possible, antigens of similar structure, those belonging to the same cross-reactive group (CREG), may be substituted with satisfactory results. The degree of antigen match between the donor and recipient is graded A, B, or C. An “A match” is a four-antigen match, in which both HLA-A antigens and both HLA-B antigens of the donor and recipient are identical. In a “B match,” three of the four antigens are the same, with the fourth antigen either identical to one of the three known antigens (i.e., the donor has homozygous inheritance of 1 of the antigens); unknown (B1u match); or coming from the same CREG (B1x match). A platelet that matches two of the antigens and has one duplicate/blank and one cross-reactive antigen (B2ux) may also successfully increase the recipient’s platelet count. In a “C match,” three of the four antigens are the same, and the fourth antigen is a complete mismatch (Box 2). When providing HLA-matched platelets, A and B matches show the most success in improving the CCI, and C matches are generally less successful.

**Box 2. Examples of HLA A, B, and C matches.**

For this example, assume the recipient’s HLA type is A2, A11, B7, B35.

<table>
<thead>
<tr>
<th>Match Type</th>
<th>Antigen Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A match</td>
<td>A2, A11, B7, B35</td>
</tr>
<tr>
<td>B1u match</td>
<td>A2, A11, B35, – (Either homozygous inheritance of B35 or blank)</td>
</tr>
<tr>
<td>B1x match</td>
<td>A2, A11, B35, B54 (B54 belongs to the same CREG as B7)</td>
</tr>
<tr>
<td>B2ux match</td>
<td>A11, –, B35, B54 (Either homozygous inheritance of A11 or blank AND B54 from B7’s CREG)</td>
</tr>
<tr>
<td>C match</td>
<td>A2, A11, B8, B35 (B8 does not belong to the CREG for either B7 or B35)</td>
</tr>
</tbody>
</table>

To provide a larger supply of platelets for patients with refractoriness due to HLA antibodies, recent transfusion strategies have focused on HLA avoidance, that is, providing platelets that lack the antigens for those antibody specificities that the recipient possesses, much like providing antigen-negative RBCs to patients with RBC antibodies. With this strategy, the donor HLA type must still be determined, but
recipient testing focuses on which HLA antibodies are present. Antibody testing should be repeated periodically to verify that no new antibodies have formed.

Flow cytometry is useful for determining the presence of HLA antibodies. Serum is incubated with a panel of microbeads, each coated with a specific HLA antigen. Fluorochrome-labelled antihuman IgG is added to detect which beads have been sensitized with HLA antibodies. When using Luminex technology, two additional fluorochromes are used to produce various colors and intensities. The flow analyzer then determines the color of the reactive beads, and computer analysis of the mean fluorescent intensity (MFI) determines which antibodies are present. The cutoff value between positive and negative reactions is set by each laboratory, usually between 1000 and 3000 MFI, with an MFI greater than the cutoff value indicating the presence of the antibody (Figure).

![MFI Graph Showing Multiple HLA Antibodies](image)

**Figure.** MFI Graph Showing Multiple HLA Antibodies.

- X-axis shows HLA antibody specificity. Y-axis shows MFI.
- The laboratory that generated these results considers a value greater than 3000 MFI a positive reaction for antibodies to HLA class I antigens.
- This patient has a moderately reactive antibody to the HLA A2 antigen and weakly reactive antibodies to HLA A1, A23, A24, A32, A36, A68, A80, and B49.

A similar enzyme-linked immunosorbent assay (ELISA)–based method uses HLA antigens bound to microtiter wells. Serum is incubated with the antigens in the wells, followed by the addition of an enzyme-labelled antihuman globulin reagent that produces a color change when the substrate is added. This
color change may be read visually or may be determined by measuring the optical density. This method is less sensitive than flow cytometry.

When it has been determined that HLA antibodies are not the source of refractoriness or while awaiting HLA test results, an attempt to find suitable platelets may be made by performing a platelet crossmatch, using either flow cytometry or solid-phase RBC adherence (SPRCA). Both methods use intact platelets from the unit intended for transfusion incubated with the recipient's serum, followed by a wash step to remove unbound antibodies. In flow cytometry, the wash step is followed by the addition of fluorescent-labelled anti-IgG antihuman globulin reagent. If antibodies are present, the anti-IgG reagent will bind to them. The fluorescence of the recipient's specimen is compared with that of a negative control to determine compatibility. In SPRCA, the platelets are bound to a microtiter well before adding the recipient's serum. After the incubation and wash steps, anti-IgG-coated indicator RBCs are added and the microtiter well is centrifuged. If patient antibodies have sensitized the platelets during incubation, the anti-IgG will attach to those antibodies, creating a diffuse pattern of indicator RBCs throughout the well. If no antibodies were present, the indicator cells will form a pellet of indicator RBCs at the bottom of the well. Both flow and SPRCA methods have the advantage of detecting both HLA and platelet antibodies. Platelet cross-matching can be accomplished in a matter of hours, whereas HLA typing may take days to obtain results. However, providing platelets that are cross-matched only, rather than HLA matched, allows the possibility that the patient's immune system may be stimulated to form additional antibodies.

When antiplatelet antibodies are suspected as the source of refractoriness, several ELISA methods can be used to determine the antibody specificity. Antigen-capture ELISA (ACE), modified ACE (MACE), and monoclonal antibody immobilization of platelet antigens (MAIPA) all involve incubating the patient's serum with a panel of platelet glycoproteins with known antigen content, either before or after capture of the antigens in microtiter wells. Presence of the patient's bound antibodies is demonstrated by the addition of an enzyme-labelled antihuman globulin reagent. These tests are highly specific, detecting platelet antibodies only. When necessary, donor and recipient antigens are determined using molecular methods.

When ABO antibodies appear to be the source of refractoriness, transfusing platelets with low anti-A and anti-B titers or platelets preserved in a platelet additive solution may reduce the incidence of refractoriness. In addition, transfusion of fresh (less than 48 hours old) ABO-compatible platelets has been used as an interim measure to reduce refractoriness while awaiting HLA and HPA test results.
Summary

Platelet refractoriness is the repeated failure of platelet transfusions to provide the expected increase in the recipient’s platelet count. Refractoriness can be monitored by determining the 1-hour post-transfusion CCI. Nonimmune sources account for approximately 80% of refractory cases. The most common immune source is HLA antibodies. In those cases, refractoriness can be countered by providing HLA-matched or HLA-avoidant platelet components. A small number of cases are the result of antiplatelet or ABO antibodies. Platelet cross-matching may provide suitable platelets while the results of HLA or HPA testing are pending. Transfusion of fresh ABO-compatible platelets may also improve the post-transfusion increment.

References


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