Title

Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis

Journal

Transplant Infectious Disease, 14(3)

ISSN

1398-2273

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Publication Date

2012-06-01

DOI

10.1111/j.1399-3062.2012.00723.x

Peer reviewed

Longitudinal management with crossmatch-compatible platelets for refractory patients: alloimmunization, response to transfusion, and clinical outcomes

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BACKGROUND: The use of crossmatch-compatible platelets (PLTs) improves posttransfusion corrected count increments (CCIs) in patients with alloimmune PLT refractoriness. However, few reports address the efficacy of utilizing this strategy for patients requiring intensive PLT transfusion therapy lasting several weeks to months.

STUDY DESIGN AND METHODS: Medical records of patients with two or more PLT crossmatch assays performed between 2002 and 2010 were reviewed. All patients were refractory to random single-donor apheresis PLT units, defined as two consecutive 1-hour posttransfusion CCIs of less than 7500. A commercial solid-phase adherence assay was used for crossmatching.

RESULTS: Seventy-one patients were included. A median of four crossmatch assays were performed per patient (range, 2-17). Mean percent reactivity in initial (58.6%) versus last (55.3%) crossmatch assay for each patient demonstrated no trend toward progressive alloimmunization ($p = NS$). A total of 738 crossmatched PLT units were administered with a mean ± standard deviation CCI of 7000 ± 7900 ($n = 443$ units with adequate 1-hr posttransfusion counts), a significant improvement over random PLTs ($p < 0.001$). Patients with an initial crossmatch reactivity of greater than 66% were significantly more likely to demonstrate at least one panreactive crossmatch assay, impacting the availability of compatible PLTs for optimum transfusion support. One patient (1.4%) developed WHO Grade IV bleeding.

CONCLUSIONS: Progressive alloimmunization to mismatched antigens does not impact medium-term transfusion support with crossmatched PLTs. Increased reactivity in the initial crossmatch assay can serve as a trigger to initiate workup for HLA-matched PLTs as a second-line approach. However, for most patients, medium-term transfusion support with crossmatched PLTs offers an effective and rapid first-line approach to management of PLT transfusion refractoriness.

Platelet (PLT) transfusion refractoriness is a common problem among patients receiving multiple PLT transfusions.[1,2] This refractoriness may be due to a variety of clinical factors such as fever, sepsis, and splenomegaly or blood bank factors such as ABO status.[3,4] In approximately 20% of patients, however, an immune-mediated mechanism is likely the major reason for transfusion refractoriness.[5] This process is thought to be primarily mediated by antibodies toward HLA A- and B- antigens leading to destruction of transfused PLTs.[1,2]

The clinical issue of PLT transfusion refractoriness is a critical one, as the inability to increase PLT counts above 10×10^9/L is associated with a significantly increased risk of major spontaneous and/or life-threatening bleeding.[6] As a result, a number of approaches have been developed to address this problem. One of the most frequently used modern methods is HLA-matching. While this method does reliably improve PLT increments in patients with alloimmune refractoriness, some studies have found that up to 40% of HLA-matched PLT transfusions remain unsuccessful.[7,8] HLA typing of patients as well as PLT donors is expensive and the long turnaround time decreases its utility in some clinical situations. In addition

ABBREVIATION: SD = single donor.

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Supported by the UCSF Department of Laboratory Medicine.

Received for publication August 22, 2011; revision received January 3, 2012, and accepted January 3, 2012.

doi: 10.1111/j.1537-2995.2012.03593.x

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to these drawbacks, even large blood suppliers periodically have difficulty identifying HLA-matched donors for some patients.[9] As a result, alternative strategies have been developed to obtain HLA-compatible, if not fully HLA-matched, PLTs for patients based on similarities in HLA antigen epitopes and specificity of HLA antibodies identified in patient sera.[10-12] While these methods have the ability to expand the available donor pool, they still require completion of HLA testing of patients and donors.

PLT crossmatching assays are a relatively low-cost and rapid alternative to the HLA-matched approach to management of PLT refractoriness.[13] In a common version of this assay, solid-phase capture method is used to screen patients' plasma for PLT antibodies directed against HLA or other antigens on PLTs. Typically, a given patient's plasma is tested against PLT samples obtained in the preceding 48 hours during plateletpheresis of ABO-compatible donors. Donor PLTs lacking reactivity on the assay are considered to be "crossmatch compatible" and are selected for transfusion support over random single-donor (SD) apheresis units.[14]

Selection of products based on PLT crossmatching has been shown to improve posttransfusion PLT increments in refractory patients.[8,15-17] However, as products expire in 2 to 3 days after arrival at the transfusion service, crossmatching must be repeated with additional donor pools at frequent intervals. Despite the routine use of PLT crossmatching at many institutions, little has been published about the safety or effectiveness of this strategy for the medium-term (several weeks–months) management of refractory patients. Here, we present transfusion-related and clinical outcomes observed at our institution that primarily uses crossmatched PLTs for managing PLT refractoriness in complex medical and surgical patients.

MATERIALS AND METHODS

Patient population

This study was approved by the Committee on Human Research at the University of California, San Francisco. All patients with a transfusion service record of two or more PLT crossmatching assays performed between November 2002 and March 2010 were identified. Our institution's standard protocol for PLT crossmatching was followed during the study period: assays were performed only after patients had demonstrated a 1-hour posttransfusion corrected count increment (CCI) of less than 7500 after at least two consecutive transfusions. Most of the patients were receiving treatment for hematologic malignancies (see Table 1). Patients with consumptive PLT disorders (i.e., fever, sepsis, and splenomegaly) were not excluded. CCIs were calculated using the formula:

$$\begin{aligned}\text{CCI} = &[\text{Posttransfusion PLT count}\,(10^9/\text{L}) - \\ &\text{pretransfusion PLT count}\,(10^9/\text{L})] \times \\ &[\text{body surface area}\,(\text{m}^2)]/ \\ &[\text{PLT dose transfused}\,(10^{11})].\end{aligned}$$

PLT counts were available for the majority of units transfused. For components without recorded data, a default count of 3.0×10^{11} was used to calculate CCI.

PLT crossmatch assays

PLT crossmatch assays were performed at the Blood Centers of the Pacific reference laboratory using a solid-phase system (Capture- P, Immucor, Norcross, GA) for the detection of IgG antibodies to the HLA-A and HLA-B antigens found on PLTs and to PLT-specific antigens. Briefly, donor PLTs are first bound to the surface of polystyrene microplate wells. Patient serum is incubated in these wells; unbound immunoglobulins are then washed away and replaced with anti-IgG–coated "indicator" red blood cells (RBCs). If anti-PLT IgGs are present, the indicator RBCs form a confluent monolayer over the test well and constitute a positive test. Only donor units demonstrating major ABO compatibility with the intended recipient are selected for crossmatching (for example, group O recipient plasma was only crossmatched against O donor PLTs; group A recipients were crossmatched against both group A and group O PLTs). In a typical assay, PLTs from 25 to 35

TABLE 1. Patient demographic and PLT transfusion data

Age at first crossmatch assay (mean ± standard deviation, years)	49 ± 17
Female sex	46 (65)
Patient primary diagnosis	
Acute myeloid leukemia	28 (39)
Acute lymphocytic leukemia	6 (8)
Multiple myeloma	5 (7)
Lymphoma, any subtype	8 (11)
Other hematologic malignancy	8 (11)
Aplastic anemia	5 (7)
Solid tumor	3 (4)
Liver transplant/end-stage liver disease	5 (7)
Other†	3 (4)
Therapy	
Stem cell transplantation	20 (28)
High-dose chemotherapy without stem cell transplantation	39 (55)
Other	12 (17)
PLT transfusions	
Total number PLT units transfused	35 (2-154)
Number of crossmatched PLT units transfused	7 (0-49)
Number of random units transfused before crossmatch	8 (2-58)
Number of days between first and last crossmatch assays during single inpatient admission	12 (1-62)

* Data are reported as mean ± standard deviation, number (%), or median (range).
† Other diagnoses include pancreatitis, congestive heart failure, and idiopathic thrombocytopenic purpura.

SD PLT units are tested against each patient sample to assess crossmatch compatibility. Crossmatch assays are performed on PLT products that will complete bacterial testing the following day or the same day (i.e., 1- or 2-day-old PLTs). In this study, for seven group O patients requiring cytomegalovirus-negative blood products, only a smaller pool (typically 7 to 20 units) was available for testing. Results were reported as a number of noncompatible (reactive) donor units present in the entire donor pool.

Clinical management of crossmatched PLT units

For patients presumed to have inadequate response to random PLT units, at our institution the clinical team typically contacts the transfusion service to consult on the administration of crossmatch-compatible units. The medical staff of the transfusion service then assesses that patient's CCI response to recent random SD PLT units. If the request is approved, patient whole blood is collected and sent to our reference laboratory at Blood Centers of the Pacific for crossmatching (see assay details above). The number of compatible PLT units requested from a single crossmatch assay varies depending on clinical need of the patient, but typically is three to four. Compatible PLTs can be obtained within 6 to 24 hours of receipt of patient sample at the Blood Center Reference Laboratory. The clinical team is notified by blood blank staff immediately upon receipt of crossmatch-compatible units to minimize time to transfusion. For patients with continuing needs for PLT crossmatching, the crossmatch assay is typically performed on both Monday and Friday. For example, four crossmatch-compatible units identified on Monday allow for transfusion on Tuesday (PLTs aged 2 or 3 days; crossmatched on Day 1 or Day 2 after collection, respectively), Wednesday (aged 3 or 4 days), Thursday (aged 4 or 5 days), and Friday (aged 5 days). To avoid PLT wastage, at noon on Day 5 of PLT age the clinical team was consulted as to their current need for crossmatched PLTs. If the patient did not require transfusion that day, the PLT unit was instead transfused to another patient in the hospital. Overall, our annual PLT wastage rate (i.e., units expiring before transfusion, including both random and crossmatched PLTs) in the years 2004 to 2010 ranged from 0.3% to 1.0% of all PLT units ordered from the blood center.

Patients received random SD units interspersed with crossmatched units in a variety of clinical scenarios. Unfortunately the exact reason is only rarely noted in the complete medical record. Based on transfusion records, this situation appeared to most frequently occur when all crossmatch-compatible units obtained from a given assay were already transfused, but additional PLT support was required before the next round of crossmatching. Alternatively, random SD units were often administered to patients with low degrees of reactivity on the crossmatch assay, particularly when crossmatch-compatible PLTs did not provide significant CCI benefit over random units. After discussion with the clinical team, for many of these patients with low reactivity on the crossmatch PLT crossmatching was discontinued and they were returned to management with random PLT units (see Table 2).

Some patients managed with crossmatched PLTs are discharged and subsequently readmitted, typically for additional rounds of chemotherapy. For these patients, the clinical team is instructed to contact the blood bank before readmission. Thereby, crossmatching can be performed as soon as possible upon patient arrival and compatible units are available once the patient becomes thrombocytopenic due to chemotherapy. For patients where this coordination does not take place, upon PLT transfusion request the blood bank administrative data file alerts blood bank staff that this patient was previously managed with crossmatched PLTs. The current clinical team is contacted and reinitiation of management with crossmatched, rather than random, PLTs is suggested by the transfusion service medical staff. These patients will receive random PLT units until the crossmatch-compatible units can be obtained.

TABLE 2. Overall crossmatch assay outcomes with patients subdivided by reactivity on initial crossmatch assay*

	Group 1	Group 2	Group 3	p (Fisher's exact test)
Percentage of all patients	46 (n = 33)	31 (n = 22)	23 (n = 16)	
Percentage of patients with 0% reactivity on at least one assay	0 (n = 0)	23 (n = 5)	25 (n = 4)	p = 0.0076 (1 vs. 2) p = 0.0086 (1 vs. 3) p = 1 (2 vs. 3)
Percentage of patients with 100% reactivity on at least one assay	45 (n = 15)	14 (n = 3)	6 (n = 1)	p = 0.019 (1 vs. 2) p = 0.0083 (1 vs. 3) p = 0.65 (2 vs. 3)
Percentage of patients returned to management with random PLT units	12 (n = 4)	50 (n = 11)	63 (n = 10)	p = 0.0043 (1 vs. 2) p = 0.0005 (1 vs. 3) p = 0.52 (2 vs. 3)

* Group 1 initial reactivity, greater than 66%; Group 2, 34% to 66%; Group 3, 0% to 33%. Return to management with random PLT units is defined by the discontinuation of crossmatch assays after transfusion of final crossmatch-compatible unit, with further PLT transfusion needs met by 2 or more random PLT units.

HLA matching

HLA-A and -B low- or intermediate-resolution genotyping (by SSO/SSP) and antibody screening for HLA Class I antibodies (enzyme-linked immunosorbent assay or bead array) were performed by the UCSF Immunogenetics and Transplantation Laboratory using standard methods. As our primary blood supplier had a limited pool of HLA-typed donors, HLA-matched or HLA-compatible (based on antibody profile of patient) donors were identified in most instances by contacting other blood collection centers with panels of HLA-typed PLT donors. Data on donor HLA types or the grades of HLA match for donor–recipient pairs were not electronically documented in the laboratory information system and paper records were not available for retrospective review.

Retrospective data collection

Retrospective review of laboratory results and the electronic medical record was performed. PLT crossmatch assay results were converted into a

$$\text{Percent reactivity} = [\text{Number of reactive donor units}/\text{Total number of donor units tested}] \times 100\%.$$

One-hour posttransfusion CCIs were calculated for PLT transfusions with appropriate data available: CCIs were considered valid if a whole blood specimen was received in the clinical laboratory for PLT count assessment up to 3 hours after the unit was issued by the blood bank. This time window was designed to account for time of blood product transport to floor, time to prepare patient, time to transfuse PLTs, and transportation and accessioning of posttransfusion blood sample. The electronic medical record was examined for patient demographic information as well as clinical notes associated with transfusion of crossmatched PLTs. The patient outcome of WHO Grade IV bleeding, associated with debilitating outcome or mortality,[18] was the primary clinical endpoint. The electronic medical record did not contain detailed enough data to consistently assess less severe bleeding events retrospectively for all patients.[19]

Statistical analysis

Fisher's exact test was used to compare categorical variables. A t-test was used for continuous variables. Pearson's correlation was used for correlation analysis. Linear regression was performed using least-squares fitting. All analysis was performed using computer software (GraphPad Prism 5.0, GraphPad, La Jolla, CA).

RESULTS

Crossmatch reactivity in successive assays

We identified 71 patients for whom two or more PLT crossmatch assays were performed. Patient demographic and baseline clinical information as obtained from the electronic medical record is provided in Table 1. A median of four crossmatch assays were performed per patient (range, 2-17). The percent reactivity in each crossmatch assay was defined as the number of noncompatible donor units divided by the total number of donor units tested. In this study, the percentage of crossmatched PLTs transfused on each day after collection was as follows: 2 days, 15%; 3 days, 38%; 4 days, 25%; and 5 days, 22%. For patients being actively managed with crossmatched PLT units, the median number of days between crossmatch assays was 4 (range, 0-7 days) and median number of crossmatch-compatible PLT units transfused per day was 0.75 (range, 0.25-2/day).

We first examined trends in crossmatch assay reactivity over multiple assays performed. Each crossmatch assay involved relatively small donor panels (typically 25-35 donor units). Except for samples from highly alloimmunized patients, other samples would not be expected to react broadly with donor PLTs from any given panel. Not surprisingly, we found relatively wide variation in reactivity, illustrated for four representative patients (initial crossmatch reactivity ranging from 20% to 80%) in Fig. 1A. This variation likely reflects differing degrees of alloimmunization against the particular antigens expressed in each successive donor pool.

We next performed a linear regression analysis of crossmatch reactivity versus number of crossmatch assays performed for each patient. In this analysis, a positive slope indicates increasing reactivity (i.e., increasing alloimmunization) with subsequent crossmatch assays, and a negative slope indicates decreasing reactivity. As shown in Fig. 1B, the slopes are largely distributed around zero, demonstrating no overall trend of increasing alloimmunization during management with crossmatched PLTs. In addition, overall reactivity in the initial versus last crossmatch assay, averaged over all patients, was 58.6% versus 55.3%, respectively ($p = NS$). As demonstrated by the representative patient data in Fig. 1A, however, even a slope of approximately $\pm 10\%$ per assay can lead to relatively large changes in the availability of crossmatch-compatible units during medium-term management.

Predictive value of reactivity in initial crossmatch assay

As one of the aims of the study was to evaluate whether reactivity in the initial crossmatch assay could predict a need to initiate workup for HLA-matched PLTs at a later stage, we stratified patients into groups based on initial crossmatch assay reactivity. Patients were divided into groups based on the tertile of initial crossmatch reactivity: Group 1, 67% to 100% reactivity; Group 2, 34% to 66% reactivity; and Group 3, 0% to 33% reactivity. This stratification means that patients in Group 1 had the fewest

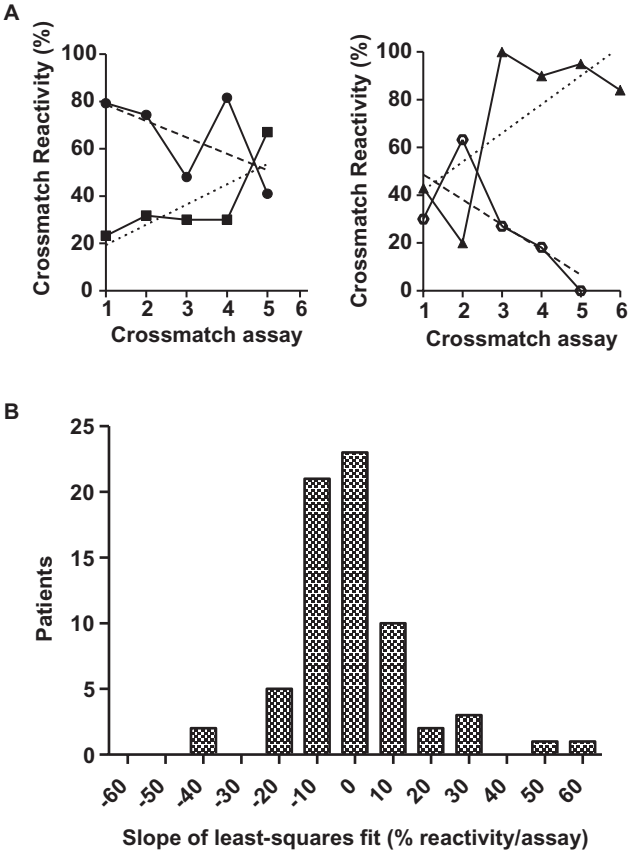

Fig. 1. Longitudinal crossmatch assay reactivity. (A, left and right panels) Percent crossmatch reactivity for four representative patients as a function of assay number performed. Frequent wide variation in reactivity in sequential assays is noted. A linear regression was also performed using a least-squares fit (dashed lines) for each patient's data. (B) Histogram of least-squares fit slopes for percent reactivity versus assay number for all patients. The large majority of slopes are near zero, indicating no significant trend toward alloimmunization over longitudinal management with crossmatched PLTs.

crossmatch-compatible units identified, consistent with the highest degree of alloimmunization, while those in Group 3 were compatible with the large majority of PLT units and presumed to have the lowest degree of alloimmunization.

This stratification permits an examination of PLT management outcomes as a function of initial crossmatch assay reactivity. For example, a crossmatch assay showing 100% reactivity in a given patient is a worrisome development as this means no crossmatch-compatible PLT units could be identified from among the tested donor pool. Another pertinent outcome is 0% reactivity, as this could mean that the patient is either not alloimmunized at all or is minimally alloimmunized with antibodies not detectable against the donor panel used in that particular assay. Additional crossmatch assays are not usually warranted in such patients and random SD PLT units were routinely recommended for transfusion support.

In Table 2, we summarize this data. Patients in Group 1 had a significantly higher probability of demonstrating at least one panreactive (100% reactivity) crossmatch assay at some point during their management. Not surprisingly, they also had a significantly lower chance of demonstrating a nonreactive assay. Interestingly, patients in both Group 2 and Group 3 showed similar likelihood of having a subsequent assay that was either panreactive or nonreactive, further demonstrating wide variation in individual assay reactivity in patients that are not severely alloimmunized.

CCI analysis of crossmatched PLT units

At our institution, crossmatched PLTs are only considered for patients demonstrating a CCI of less than 7500 in response to at least two consecutive random PLT units. For the patients included here, the mean (± standard deviation) CCI measured for random SD units transfused just before the request for PLT refractoriness workup was 710 (±2700), well below our threshold for triggering a crossmatch assay. At our institution, HLA typing and HLA antibody screen with determination of antibody specificities is initiated only after a decision is made to search for HLA-matched donors. This is typically reserved for the small number of severely alloimmunized patients for whom crossmatch-compatible units cannot be identified even after screening large (>30) donor panels or for those rare patients that appear not to respond to crossmatch-compatible units and where other nonalloimmune causes of PLT destruction have been reasonably ruled out.

Of the 71 patients in the study, five did not eventually receive crossmatched PLTs. Of these, three had crossmatch assays with repeatedly low (0%-15%) reactivities and hence required only random SD PLTs. The other two patients had repeatedly panreactive assays; crossmatch-compatible PLTs could not be identified. These patients were instead transfused with HLA-matched PLTs. The remaining 66 patients received a total of 738 crossmatched PLT units, with a median of 7 units transfused per patient (range, 1-49 units). Of these transfused units, 443 had posttransfusion counts adequate for calculation of a 1-hour CCI. The mean (± standard deviation) CCI in response to each crossmatched unit transfused was 7000 (±7900), significantly higher than that for random PLT units ($p < 0.001$). Figure 2A shows a histogram of the mean CCI in response to crossmatched units for each patient. In addition, we plotted the mean CCI for first through 15th PLT unit transfused, averaged over all patients (Fig. 2B). Our data do not show any evidence for increasing alloimmunization in response to repeated transfusions of crossmatch PLTs.

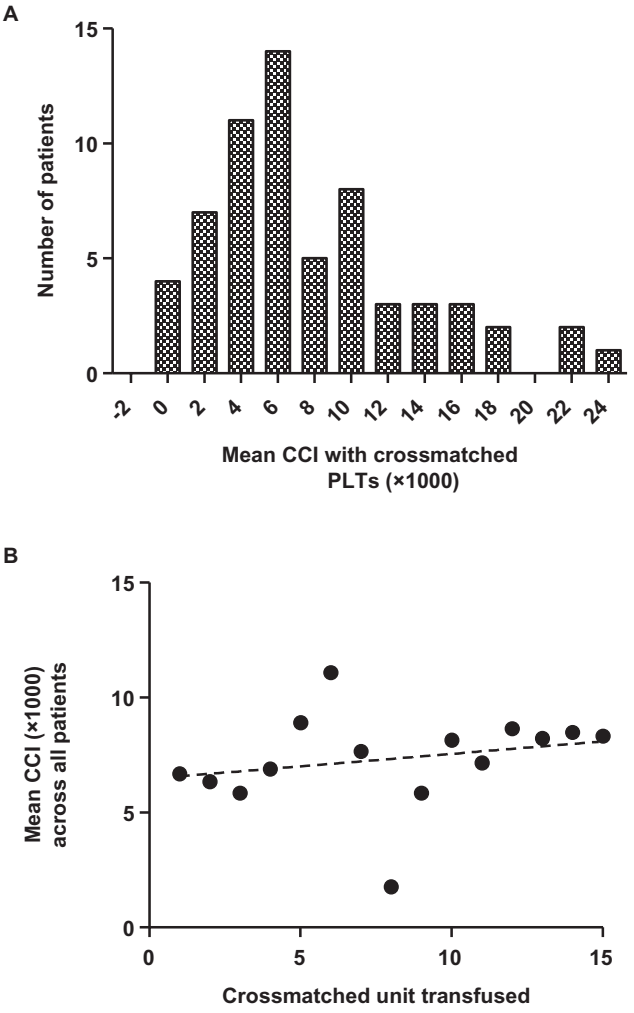

Fig. 2. CCIs in response to crossmatched units. (A) Mean CCI in response to crossmatched PLTs for each patient. (B) CCI averaged across all patients for the first through 15th crossmatched PLT unit transfused (minimum number of units transfused at each data point, 10). Linear fit shows no evidence of decreased response to transfusion with successive PLT units.

We also investigated whether there was any correlation between mean CCI response to crossmatched PLTs and the degree of reactivity observed on the initial crossmatch assay. One may suppose that for patients with a low degree of crossmatch reactivity, the large majority of random PLT units would actually be crossmatch compatible; thus, obtaining crossmatched PLTs would not lead to much improvement in CCI over prior ineffective response to random units. Conversely, for patients with a high degree of reactivity, it may be assumed that the large majority of randomly transfused PLTs were rapidly consumed due to alloimmunization, so obtaining crossmatched units may provide the most CCI benefit. In exploring this hypothesis (Fig. 3), we found that the few

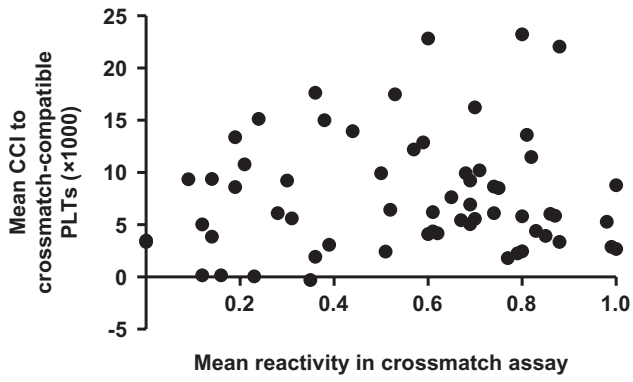

Fig. 3. Lack of correlation between mean CCI and mean crossmatch assay reactivity. While the few patients who benefited the most (i.e., highest mean CCI) from crossmatch-compatible units had higher degrees of mean crossmatch assay reactivity, overall the correlation between mean CCI and mean reactivity was weak (Pearson r = 0.08).

patients with the largest mean CCI benefit in response to crossmatched PLTs (CCI > 20,000) did demonstrate higher degrees of average crossmatch reactivity (>60% reactivity). However, overall we found only a very weak correlation (Pearson r = 0.08) between mean CCI response and mean degree of crossmatch reactivity. This finding indicates that for the majority of patients, other nonalloimmune causes of PLT refractoriness may play an important role in determining response to transfusion. Variability in response to individual PLT units or variation in successive crossmatch assay reactivity also may not be reflected in these mean values.

Twelve patients (17%) received HLA-matched units. Eight of these patients had HLA antibody screen testing performed during the same hospitalization as HLA-matched PLTs were administered. Seven of eight patients demonstrated HLA Class I antibody reactivities over 85% (five with 100%); all eight demonstrated a panreactive crossmatch assay during the same hospitalization. These results suggest that reactivity in the crossmatch assay may correlate with antibodies directed toward HLA antigens. Of the 10 patients receiving both crossmatched and HLA-matched PLTs, three demonstrated no significant benefit with HLA matching, with CCI differences (= mean CCI of HLA-matched units – mean CCI of crossmatched units) of –3300, –2400, and 1500. Two demonstrated moderately improved response, with CCI differences of 4500 and 6500. The remaining five patients demonstrated markedly improved response, with CCI differences of 9400, 11,100, 12,500, 24,900, and 25,200, respectively.

Complications of thrombocytopenia during management with crossmatched PLTs

We also examined the electronic medical record during the time period the patients were managed with cross-

matched or HLA-matched PLTs provided under our institutional policies. One patient, with acute myeloid leukemia undergoing high-dose chemotherapy, was initially responsive to crossmatched units. Later, he showed poor increments to PLT transfusions (PLT counts remained between 1×10^9-3×10^9/L), developed pulmonary hemorrhage, and expired. Two crossmatch assays performed immediately before expiration were panreactive. He did not receive HLA-matched PLTs. No other patients developed a WHO Grade IV bleed.[18]

DISCUSSION

Crossmatch-compatible PLTs are known to provide good posttransfusion increments in the short term for alloimmunized patients refractory to random PLT units. We examined the effectiveness of the crossmatch-compatible method of PLT selection as the first-line approach for medium-term transfusion support of patients with PLT refractoriness.

Sixty-six of 71 (93%) patients included in the study were transfused with crossmatched PLTs during the course of their illness. Only one patient (1.5%) developed a WHO Grade IV bleed, a rate similar to that observed in studies examining transfusion thresholds in patients with thrombocytopenia.[20] The mean CCI of 7000 achieved with crossmatched PLTs in this study corresponds to a PLT count increase of 14×10^{15}/L in a standardized patient (assuming a mean adult body surface area of 1.73 m^2 and a typical PLT dose of 3.5×10^{11}), which is sufficient to avoid significant spontaneous bleeding. Thus, while many patients required transfusions on a daily or every-other-day schedule, the beneficial effect of crossmatched PLTs likely prevented serious bleeding complications. Our study did not specifically exclude patients with fever, splenomegaly, coagulopathy, or other potential clinical causes of refractoriness, as such patients reflect the reality of PLT management at a quaternary care center with both active hematology or oncology and organ transplantation services. Overall, only 41% of crossmatched units resulted in a CCI of greater than 7500. However, this CCI response to crossmatched units was still significantly higher than comparable random PLT units for these patients, demonstrating benefits with crossmatch compatibility despite not reaching a mean CCI over our standard threshold for a successful transfusion. The modest response to crossmatched units seen in our study is also consistent with prior studies[3,21] and likely points to in vivo, nonimmune causes for PLT refractoriness trumping crossmatch compatibility established in vitro. Of note, however, the retrospective nature of this study and heterogeneous patient population preclude a comprehensive analysis of the effectiveness of crossmatched PLTs in patients with immune-mediated refractoriness to transfusion.

One of the goals of this study was to establish trends in transfusion response over time with the use of crossmatched PLTs. Given that crossmatch-compatible PLTs are not necessarily selected or matched for HLA antigens, one may hypothesize that patients receiving multiple transfusions may eventually develop HLA antibodies to the mismatched antigens. Our results showed that while patients demonstrated wide variation in reactivity in individual crossmatch assays, there was no overall trend toward increasing alloimmunization during medium-term management with crossmatched PLTs. The CCI response to crossmatched PLTs averaged across all patients stayed relatively constant for 15 consecutively transfused PLT units (Fig. 2B). A previous study has demonstrated the effectiveness of a donor selection strategy based on matching for HLA antibody specificities rather than matching for HLA antigens.[16] Similar to our findings, this study also found no evidence for a significant increase in alloimmunization to unmatched HLA antigens over time and showed that the selection of compatible PLTs based on the patient's HLA antibody specificity provides an alternative to the gold standard of matching for HLA antigens. However, the need to perform periodic HLA antibody testing for changing specificities of antibodies is a potential limitation. Patients in both studies benefitted from leukoreduced, SD apheresis PLTs. Leukoreduction decreases alloimmune PLT refractoriness by decreasing exposure to donor antigen-presenting cells.[22] Interestingly, a large proportion of patients in our study (39%; n = 28) appeared to demonstrate decreasing alloimmunization over successive assays, with mean reactivity decreasing by 10% or more per assay. This finding is consistent with other studies that showed the disappearance or decrease in titers of HLA antibodies over time.[23,24] The knowledge that most patients demonstrated, on average, a consistent response to crossmatched PLTs is reassuring from the perspective of using this strategy for the mid-term transfusion support of refractory patients.

For institutions using the crossmatch strategy, the ability to predict in advance the likelihood of not identifying a single compatible unit after a crossmatch assay is important for patient management. We stratified study patients based on reactivity in their initial crossmatch assays. For patients with initial crossmatch reactivity of not more than 66%, subsequent assay reactivity was difficult to predict, with similar likelihood of progressing toward either panreactivity or zero reactivity. However, we found that for patients with an initial crossmatch assay reactivity of greater than 66%, there was a 45% likelihood that at least one subsequent crossmatch assay would not yield any compatible units, prompting a workup for HLA-matched PLTs. In light of the findings presented here, we have changed our institutional protocol for PLT refractoriness. For patients with an initial crossmatch reactivity of greater than 66% and a high clinical probability of contin-

ued thrombocytopenia, we now initiate patient HLA typing and antibody testing in anticipation of difficulty supporting future transfusion needs exclusively with crossmatched PLTs. This contrasts with our prior practice of initiating such a workup only after the first panreactive crossmatch assay.

Twelve of 71 (17%) patients required HLA-matched PLTs. Ten of these patients initially received crossmatched PLTs; only later did they receive HLA-matched products. Two other patients required HLA-matched products from the outset as crossmatch-compatible units could not be identified. In our very limited sample, we found that more than half of patients receiving HLA-matched PLTs demonstrated markedly improved (CCI difference > 7500) response to HLA-matched PLTs when compared with their response to crossmatched PLTs. While in our retrospective study grades of HLA matching were not available for review, a small prospective study found that crossmatched PLTs provided similar transfusion response to HLA-matched PLTs overall, although the highest grades of HLA-matching (A and BU) provided an appreciably greater benefit than crossmatched PLTs alone.[8] Other studies have found that crossmatch compatibility is a key determinant of response to PLT transfusion, although HLA matching may further improve response to crossmatch-compatible units.[25,26]

It is important to note, however, that for new patients, the process of HLA typing, testing for HLA antibodies, and the identification and collection of PLTs from HLA-matched donors could take several days to complete. For institutions that restrict themselves to an HLA-matching strategy, this could mean an initial delay in finding compatible PLTs and dealing with the risks attendant with uncorrected severe thrombocytopenia. This delay could be accentuated for smaller facilities without HLA testing capabilities or for transfusion services that primarily depend upon blood suppliers without a local pool of HLA-typed donors. In contrast, most blood suppliers have the expertise to perform PLT crossmatch assays and provide compatible PLTs, often within 6 to 24 hours. An earlier comparison of cost-effectiveness, from the perspective of the supplying blood center, suggested that the high fixed costs associated with HLA typing of patients and donors makes providing HLA-matched PLTs more expensive than crossmatched PLTs.[13] However, for patients with prolonged transfusion needs, frequent crossmatch assays increase the variable costs, while there is relatively little marginal cost associated with long-term support with HLA-matched PLTs.[13] As charged by our blood suppliers to our institution's blood bank, random SD apheresis PLTs cost $622 per unit, crossmatched PLTs cost on average approximately $836 per unit, and HLA-matched PLTs cost between $950 and $1200 per unit. However, these relative costs may vary widely across medical centers.

In summary, our study showed that longitudinal management with crossmatch PLTs provided an overall effective regimen for the medium-term management of patients refractory to random SD apheresis PLTs, with improved CCIs and sustained response over time. In addition, PLT crossmatching may provide a potentially more rapid first-line alternative to HLA-matched or HLA-compatible PLT transfusions for refractory patients who do not yet have HLA typing performed. Importantly, the prolonged use of crossmatched PLTs did not worsen the degree of alloimmunization over time. We identified a threshold for high reactivity in the initial crossmatch assay that helps predict the need to switch to HLA-matched PLTs for some patients; this has now been incorporated into our protocol to further enhance safety of our approach for severely alloimmunized patients. The primary limitation of the crossmatch method is the need to perform frequent assays for patients requiring ongoing transfusion support. We anticipate that this study will help transfusion services evaluate the safety and effectiveness of a crossmatched PLTs strategy for the mid-term transfusion support of complex patients with PLT refractoriness.

ACKNOWLEDGMENT

We thank the staff of the reference laboratory at the Blood Centers of the Pacific for performing platelet crossmatch assays.

CONFLICT OF INTEREST

None.

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