

Platelet Transfusions in Immunologically Refractory Patients

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There are two main indications for platelet transfusions: i) therapeutic, for actual bleeding; and ii) prophylactic, for patients with bone marrow suppression or bone marrow failure, when the platelet count falls below a pre-agreed trigger, which used to be $20\text{--}30 \times 10^9/\text{L}$. This trigger has definitely decreased in recent years. In fact, many large bone marrow transplant centres in London have reduced the trigger for prophylaxis to $10 \times 10^9/\text{L}$ with no adverse consequences and considerable savings in patient donor exposure and in cost. In large cities with large numbers of hospitals and advanced medicine, most of the platelets produced are used prophylactically on a temporary basis, especially following bone marrow (BMT) or peripheral blood stem cell transplantation (PBSCT).

The provision of platelet supportive therapy is essential for the successful outcome of BMT, PBSCT and umbilical cord blood transplantation. The majority of recipients of BMT need platelets for a minimum period of 2-3 weeks, but in a large proportion this period is prolonged to 5 or more weeks. On the other hand, recipients of PBSCT seem to require platelet support in smaller quantity and for a shorter time, often one–two weeks. For example, at University College Hospital in London, recipients of autologous BMT require, on average, 20-30 adult platelet doses (300×10^9 platelets/dose) for support during the pancytopenic period whereas recipients of PBSCT require only 4 adult platelet doses on average (David Linch, personal communication). The period of thrombocytopenia after cord blood transplantation seems to be longer than after BMT. Most patients will fare well with random donor platelet concentrates (RDPC) obtained from routine donations of whole blood and containing not less than 55×10^9 platelets in 50 ml of plasma. Better quality platelet concentrates with less leucocyte contamination can be obtained from routine donations of whole blood that are processed into buffy coats by 'top and bottom' systems which are pooled in a closed system using a sterile connecting device. The pools are then processed into buffy coat residues and high quality platelet concentrates. Such pools, from 4 donations, provide an adequate adult dose of $250\text{--}300 \times 10^9$ platelets. Platelet concentrates of an even higher quality can be obtained using third-generation cell separators, allowing the collection of one or two doses from a single donor.

In general, T-cell depletion, graft-versus-host disease (GVHD), alloimmunisation, sepsis, fever, disseminated intravascular coagulation (d.i.c.), and ABO incompatibility of platelet concentrates considerably increase the need for platelet support. Platelets should have as low a content of white cells as possible; febrile reactions, HLA alloimmunisation, and immunological refractoriness to the transfusion of RDPCs are correlated directly with the de-

gree of white cell contamination in transfused components. There is a leucocyte threshold for immune-mediated adverse effects, which varies depending on the effect and on the individual patient; for example, the level of white cell contamination that leads to HLA immunisation is significantly lower than the level that induces febrile transfusion reactions.

Refractoriness to the transfusion of platelets is the failure to achieve the expected platelet increment after two consecutive platelet transfusion episodes. Several formulas have been developed to monitor the effectiveness of platelet transfusions. The platelet count in peripheral blood immediately before and 15 minutes, 60 minutes, and/or 24 hours after transfusion must be measured. In addition, the body surface area or blood volume of the recipient must be known. One of the accepted standard approaches to estimating post-transfusion platelet increment is to calculate the corrected count increment (CCI) with knowledge of the patient's body surface area (BSA), as follows:

$$\text{CCI} = \frac{\text{platelet increment (}10^9/\text{L)} \times \text{BSA (m}^2\text{)}}{10^{11} \text{ platelets transfused}}$$

The response to platelet transfusions can also be measured as the % recovery of platelets transfused at a defined time post-transfusion:

$$\% \text{ recovery} = \frac{\text{platelet count increment / ml} \times \text{blood volume}}{\text{No. platelets transfused}}$$

The platelet transfusion outcome is considered a failure if the CCI at 1h is <7.5 or the % recovery at 1h is $<15\text{--}20$, or if the CCI at 20–24h is <4.5 .

Pregnancy and especially transfusion before BMT are major causes of primary HLA alloimmunization and the consequent immunological refractoriness to further platelet transfusions; in those patients with aplastic anaemia who are transplanted soon after diagnosis, the incidence of refractoriness (and graft rejection) is lower than in those who receive multiple transfusions prior to transplantation. The main factors affecting development of refractoriness due to alloimmunisation are:

a) the time required for antibody formation: in a previously unexposed recipient it is unlikely that immunological refractoriness will develop within the first two weeks of starting platelet transfusions;

b) the number of transfusions and the white cell load in blood components: as stated above, there appears to be a leucocyte threshold for HLA alloimmunisation. It is accepted that primary HLA alloimmunisation can be prevented if the white cell load per transfusion episode is $<5 \times 10^6$.

c) immune status of the recipient: immunosuppressed patients on chemotherapy have a significantly lower rate of alloimmunisation than patients with aplastic anaemia who are not immunosuppressed. In addition, if patients have been immunised before by pregnancy or transfusion, secondary alloimmunisation ensues more rapidly and more easily, requiring lower white cell contamination in blood components.

Diagnosis and etiology

The diagnosis of immunological refractoriness to platelet transfusions is established after excluding non-alloimmune causes of refractoriness such as:

- transfusion of poor quality, non-viable platelets (difficult to prove prospectively)
- splenomegaly and hypersplenism
- disseminated intravascular coagulation caused by infection, septicaemia, or malignancy
- infection and fever, in particular CMV infection
- drug-related thrombocytopenia
- poor platelet transfusion responses in patients treated with Amphotericin B
- BMT in itself can lead to increased platelet consumption due to GVHD, CMV infection, formation of autoantibodies, drugs, and microangiopathic associated syndromes.
- platelet autoantibodies
- circulating immune complexes which bind to platelet Fc receptors leading to clearance by the mononuclear phagocytic system

The most common cause of immunological refractoriness to platelet transfusions is alloimmunisation to HLA class I antigens. Immune refractoriness can also be due to ABO incompatibility (both *major*, i.e., group A platelets transfused to O recipients and *minor*, O platelets to A recipients), and to platelet-specific antibodies. The diagnosis is made using both laboratory and clinical criteria:

a) Laboratory: As HLA antibodies are the predominant cause of immunological refractoriness to platelet transfusions, HLA antibody screening by lymphocytotoxicity is used as an indirect method for detecting platelet alloimmunisation. If complement-fixing HLA antibodies are not found in a refractory patient, a search should be undertaken for HLA antibodies reacting by the indirect antiglobulin technique (usually by flow cytometry) and for platelet-specific antibodies (usually by ELISA or flow cytometry). Some centres such as in Leiden, the Netherlands, screen for HLA antibodies and for platelet-specific antibodies as soon as a diagnosis of immunological refractoriness is suspected. IgM platelet autoantibodies should also be investigated since they interfere with the screening tests but do not seem to play a role in the outcome of platelet transfusions.

b) Clinical criteria: A poor response to transfused allogeneic platelets, is demonstrated either by a reduced or absent post-transfusion platelet increment or recovery on at least two occasions, or a shortened post-transfusion platelet survival, or both.

Management

The vast majority of BMT patients supported with platelets from our Service do not become immunologically refractory to the transfusion of RDPCs. In addition, not all patients with HLA antibodies become refractory to the transfusion of RDPCs. In fact, reported figures in the literature for HLA immunisation in platelet-transfusion dependent patients range from 30-70% whilst the incidence of immunological refractoriness ranges from 8-40%. Most patients who are immunologically refractory have HLA antibodies reacting with > 60% of the lymphocyte screening panel. Hence, it is clear that HLA alloimmunisation is not synonymous with immunological refractoriness. Sometimes the HLA antibodies are of restricted specificity and then most RDPCs are compatible; on other occasions, the HLA antibodies are directed against class I antigens expressed weakly on platelets. Nevertheless, for those patients who become immunologically refractory, two options are available for the provision of platelet concentrates with a satisfactory outcome: a) HLA-matched or, b) crossmatch-compatible platelets.

a) HLA-matched: In the presence of HLA antibodies, the complexity of the HLA system makes it very difficult to provide multiple HLA-matched donors required to meet the transfusion needs of chronically thrombocytopenic patients. Such platelets can only be obtained from a large (2,000-20,000) panel of HLA-typed donors and/or patients' relatives willing to donate single donor platelets (SDPs) by apheresis. These procedures are expensive, time consuming, and demanding on donor and operator. SDPs should have no less than 2.5×10^{11} platelets. At our centre in Colindale, due to the low white cell contamination of our red cell and platelet concentrates, not more than 8% of the total number of platelet concentrates requested are for immunologically refractory patients. In our panel of over 3,000 HLA-typed donors, those homozygous at the HLA-A and -B loci (e.g. A1-B8/A1-B8) are particularly useful. Some centres select HLA-typed platelet concentrates based on the Leiden system which considers split HLA-A and -B antigens: *A match* (all HLA split antigens identical for donor and recipient); *BIU* (one blank or homozygous antigen in the donor and the other three antigens identical); *B2U* (two blank or homozygous antigens in the donor, the other two antigens identical for donor and recipient); *BIX* and *B2X* (one or two crossreactive antigens within the broad groups and the rest identical between donor and recipient). If no HLA-matched donors are available and the specificity of the HLA antibodies is known, donors compatible with the antibodies are selected, if available. In other centres, acceptable HLA mismatches are considered in the selection procedure. From the above, and regardless of the method used to provide platelets for immunologically refractory patients, it is obvious that it would be helpful if patients who are likely to become refractory to the transfusion of platelet concentrates were typed for HLA A and B at the time of diagnosis. In some of the centres that provide HLA-matched platelets as first-line therapy, a panel of HPA-typed

donors is available for the provision for platelet concentrates for that small number of patients who develop HPA antibodies with or without HLA antibodies.

b) Crossmatch compatible: In the absence of an HLA-typed panel of donors or of compatible relatives, or if patients become refractory to HLA-matched platelets, platelet-specific antibodies should be investigated again and the "platelet crossmatch" using patient's serum against donor platelets by ELISA, solid-phase or by flow cytometry will be helpful for the provision of platelet supportive therapy. The occurrence of immunisation to platelet specific antigens is unusual in the absence of HLA alloimmunisation, despite the fact that platelet-specific antigens have been shown occasionally to be immunogenic in leucodepleted blood components. Several centres provide crossmatch-compatible platelets as a first-line approach to dealing with all types of immunological refractoriness (i.e. mostly due to HLA antibodies), thus avoiding the need for a panel of HLA-typed apheresis donors.

With the above two platelet transfusion approaches (a and b), successful outcomes in immunological refractoriness range from 50-90% of platelet transfusions. It remains to be decided which of the two approaches is more successful at achieving satisfactory platelet increments.

When patients become refractory to all available platelets, massive ABO-identical platelet transfusions, intravenous IgG (IVIG), plasma exchange, acid-treated platelets to strip HLA antigens, or EACA (epsilon amino caproic acid) can be tried. Of all these alternatives, massive platelet transfusion seems to be the most successful. However, a number of workers have shown that despite the lack of satisfactory post-transfusion platelet increments in immunologically refractory patients, it is possible to activate haemostatic mechanisms after the transfusion of HLA-incompatible platelets.

Patient follow-up

Immunological refractoriness to platelet transfusions can disappear in a matter of weeks, and patients become responsive again to the transfusion of RDPCs. For this reason, it is important to monitor the presence, specificity, and potency of HLA antibodies at least monthly throughout the period of platelet transfusion support.

Prevention of HLA alloimmunisation and immunological refractoriness

Leucodepletion: White cell contamination should be reduced to a minimum in cellular blood components transfused to platelet transfusion-dependant patients, since it is alloimmunisation to leucocytes which is the major cause of febrile transfusion reactions and of immunological refractoriness to platelet transfusions. It is possible, with the top and bottom systems, to produce platelets with white cell counts significantly lower than those found in platelet concentrates prepared by the "PRP" (platelet rich plasma) method.

Although it is impossible to prevent primary HLA alloimmunisation totally, in view of previous allogeneic exposure by pregnancy or transfusion in many patients, some transplant centres aim at preventing immunological refractoriness altogether by depleting platelet concentrates of white cells either by filtration or by collecting single donor platelet concentrates with third-generation cell separators such as the Spectra-COBE with the leucoreduction system (LRS). It has been well documented that alloantigen recognition requires the expression of both class I and class II HLA antigens on the surface of the transfused cells; hence, it appears that dendritic cells, as antigen-presenting cells (APCs), are particularly important in this respect. As platelets, in contrast to white cells, express only class I but not class II HLA antigens, leucocyte-poor cellular components (red cells and platelets) will prevent immunisation to HLA antigens and, consequently, avoid the vast majority of cases of immunological refractoriness.

Leucopoor blood components, prepared by centrifugation methods (e.g. Optipress, Compomat) will decrease but not prevent HLA alloimmunisation. Such leucopoor red cell or platelet concentrates will prevent non-haemolytic transfusion reactions and the release of cytokines (IL-1, -6, -8, TNF) during storage. On the other hand, *leucodepleted* components, prepared either by filtration or third generation cell separators will prevent HLA alloimmunisation in previously untransfused patients and in women without a history of pregnancy. It has been proposed that the threshold for HLA alloimmunisation is 5×10^6 leucocytes per transfusion episode.

UV irradiation of platelets: As stated above, a major route of alloimmunisation is by means of APCs. Donor APCs interact with T-helper cells of the transfused recipient to induce the formation of alloantibodies in the recipient. UV irradiation has been documented to interfere with the function of APCs by one of several possible mechanisms:

- a) by preventing the APCs from releasing immunoregulatory substances such as interleukins
- b) by interfering with a receptor or decreasing the expression of a membrane antigen on the surface of the APCs resulting in an inability of the cell to participate in the immune recognition process
- c) by inducing the formation of suppressor cells in the transfused recipient, hence preventing antibody formation.

Platelet substitutes, such as synthetic phospholipids or fibrinogen-coated albumin micro-particles, are not at the advanced stage needed to be used in prophylaxis and are only being used experimentally to arrest bleeding.

The prophylactic platelet transfusion trigger of 20–30 $\times 10^9/L$ has been lowered by many clinicians in recent years to 10 and even 5 $\times 10^9/L$ following clinical audit of platelet usage. In addition, the advent of growth factors and stem cell therapy is significantly reducing the period of platelet transfusion dependency in patients requiring marrow ablation therapy. These developments are reducing the clinical problem of immunological refractoriness to platelet transfusions.