A mini-review on platelet refractoriness

Several extensive reviews have recently been published on the management of patients – most frequently in the onco-hematologic setting – who do not seem to benefit from the administration of platelet concentrates from random donors, as indicated by the lack of an adequate post-transfusion platelet count increment. This condition goes under the term of platelet refractoriness.1 Although a certain level of theoretical consensus on the definition and management of platelet refractoriness exists – as witnessed by a number of publications, guidelines and reviews2–5 – clinicians’ approach to the diagnosis and resolution of this important transfusion complication shows significant variance. An audit of practice in platelet refractoriness performed in UK by surveying 56 consultant hematologists6 showed that clinicians differed on the definition of platelet refractoriness7,8 and on the importance given to its immune versus non-immune causes.9

This mini-review summarizes the most recently acquired evidence on platelet refractoriness, with regard to its frequency, methods for its diagnosis and management, and the costs it can generate. The literature search strategy was based on the use of Pubmed with platelet refractoriness and platelet transfusion as key words. Prevalent attention was given to articles published in English during 2000-2004.

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Frequency in medical and surgical recipients

Refactoriness to random donor platelet support mainly affects patients suffering from bleeding disorders and cancer.10,11 This is largely due to (i) the presence of co-morbidity and/or severely compromised clinical conditions in a large proportion of these patients (the non-immune detrimental factors, namely infection, high body temperature, splenomegaly, use of antibiotics and antifungal drugs),12 and (ii) the intensive and numerically high transfusion requirements of these patients, in turn due to prolonged periods of thrombocytopenia.
nia caused by their primary diseases, or induced by pharmacological treatment. Repeated challenge with allogeneic blood products frequently triggers the development of anti-human leukocyte antigen (HLA) antibodies by platelet recipients. Less frequently alloantibodies reacting to human platelet antigens (HPA) have been attributed a causative role in alloimmune platelet refractoriness. Both types of antibodies, which may react with donor platelets and decrease their in vivo function and survival, are collectively known as the immune detrimental factors.

The relative role of immune versus non-immune factors was carefully evaluated in a fairly small prospective study performed before the regular use of leukoreduced blood components. This study showed that 116 (44%) of 266 platelet transfusions given to 26 consecutive recipients being treated for hematologic malignancies failed to produce a satisfactory response and that non-immune factors were present in 88% of the 116 unsuccessful transfusions. These and current clinical observations indicate that non-immune factors have a prevalent role in decreasing the effectiveness of platelet support. Nonetheless, the study and management of immune factors has an important role in the patients’ management because of the possibility of preventing the formation of the alloantibodies and of selecting compatible platelets for the alloimmunized recipients.

The incidence, specificity and persistence of the antibodies have been the object of a number of investigations. A recent study performed in a cohort of 252 oncology and hematology recipients found that platelet-reactive antibodies were detected in the sera of 113 patients (44.8%). Anti-HLA and anti-HPA specificities were found in the sera of 108 and 20 patients, respectively. This study supports the belief that antibodies to the HLA system, rather than antibodies with specificities against other antigen systems, are the major cause of platelet refractoriness. Another multicenter study performed in 150 multi-transfused, untransplanted patients with acquired aplastic anemia from eight European centers showed that 62% of patients were alloimmunized, that antibody production persisted for many years after the last transfusion, and that anti-HLA antibodies were focused on a few specific class I epitopes, mostly mapped to the HLA-A molecule. Besides the identification of the causative antibodies, recent elegant studies have provided novel insights into the complex immune cellular pathways regulating recipient T- and B-cell alloreactivity, NK-cell mediated allore cognition, antigen processing and IgG antiplatelet immunity. In particular, some of these studies support the concept that antigen-processing pathways can be targeted for specific immunotherapies designed to further reduce the alloimmune response to transfused WBC-reduced platelets. With regard to the frequency of alloimmune refractoriness observed with current blood component preparation procedures, recent evidence suggests that this complication is shown by about 15% of medical platelet recipients undergoing modern chemotherapy treatment. This proportion decreases to 5% or less in patients given blood components in which most white cells have been removed or inactivated.

As expected, the type of blood component seems to be related to the frequency of platelet alloimmunization and alloimmune refractoriness. Following and supporting the previous evidence reported in the Trial to Reduce Alloimmunization to Platelets (TRAP), a very recent investigation from Canada not surprisingly showed that the routine adoption of filtration leukoreduction in that country was associated with a significant reduction of both platelet alloimmunization and alloimmune platelet refractoriness. Values decreased from 19% to 7% and from 14% to 4%, respectively. Another recent publication mainly addressing the management of alloimmune refractoriness by platelet cross-matching provided additional information on the relation between white cell content of blood components and the rate of alloimmune refractoriness. The latter study, carried out on a consecutive series of non-surgical platelet recipients, showed that the routine use of Buffy-coat-deprived red blood cells (RBC), a popular RBC preparation in Europe with a white cell content intermediate between those of non-filtered, Buffy-coat-rich RBC (i.e. the traditional standard RBC) and of filtered RBC, was associated with an 8.3% frequency of alloimmune refractoriness, i.e. a value which is intermediate between the 14% and 4% frequencies of the Canadian study.

With regard to the degree of leukoreduction needed to prevent the development of alloimmunization, current standards require that leukoreduced red blood cells and platelets contain fewer than 1 million (European standards) or 5 million (US standards) white cells. Leukoreduction is usually obtained by filtration of red blood cells and whole blood derived platelets or by platelet apheresis.

Although platelet refractoriness does not seem to represent a frequent complication in the surgical setting, there are few accurate estimates on this matter in surgical patients. One recent, randomized, prospective clinical trial showed that anti-HLA alloimmunization was detected between 20 and 50 days after surgery in 12.6% of 317 cardiac surgery patients undergoing a single transfusion event with multiple RBC units partially or almost totally deprived of their WBC content by Buffy-coat removal or Buffy-coat removal plus filtration, respectively. Of note, in this study the difference in anti-
The latter element is used to per-

Table 1. Formulae used for the identification of refractory patients.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Mode of computation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Platelet Increment (API)</td>
<td>Post-minus pre-transfusion platelet count</td>
</tr>
<tr>
<td>Corrected Count Increment (CCI)</td>
<td>(Post-minus pre-transfusion platelet count) divided by no. of administered platelets, multiplied by patient’s body surface area</td>
</tr>
<tr>
<td>Percent Platelet Increment (PPI)</td>
<td>Observed/expected platelet count increment</td>
</tr>
</tbody>
</table>

WBC alloimmunization rates in recipients of RBC partially or almost totally deprived of their original WBC content did not reach statistical significance.

**Diagnosis**

Part of the difference in the prevalence of refractoriness reported in the different studies performed in the oncology/hematology settings may be due to the incomplete consensus on the definition of refractoriness. In general, the diagnosis of refractoriness is based on a computation which takes into account the pre- and post-transfusion platelet counts of the recipient, the number of infused platelets and a correction factor for the patient’s size, which may be an estimate of the patient’s blood volume (BV) or body surface area (BSA). The latter element is used to permit comparison of the outcome of transfusions given to patients of different body size. Some formulae reported in the literature that have been used for the identification of refractory recipients are shown in Table 1. A critical review of the large data set from the TRAP study suggests that these formulae are not suitable for comparing the efficacy of different platelet preparations (for example, UVB-irradiated platelets versus leukoreduced platelets) because, in the opinion of the authors of the review, they ‘are biased in favor of platelet preparation techniques that provide fewer platelets’. Accordingly, their use should be limited to the evaluation of different transfusion episodes in which the same platelet preparation procedure is used.

Clinicians and nurses do not always comply with the general recommendation contained in most platelet transfusion guidelines of determining the patient’s platelet count 10-60 minutes or 18-24 hours after transfusion. At our Institution, for example, an audit performed in 2000 showed that the post-transfusion platelet count was diligently determined (either at 10-60 minutes or at 18-24 hours) in no more than two-thirds of platelet transfusions. Although data have been published supporting the equivalence of evaluating the post-transfusion increments at 10 rather than at 60 minutes—a procedural facilitation in a busy clinical ward—a careful study challenged the validity of the 10-minute count. In the latter investigation the post-transfusion redistribution of platelets was evaluated in 16 healthy volunteers and in 12 thrombocytopenic patients given indium-111-labeled platelets. The authors concluded that transfused platelets do not reach intravascular equilibrium for 60 minutes post-infusion and that the count of 10 minutes cannot detect platelet refractoriness, thus suggesting that additional investigation is necessary to reach firm conclusions on this practically important issue.

Minimum levels for the results of the different formulae that may be expected in the non-refractory patient have been proposed to facilitate the identification and management of the refractory ones, who show lower increments and increased bleeding risk. For the corrected count increment (CCI), refractoriness is usually defined as a CCI value at 10-60 minutes and at 18-24 hours post-transfusion below 4,500-5,000 and 2,500 platelets per microliter, respectively. Values indicating refractoriness with the percent platelet increment (PPI) have been set at less than 20% at one hour or less than 10% at 16 hours. The specifications of ‘16 hours’ or ‘18-24 hours’ generally indicate the morning platelet count performed the next day after transfusion. Because a number of clinical and pharmacological factors can decrease the outcome of platelet support, it is usually accepted that a patient is not considered refractory until low CCI or PPI values have been confirmed after at least 2-3 consecutive transfusions of fresh, ABO-compatible platelets. The latter specification derives from the observation that major ABO incompatibility, although neither required nor usually associated with significantly decreased post-transfusion increments, can have a negative impact in some cases. This prudent approach is justified by balancing the need to prevent the risk of hemorrhage against the high cost of providing HLA-compatible platelets to alloimmunized recipients.

The accurate definition of threshold values for the different formulae used to identify refractory patients, although methodologically correct and necessary in some cases, has more value in the context of scientific studies than in that of the patient’s daily management. In fact, the post-transfusion platelet count increment—the numerator in all formulae—is frequently close to zero in the difficult case of refractoriness. In addition, although there is a prevalent consensus that the platelet count should be maintained above 10,000 platelets per microliter, it must be pointed out that basic laboratory tests, including the platelet count, are of limited value in predicting...
the bleeding risk and that many other factors including fever, infection, coagulopathy, vascular lesions and high white cell count play important roles. In this regard, a retrospective 10-year multivariate analysis of all thrombocytopenic adult patients (n=2,942) admitted to the John Hopkins Oncology Center in Baltimore, MD, “showed no relationship between either the first morning platelet count or the lowest platelet count of the day and the risk of hemorrhage.” Interestingly, the findings of this and of another study challenge the consolidated practice of bleeding prophylaxis in oncohematology patients, as opposed to a policy based on limiting the use of blood products to the aggressive treatment of actual bleeding episodes.

A novel instrument for improving the definition of the risk of bleeding in patients with lymphoma or solid tumors was recently described by Elting et al. The instrument, named the Bleeding Risk Index (BRI), was developed from logistic regression analysis of 750 chemotherapy cycles randomly selected from 1,262 cycles in 608 patients treated at the M. D. Anderson Cancer Center during January 1994-December 1995. Like the results reported by Friedmann et al., this study also challenges the clinical significance of the relation between present platelet count and bleeding risk, i.e. the validity of a prophylaxis policy based on a pre-defined platelet count threshold. In fact, the study showed that factors that had a statistically significant value in predicting bleeding were: any prior episode of bleeding (OR 5.6), treatment with a drug affecting platelet function (OR 5.1), bone marrow metastases (OR 4.3), a baseline (day-1 of chemotherapy) platelet count below 75,000 per microliter (OR 3.5), genitourinary or gynecologic malignancy (OR 3.3), a Zubrod performance status score greater than 2 (3 defining a symptomatic patient, in bed > 50% of the day but not bedridden) (OR 3.4), and treatment with bone marrow toxic agents (OR 2.2). Based on the evidence that in comparison with the traditional 20,000 and 10,000 platelet threshold strategies, the BRI-based strategy “provided the best trade-off between sensitivity for major bleeding episodes (80%) and specificity for any bleeding (84%)”, the authors of this study concluded that “an individualized, BRI-based approach to bleeding prophylaxis provides a highly sensitive and specific alternative to traditional, nonindividuated platelet threshold strategies”. A validation study of the BRI approach would be desirable in leukemia patients, who represent a large proportion of platelet recipients.

In addition to using the post-transfusion platelet count as a method to detect platelet refractoriness and evaluate platelet transfusion effectiveness, some investigators have recently tested the Platelet Function Analyzer (PFA-100) device in a small group of patients. In this machine, the time required in vitro for platelets to close a disposable tube is considered to be inversely proportional to platelet function in vivo. Based on the evidence that the 9 patients with improved PFA values post-transfusion had more frequent hemorrhage resolution than did the 7 patients who showed no improvement in PFA values, the authors concluded that the PFA machine can be an effective aid for supporting platelet transfusion decisions. Additional series of patients are required to determine the importance of this approach.

A cellular approach for predicting the outcome of platelet transfusion was developed by Lim and colleagues, who tested the ability of monocytes to phagocytize platelets labeled with 5-chloromethyl fluorescein diacetate after incubation with the patient’s serum. The proportion of monocytes that phagocytized opsonized and fluorescent platelets correlated well with the 1-hour and 24-hour post-transfusion CCI.

Management

Based on the prevalence of anti-HLA antibodies and their relevance, methods to overcome alloimmune platelet refractoriness rely on the selection of HLA-compatible platelets. This has been traditionally pursued with two approaches: (i) the selection of HLA-typed donors compatible with the patient’s HLA type; and (ii) platelet cross-matching.

The value of alloantibody detection in predicting response to HLA-matched platelet transfusions was recently investigated by Levin et al. In their retrospective study, the outcome of the first HLA-matched platelet transfusion was evaluated in 72 hematologic platelet recipients, 54 of whom alloimmunized to HLA. HLA-matched platelets had been ordered for all 72 patients because all of them had been considered refractory by their physicians. The purpose of the study was to evaluate the outcome of the matched platelet transfusions in two ways: first, according to the decision strategy by which the clinician had requested an HLA-matched platelet transfusion. The possible strategies were: (i) results of alloantibody detection unavailable (n=17); in retrospect, 82% of the transfusions following this strategy showed a positive HLA test; (ii) a positive alloantibody test (n=39); (iii) a negative alloantibody test (n=15). In one case, omitted from this part of the analysis, the strategy was unclear. Secondly, the authors investigated the outcome of the 72 first HLA-matched platelet transfusions in relation to the results of alloantibody testing. The decision strategies did not show a significant relation with the HLA-matched platelet transfusion outcome. The sec-
The second part of the study showed that a positive alloantibody test predicted a better transfusion outcome than that in patients with a negative alloantibody test. In addition, and perhaps unexpectedly, the study showed that a significant proportion of refractory patients without either non-immune or immune detrimental factors may also benefit from HLA-matched transfusions. The authors report that the latter finding cannot be easily explained, although it could be attributed to “transient non-immunological factors playing a role in the lack of response of these patients to earlier random platelet transfusion or to restricted sensitivity of HLA-tests in detecting alloantibodies”. Rather than providing clear and definitive answers to the old problem of the relation between immune versus non-immune factors and refractoriness, this elegant study documents the complexity of the management of the different strategies used to overcome it and provides some evidence that patients apparently free of alloantibodies may also benefit from HLA-matched platelet transfusions.

At the author’s institution the use of HLA-typed donors was abandoned several years ago, in spite of local availability of excellent HLA typing skills. The main reason for this choice was the limited number and high cost of the local HLA-typed platelet donor panel and the frequent delay between notification of platelet refractoriness and provision of typed and effective platelets. The delay was mainly due to the practical aspects of donor availability and the platelet apheresis procedure. The currently used approach is based on automated cross-matching of random platelets with a solid-phase assay. The results of the routine use of this approach for the management of refractoriness were published recently. The cohort examined in this study included 480 consecutive recipients of random donor platelets. During 33 months of observation, 40 patients (8.3%) became refractory to platelet support and received 569 cross-match-negative pools of platelets each obtained from 5-6 buffy-coats of whole blood donations. The mean number of days from first transfusion to detection of refractoriness was longer in the 13 men (219 days) than in the 27 women (119 days), who reported previous pregnancies – a well known factor able to trigger anti-HLA alloimmunization - in 80% of the cases.

Absolute post-transfusion platelet count increments greater than 10,000 per microliter were obtained in 68% of cross-matched transfusions. The platelet counts associated with the 569 cross-matched transfusions before the transfusions and 1 and 24 hours after were 7.7±5.5, 32±21 and 16.8±15.5×10^9/L, respectively. The increments were significantly higher than those observed in the same patients during the month before detection of refractoriness, when pre-transfusion, 1- and 24-hour post-transfusion platelet counts associated with 303 random donor platelet pools were 7.0±8.6, 15.9±16.1 and 9.6±12.8×10^9/L, respectively. These recent observations and other data from the literature indicate that the HLA typing strategy and the cross-matching strategy show similar effectiveness and suggest that the strategy to use must be chosen based on local operational convenience and cost analysis.

Refinements of the HLA-compatible donor selection strategies include the molecularly based algorithm for histocompatibility determination named the HLA Matchmaker, which was described in 2002 by Duquesnoy and the Antibody Specificity Prediction (ASP) method described and tested in 114 patients by Petz et al. Both approaches allow the identification of permissible platelet donors, thus substantially enlarging the compatible donor panel predicted solely on the basis of patient and donor HLA types.

Other options which have been used to overcome alloimmune platelet refractoriness include administration of intravenous IgG, transfusion of vinblastine-loaded platelets, treatment with cyclosporine A, immunoadsorption with staphylococcal protein-A columns, and citric acid platelet treatment to remove class I HLA epitopes. Despite successful reports in some patients, most trials have yielded negative or inconclusive results and none of these strategies have become validated clinical practice.

### Costs

Several elements for a cost analysis of platelet transfusion and platelet refractoriness have been reported in the recent literature, both in adults and in neonates. These studies indicate that platelet transfusion is an expensive component of patient therapy. More specifically, the cost of managing a patient developing platelet refractoriness is very high. Meehan et al. reported that refractory and non-refractory patients had median hospital stays of 35 and 14.4 days and inpatient hospital costs of US$103,956 and 37,818, respectively.

Other cost data can be derived from our study, in which 40 refractory patients received a mean of 14 cross-matched platelet transfusions during 33 months. The cost of commercial kits to select cross-match-negative platelets for these patients amounted to 173,000 Euros. From these data it can be determined that each refractory patient generated an average expense of 4,325 Euros just for the purchase of the solid-phase, disposable kits required for the selection of compatible platelets. Labor costs should be added to this sum.

The above data indicate that it is appropriate not
only for medical but also for financial reasons to develop strategies aimed at reducing the incidence of refractoriness and/or at developing cost-effective systems to provide effective platelet support to these patients. Other economic aspects need consideration as well, as they may be expected to have similar impacts on the refractory and the non-refractory patient. In this regard, an interesting study on non-refractory patients undergoing haemopoietic stem cell transplantation was published by Ackerman et al. This study was triggered by the observation that decreasing financial resources had led to the use of lower-dose platelet components, although the economic consequences of such a policy had not been determined. The study showed that a 58% reduction in mean platelet dose would be associated with 60% more platelet transfusions in the post-transplant period, with a corresponding increase in the median cost to the hospital from US$ 2,804 to US$ 4,486/patient.

**Conclusions**

In spite of significant strides towards its resolution, platelet refractoriness is still a transfusion complication which cannot be prevented or corrected in a relatively small but not negligible proportion of recipients. In the hematology and oncology settings it may be estimated that approximately 5% of patients regularly transfused with leukoreduced or inactivated blood components show platelet reactive antibodies in the serum and insufficient platelet count increments after the transfusion of random-donor platelets. Although this proportion, which increases to about 15% in recipients of non-leukoreduced blood components, is relatively limited, refractory patients are exposed to an increased risk of developing clinically significant bleeding and need special attention. Moreover, platelet refractoriness generates a significant economic burden and requires organizational efforts in the clinic and the laboratory to ensure its proper management. The objective of such efforts is to promptly re-establish effective platelet support. When the most popular strategies to overcome alloimmune platelet refractoriness – donor HLA-typing or platelet cross-matching – are used by expert operators, this blood transfusion complication can be resolved in approximately two-thirds of the cases. It is expected that recent insights into cellular mechanisms governing alloimmune responses and their humoral counterparts may facilitate the identification of novel ways of managing alloimmune platelet refractoriness in patients who do not benefit from current therapeutic approaches. In spite of the advances in the management of the alloimmune causes of platelet refractoriness, the management of the non-immune factors – poor clinical conditions and use of drugs affecting platelet survival and function – remains largely unresolved.

**References**

Platelet refractoriness


