

EDITORIAL**The Leukodepletion of Cellular Blood Products in the Prevention of HLA-Alloimmunization and Refractoriness to Allogeneic Platelet Transfusions**

By Nancy M. Heddle and Morris A. Blajchman

FOUR YEARS AGO an editorial in *Blood* identified a number of issues concerning policy decision-making about the clinical use of leukodepleted cellular blood products to prevent HLA-alloimmunization and refractoriness to transfused allogeneic platelets.¹ In that editorial, Schiffer concluded that it was premature to recommend platelet leukodepletion for routine use. The issues identified in that editorial included the following: (1) The applicability of the available results to the general leukemic population, because most studies had been done using highly selected patient populations. (2) The lack of available data on the effectiveness of leukodepletion to prevent HLA-alloimmunization and platelet refractoriness in individuals who were already alloimmunized, including those who had been transfused previously and/or females with a history of prior pregnancies. (3) The inconsistency in the degree of leukodepletion of the cellular blood products used in the various studies reported. (4) The economic implications of leukodepleting all cellular blood products when the potential clinical benefit might be available to only a small percentage of patients who would be receiving such products.

During the 4 years since that editorial, three more clinical trials reporting on the use of leukodepleted cellular blood products have appeared.²⁻⁴ These include the article by Sintnicolaas et al in this issue of *Blood*.⁴ In addition, several international forums and consensus conferences have been held to try to deal with the indications for the clinical use of leukodepleted cellular blood products.⁵⁻⁷ Are the answers to the unresolved issues, previously identified by Schiffer, on which to make policy decisions about the use of leukodepleted cellular blood products now available? As will be outlined below, even though much of the available data are tantalizing, the actual clinical benefit for the leukodepletion of cellular allogeneic blood products has not been established for most indications suggested for their use.

Research methodologists and epidemiologists have provided a very useful framework that can be applied to try to address the issues relating to the clinical use of leukodepleted cellular blood products.^{8,9} This approach involves the careful assessment of the published data to determine: the validity of the methodology used; the magnitude of the treatment effect; and whether the results of a particular study are relevant to the care given to a specific patient. This methodologic framework for critical appraisal has been used to evaluate

five randomized controlled trials (RCTs), published between 1983 and 1991, which investigated the efficacy of leukodepletion to reduce the frequency of HLA-alloimmunization and platelet refractoriness in patients who required cellular blood product transfusions.¹⁰

Validity of the Methodology

The critical appraisal of the five RCTs published highlighted several methodologic issues that might have biased the final results of the studies. These issues included the failure to blind patients and study personnel to the treatment allocation; a lack of similarity between patients in each treatment group, which usually resulted from exclusion of patients after randomization; and the possibility of patients receiving nonexperimental treatments that could affect the study results. However, even when the impact of these issues on the treatment effect were considered, there was still good evidence that the leukodepletion of the transfused cellular allogeneic blood products to a level below 5×10^6 leukocytes per product could prevent HLA-alloimmunization.

The Magnitude of the Treatment Effect

The magnitude of the effect of leukodepletion was ascertained by performing a meta analysis on the data from the five studies. Using this approach, the common odds ratio for HLA-alloimmunization was estimated to be 0.27 (95% confidence interval [CI] 0.13 to 0.55) whereas that for platelet refractoriness was estimated at 0.28 (95% CI 0.13 to 0.54), suggesting that the frequencies of both HLA-alloimmunization and platelet refractoriness were lower in patients who received leukodepleted cellular blood products compared with those who did not.

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0006-4971/95/8503-0039\$3.00/0

Relevance to Patient Care

To determine whether study results are relevant to patient care, three questions must be considered: (1) Are the results of the published studies generalizable? (2) Were clinically important outcomes considered? and (3) Are the benefits of leukodepletion cost-effective? These three questions must be addressed satisfactorily before the routine leukodepletion of all cellular allogeneic blood products can be recommended.

Are the results generalizable (ie, can they be applied to all patients)? In his 1991 editorial, Schiffer identified two issues that prevented generalizability of the data then available.¹ These included (a) the heterogeneity of the subjects used in the reported studies, and (b) the absence of information on study subjects who may have been alloimmunized previously. Two of the three clinical studies published over the past 4 years also involved heterogeneous patient populations, thus the first of these two issues remains unresolved. However, the study by Sintnicolaas et al in this issue of *Blood* provides very useful information on the effectiveness of leukodepletion in the group of patients with a high risk for prior alloimmunization; ie, females with a history of prior pregnancies.⁴

Using a randomized controlled double-blind design, the effect of leukodepletion on HLA-alloimmunization and platelet refractoriness was investigated in 75 female patients with hematologic malignancies who had a prior history of one or more pregnancies. In contrast to most of the other published studies, no statistically significant difference was seen in this cohort of patients in the frequencies of HLA-alloimmunization or refractoriness to allogeneic platelet transfusions. The authors speculate that prior exposure to HLA antigens, during a previous pregnancy, resulted in a boosting of previously formed HLA alloantibodies, causing the refractoriness. The authors raise the possibility that this secondary HLA-alloimmunization could have been caused by residual leukocytes, and/or by soluble antigens or microparticles present in the transfused allogeneic blood products, which escaped the leuko-filtration process. Indeed, there is experimental animal as well as human data to support the latter hypothesis.¹¹⁻¹³ However, it is important to note that the power of the study of Sintnicolaas et al to detect a statistically significant difference was low because there were only 62 evaluable patients in this study. For this study to have shown a 50% reduction in the frequency of platelet refractoriness (ie, a decrease from 41% to 20%), approximately 90 subjects per treatment group would have been required.

Were all clinically important outcomes considered? HLA-alloimmunization and platelet transfusion refractoriness have been used, for a long time, as surrogate laboratory measurements for morbidity or mortality caused by bleeding in thrombocytopenia patients. Recently, the clinical relevance of these laboratory endpoints have been brought in question for several reasons.¹⁰ Alloimmunization is defined by the ability to detect HLA antibodies; thus, it is a laboratory outcome measure. Refractoriness is used as the clinical outcome measure and is based on the posttransfusion platelet

count. There are several potential problems with these surrogate outcomes, even though there is evidence indicating that the likelihood for clinical bleeding correlates inversely with the peripheral blood platelet count.¹⁴

To our knowledge, there have not been any formal studies that validate the correlation of bleeding with either platelet refractoriness to allogeneic platelet transfusions or the presence of HLA-alloantibodies in a patient's serum. Refractoriness usually involves the enumeration of posttransfusion platelet increments but only in situations where other clinical factors known to adversely affect the posttransfusion platelet response are absent. The relevance of such an assessment is questionable because the majority of patients with significant morbidity or mortality associated with bleeding are often septic, febrile, and/or are receiving antibiotic agents, such as amphotericin B. Moreover, most reported studies censor patients once they have been defined as being refractory; thus, clinical follow-up throughout a particular patient's entire clinical course usually does not occur. Such an approach cannot be justified because some patients become refractory to allogeneic platelet transfusions early in their illness whereas others, over time, move in and out of the refractory state during the course of their treatment.¹⁵

Further confusion results from variability in the definition of the term 'refractory' used by different investigators. Sintnicolaas et al define refractoriness as "two consecutive platelet transfusions with 1-hour posttransfusion platelet recoveries less than 20% in the absence of clinical factors known to adversely affect platelet recovery; or, a single platelet transfusion with less than 20% recovery in the presence of HLA antibody."⁴ This definition differs from that used in other studies in which corrected count platelet increments of $<7.5 \times 10^9/L$ or $<2.5 \times 10^9/L$ were used to define platelet refractoriness.^{16,17} Moreover, in comparing each study's assessment of refractoriness, poor responses were not required to be consecutive in one study¹⁷ and, in another, information was not provided about the presence of clinical factors known to affect the posttransfusion platelet increment at the time of assessment.¹⁸ For all of these reasons, the use of refractoriness to platelet transfusions as a clinical outcome measure should be reassessed and perhaps a more clinically relevant outcome measure implemented.

The rationale for the clinical use of allogeneic platelet transfusions is to prevent or control bleeding. The use of clinical bleeding as a primary outcome measurement has not been widely used. This is because mortality due to bleeding is very infrequent; thus, clinical studies using bleeding as an endpoint would require a large number of study subjects to show a statistically significant treatment effect. Moreover, bleeding is difficult to quantitate, although some investigators have used a bleeding measurement scale to try to address this issue.^{19,20} For all of the reasons detailed above, there is general consensus that morbidity and mortality caused by bleeding cannot be used as the clinical outcome measure to assess the effectiveness of allogeneic platelet transfusions.

Is there an alternative that might be more clinically relevant than to continue using HLA-alloimmunization and/or platelet transfusion refractoriness as surrogate outcome measures for bleeding? We have suggested recently the use of

an index evaluating the *percentage of days at risk of bleeding* as a possible alternative outcome measure for bleeding.¹⁰ If allogeneic platelets are transfused and the patient has a good posttransfusion platelet count increment, then the patient is at lower risk for bleeding. Although this index is still a surrogate measure for bleeding, it would evaluate patients throughout their entire clinical course, even when other factors known to affect the posttransfusion platelet response might be present. Thus, the effectiveness of leukocyte filtration over time to improve the posttransfusion platelet response could be evaluated rather than the efficacy of only a few platelet transfusion episodes.

Are the benefits of leukodepletion cost-effective? The effectiveness of an intervention (measured in a realistic clinical setting) must be known to answer this question: Most would agree that potential harm caused by the use of leukodepleted allogeneic blood products is not an issue, because there are probably no side effects associated with their use compared with the use of nonleukodepleted cellular allogeneic blood products.²¹ However, the cost-benefit issue has not been adequately addressed. Schiffer has estimated that only 10% to 15% of individuals receiving leukodepleted blood products would benefit from such an intervention.¹ To provide an estimate of potential benefit using the concept of "number needed to treat" we have estimated a relative risk reduction for platelet refractoriness of 70% and a 4.1% frequency for a clinically significant bleed.¹⁰ Based on these estimates, we have calculated that one would have to administer leukodepleted blood products to 47 patients to prevent one clinically significant bleeding episode. It is important to emphasize that this analysis is based on a relative risk reduction for platelet refractoriness and not risk reduction for bleeding. Because morbidity and mortality caused by bleeding does not occur in all refractory patients, this "number needed to treat" is probably underestimated.

CONCLUSIONS

It appears that the necessary information on which to make a policy decision regarding the routine use of leukodepletion for cellular blood products to prevent HLA-alloimmunization and/or platelet refractoriness is still lacking. Moreover, it would be unreasonable to make such a policy decision only on the alloimmunization issue because there are a number of other potential clinical benefits to patients that might accrue if leukodepleted cellular allogeneic blood products were available for regular clinical use. These include the prevention of cytomegalovirus infection,²² the prevention of nonhemolytic febrile transfusion reactions,²³ the prevention of the immune suppression in recipients which may affect the frequency of postoperative infections and even tumor recurrence,²¹ and the potential prevention of graft-versus-host disease.²¹ Although considerable data exist relating to the efficacy of leukodepletion for some of these clinical indications, cost-benefit analyses are lacking. The decision as to when to recommend the widespread use of leukodepletion of cellular blood products is thus a rather difficult one and should be based on data from studies of all potential benefits for such a manoeuvre.

Finally, the issue of when to perform the leukodepletion

of cellular allogeneic blood products must still be addressed. Technology is now readily available to enable the leukodepletion of cellular blood products at the time of collection (prestorage leukodepletion) rather than poststorage (ie, bedside leukodepletion). Available data from studies in experimental animals suggest that prestorage leukodepletion of cellular blood products might be more effective at preventing alloimmunization and platelet refractoriness than poststorage leukodepletion.^{11,12} Clinical data in humans are not yet available that validate this interesting observation. Moreover, data on the extent of leukodepletion for the various proposed clinical indications have not yet been established.

It is critical that individuals working within each of these research areas provide valid and clinically relevant data on which the decision to leukodeplete cellular allogeneic blood products can be made. Properly designed prospective clinical trials are still needed to address many of the issues outlined above, as well as to help define the optimal conditions for the preparation of leukodepleted cellular blood products for clinical use. Sound data are essential to enable sound policy decisions to be made about the clinical value of leukodepletion. When such data become available, the decision to routinely leukodeplete all allogeneic cellular blood products should be made by an appropriate body of experts. This body should include methodologists, health care economists, policy decision analysts, as well as the investigators.

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