Leukocyte Reduction of Red Blood Cell Transfusions Does not Decrease Allosensitization Rates in Potential Kidney Transplant Candidates

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Abstract. A significant proportion of potential kidney transplant candidates continue to periodically require blood transfusions that carry a risk of allosensitization. Leukocyte reduction (leukoreduction) of blood products has been proved to reduce transfusion-associated allosensitization in patients with hematologic malignancies; however, the effect in potential kidney transplant candidates is unknown. A total of 112 kidney transplant candidates who received red blood cell transfusions while on the transplant waiting list were identified retrospectively. Sixty received a transfusion before leukoreduction (non-LR), and 52 received a transfusion after the local implementation of universal leukoreduction of blood products (LR). There was no difference in transfusion-associated allosensitization rates in patients who received a transfusion during the two eras (non-LR 27% [16 of 60] versus LR 33% [17/52]; NS). Likewise, no difference was observed in subgroups identified as being at high risk of allosensitization (previous pregnancy, transplant, or five or more previous transfusions) or at low risk (no previous allogeneic exposures) (high risk: non-LR 52% versus LR 55%; low risk: non-LR 10% versus LR 8%). Multivariate analysis revealed previous pregnancy to be the only significant risk factor associated with transfusion-associated allosensitization (relative risk, 8.2; 95% confidence interval, 2.4 to 24.0; \( P = 0.0001 \)). Leukoreduction, in particular, was not associated with any protective effect. In summary, leukoreduction of red blood cell transfusions does not confer any protection against transfusion-associated allosensitization for potential kidney transplant candidates. Physicians who care for patients with ESRD must continue to practice careful transfusion avoidance while alternative strategies to minimize transfusion associated allosensitization are sought.

Allosensitization is associated with significant barriers to successful transplantation in patients with ESRD, including prolonged waiting times and inferior graft outcomes (6–8). Accordingly, any measure to limit allosensitization would represent a substantial advance for ESRD patients. Of the three principal causes of allosensitization—pregnancy, transplantation, and transfusions—only the last is perhaps modifiable. Leukocyte reduction of blood products (leukoreduction) reduces the transfused load of allogeneic leukocytes and has been proved to limit transfusion-associated allosensitization in patients with hematologic malignancies undergoing chemotherapy (9).

The impact of RBC leukoreduction on allosensitization in ESRD patients is unknown. The few studies that have examined this practice either have been uncontrolled or have screened for allosensitization using technically inferior anti-HLA antibody screening techniques (10, 11). Several recent studies have highlighted the superior sensitivity of flow cytometric anti-HLA antibody screening (FlowPRA) (12–14). We thus set out, in this retrospective cohort study, to use sensitive flow cytometric techniques to determine whether universal RBC leukoreduction has reduced the incidence of transfusion-associated allosensitization in potential kidney transplant candidates within our center.
Materials and Methods

Universal Leukoreduction in Canada

All blood products within Manitoba are distributed by a single agency, Canadian Blood Services, and since September 1999, all RBC units distributed within Manitoba have been leukoreduced in compliance with a nationwide Health Canada directive (15). This directive was issued in response to numerous lines of evidence indicating that leukoreduction of blood products likely reduces the incidence of several adverse transfusion reactions, including allosensitization. The Winnipeg Blood Centre now performs universal prestorage leukoreduction of RBC units with commercially available in-line filtration systems (Leukotrap WB and RC PL; Pall Medical, East Hills, NY), and the maximum accepted residual white blood cell (WBC) count is \(<5 \times 10^6/\text{unit}\) (normal WBC content approximately \(5 \times 10^7/\text{unit}\)). Internal quality control testing is applied to at least 1% of all units, and the actual residual WBC content is observed to be approximately \(3 \times 10^7/\text{unit}\) (unpublished data, Canadian Blood Services/Pall Corp.).

Study Procedures

This study was approved by the University of Manitoba Biomedical Research Ethics Board. The study population consisted of patients who were on the Manitoba renal transplant waiting list and had received RBC transfusions while wait-listed for transplantation. None of the transfusions administered was prescribed as deliberate pretransplant transfusions aimed at optimizing graft outcomes. Serum collection and transfusions are tracked meticulously by local transplant coordinators and Immunogenetics Laboratory technologists. Adult transplant candidates who received RBC transfusions between January 1996 and June 2003 thus were identified for retrospective study, and of 112 wait-listed ESRD patients identified, 60 received RBC units before universal leukoreduction (non-LR) and 52 thereafter. Individuals who were broadly sensitized (FlowPRA \(\geq 80\%\)) before transfusion were excluded (\(n = 3\)). Patient data and transfusion records were abstracted from Manitoba Renal Program database.

Anti-HLA Antibody Screening

Transfusion-associated allosensitization was determined by anti-HLA panel reactive antibody (PRA) screening pre- and posttransfusion. Sera were batched and then screened concurrently by both the anti-human globulin cytotoxicity technique (AHG-CDC PRA) and a flow cytometric technique (FlowPRA; OneLambda). Both screening assays were performed in the Immunogenetics Laboratory at the Winnipeg Blood Centre using standard techniques previously described (13). A patient was considered sensitized before a transfusion when the AHG-CDC PRA was \(\geq 10\%\) and/or when the FlowPRA assay revealed any detectable anti-HLA antibodies. Transfusion-associated sensitization was defined as the de novo appearance of a positive FlowPRA or as an increment in the FlowPRA value of \(\geq 10\%\).

Statistical Analyses

Statistical analysis was performed using Statview 5.0 software (SAS Institute, Cary, NC). Values are reported as mean ± SEM or, where indicated, as medians and ranges. The \(\chi^2\) test was used for comparison of categorical variables, whereas the \(t\) test was applied to comparisons of continuous variables. \(P \leq 0.05\) was considered to be significant, and values \(>0.10\) are reported as nonsignificant (NS). In the multivariate analysis of risk factors for allosensitization, univariate risk factors associated with the outcome with \(P \leq 0.10\) were allowed into the final model. These included a +ve FlowPRA before transfusion, previous pregnancy, previous transplantation, previous transfusions, and the number of RBC units transfused in the episode under study. Leukoreduction was considered in the models despite being found to be nonsignificant in univariate analysis. Pregnancy and previous transfusions were considered as both categorical and continuous variables in the models analyzed. There was no demonstrable relationship between increasing numbers of pregnancies or transfusions and an increasing incidence of allosensitization, and the overall strength of the model was superior when these were considered as categorical variables. For these reasons, five or more previous transfusions was chosen as the transfusion variable, and this cutoff is also supported by previous studies (16).

Results

During the period of study, 112 individuals on the renal transplant waiting list received RBC transfusions and had appropriate pre- and posttransfusion serum samples collected for anti-HLA antibody screening. Sixty patients received a transfusion before universal leukoreduction (non-LR) and 52 thereafter (LR). There were significant baseline demographic differences between these two groups (Table 1). Patients who received leukoreduced transfusions were more likely to have

<table>
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<tr>
<th>Table 1. Baseline demographicsa</th>
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<tr>
<td>Gender (male/female)</td>
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<td>Age when transfused</td>
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<tr>
<td>Pregnancy</td>
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<tr>
<td>No. of pregnancies (median, range)</td>
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<tr>
<td>Previous transplant</td>
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<tr>
<td>Previous RBC transfusion</td>
</tr>
<tr>
<td>≥5 Previous RBC transfusions</td>
</tr>
<tr>
<td>+ve FlowPRA pretransfusion</td>
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<tr>
<td>Units transfused</td>
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a RBC, red blood cell.
had a previous transfusion, were more likely to have received five or more RBC transfusions in the past, and were more likely to be allosensitized before the transfusion episode under examination (Table 1). Both groups received the same mean number of RBC units in the transfusion episode under study.

**Transfusion-Associated Allosensitization and Leukoreduction**

The overall rates of transfusion-associated allosensitization were 27% (16 of 60) in the population that received standard RBC units and 33% (17 of 52) in those who received leukoreduced RBC (NS; Table 2). Of the 33 individuals who met the definition of transfusion-associated allosensitization, 16 were previously unsensitized and 17 demonstrated a +ve FlowPRA before transfusion. Fifteen of the 33 individuals developed isolated new HLA class I antibodies, six developed isolated class II antibodies, and 12 developed new class I and II antibodies. In previously unsensitized patients, the mean HLA class I and class II FlowPRA posttransfusion became 53 ± 8% and 34 ± 11%, respectively, whereas for previously sensitized patients, the mean increment in the class I and class II FlowPRA was 35% and 39%, respectively (class I pre, 44 ± 8%; post, 79 ± 5% [P < 0.01]; class II pre, 35 ± 7%; post, 74 ± 9% [P < 0.01]). There was no significant difference in either the degree (% PRA) or the nature of allosensitization (HLA class I and/or class II) that developed in patients who received standard versus leukoreduced transfusions (data not shown).

Fifty-two of 112 patients were considered to be at high risk of transfusion-associated allosensitization on the basis of having had previous allogeneic exposures (previous pregnancy, previous transplantation, and five or more previous RBC transfusions), whereas 44 of 112 were considered to be at low risk (no previous allogeneic exposures). No effect of leukoreduction on allosensitization rates was seen in either of these two subgroups (high risk, 52% non-LR versus 55% LR [NS]; low-risk, 10% non-LR versus 8% LR [NS]; Table 2). AHG-CDC PRA was positive in only six (19%) of 32 patients who displayed a +ve FlowPRA before transfusion. Similarly, AHG-CDC PRA detected new anti-HLA antibodies in only 22 (67%) of the 33 patients who developed transfusion-associated allosensitization as determined by FlowPRA.

**Risk Factors for Transfusion-Associated Allosensitization**

In univariate analysis, factors that correlated with an increased likelihood of transfusion-associated allosensitization included a +ve FlowPRA before transfusion, previous pregnancy, and five or more previous RBC transfusions (Table 3). In multivariate regression analysis, only previous pregnancy was associated with an increased risk of transfusion-associated allosensitization (relative risk, 8.2; 95% confidence interval, 2.8 to 24.0; P = 0.0001). Leukoreduction per se was not found to be protective in either univariate or multivariate analyses. Rates of allosensitization were similar for women who had a history of pregnancy and received either standard or leukoreduced transfusions (9 [69%] of 13 non-LR versus 11 [55%] of 20 LR; NS).

**Discussion**

A significant proportion of patients with ESRD are denied the full potential benefits of transplantation as a result of allosensitization. Allosensitized patients experience longer waiting times for finding compatible donors and are at risk of inferior graft outcomes transplanted (6). The barrier created by allosensitization is exemplified by the fact that approximately 30% of UNOS wait-listed renal transplant candidates are allosensitized yet only approximately 10% of transplants are performed in sensitized recipients (6). Of the three principal causes of allosensitization—pregnancy, previous transplantation, and transfusions—only the last is potentially modifiable.

Nephrologists who care for ESRD patients are well aware of the risk of deleterious allosensitization and are careful to avoid unnecessary transfusions; however, this patient population remains at risk of periodically requiring allogeneic transfusions. The UNOS database indicates that approximately 30% of wait-listed transplant candidates continue to require blood transfusions at some point before transplantation (2).

Recently, several groups reported their experience with novel immunsuppressive protocols incorporating intravenous immunoglobulin and plasmapheresis to enable the successful transplantation of sensitized recipients (17–19). Although encouraging, these protocols are available to only a small proportion of sensitized potential recipients and will likely do little to address the disparity in access to transplantation. Strategies to prevent allosensitization are likely to have a greater impact for ESRD patients.

Leukoreduction of blood products reduces the load of allogeneic HLA in transfusions and has been proved to diminish allosensitization rates in patients who have hematologic malignancies undergoing chemotherapy (9). Several randomized trials have reported a benefit in this population (20–27). The TRAP study, most notably, randomized >200 patients with acute leukemia to receive leukoreduced RBC and either modified platelet preparations or irradiated, filtered, or apheresed platelet concentrates (27). Patients who received any of the modified platelet products were significantly less likely to

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**Table 2. Transfusion-associated allosensitization rates**

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<tr>
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<th>Transfusion-Associated Allosensitization</th>
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<tr>
<td></td>
<td>Pre-leukoreduction</td>
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<tr>
<td>All patients (n = 112)</td>
<td>16/60 (27%)</td>
</tr>
<tr>
<td>High risk (n = 52) (previous pregnancy, Tx, ≥5 tf)</td>
<td>12/23 (52%)</td>
</tr>
<tr>
<td>Low risk (n = 44) (no previous pregnancy, Tx, or tf)</td>
<td>3/31 (10%)</td>
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* Tx, transplant; tf, transfusion.
develop new anti-HLA antibodies and become refractory to platelet transfusions (17 to 21% versus 45%; P < 0.001). It must be noted, however, that studies that have examined leukoreduction and allosensitization have almost exclusively been performed in this patient population. Patients therein have also received large absolute numbers of transfusions with both platelet and RBC preparations (e.g., 14 ± 11 platelet and 15 ± 7 RBC transfusions in the TRAP study).

There are comparatively few data on the impact of leukoreduction on allosensitization as a result of RBC transfusions in patients with ESRD. In the 1980s, SanFilippo et al. (10) conducted a randomized study transfusing renal transplant candidates with either standard or leukoreduced RBC units and found no difference in allosensitization. Importantly, there was no assessment of the extent and consistency to which leukoreduction was achieved with the techniques applied, and anti-HLA antibody screening was performed with the AHG-CDC technique, which is less sensitive than current flow cytometric techniques. Christiaans et al. (11) examined potential transplant candidates who were given leukoreduced RBC transfusions and reported that de novo HLA class I antibodies developed in only 6% when screened by flow cytometry. This study, however, was uncontrolled and examined only low-risk, previously unsensitized patients. Limited literature also exists in other, nonrenal patient populations. Recently, Van de Watering et al. (28) randomized >400 patients who were undergoing cardiac surgery to receive either standard or leukoreduced RBC transfusions and observed no difference in allosensitization rates in either unsensitized or previously sensitized patients.

In the current study, we found no significant difference in the rate of transfusion-associated allosensitization in renal transplant candidates who received either standard or leukoreduced RBC transfusions (27% versus 33%, respectively; mean, 3 ± 0.3 transfusions). Furthermore, similar rates and degrees (i.e., Δ%PRA) of allosensitization were seen in both low-risk and high-risk patients who received transfusions of leukoreduced blood. The observed rate of allosensitization in high-risk patients is slightly higher than previously reported, and this is likely attributable to the superior sensitivity of the flow cytometric screening technique that we used. Studies using CDC techniques have reported allosensitization rates of approximately 30% in high-risk recipients, in contrast to the approximately 50% rate that we observed with FlowPRA screening (29–31). These anti-HLA antibodies detected solely by flow cytometry are clinically relevant and have been increasingly associated with adverse outcomes posttransplantation (12–14, 31–34).

We observed previous pregnancy to be the strongest risk factor for transfusion-associated allosensitization, a finding in keeping with previous observational studies (35, 36). Other allogeneic exposures, such as a previous transplant or previous transfusions, have also been reported to be risk factors for transfusion-associated allosensitization, although did not reach statistical significance in multivariate analysis herein (35, 36). This may represent a limitation of the size of our data set or, alternatively, an accurate representation of risk factors within our patient population. Notably, leukoreduction was not associated with any protective effect in either univariate or multivariate analysis.

This study is retrospective, and ideally a randomized, controlled trial would be performed to evaluate the impact of leukoreduction on allosensitization in potential renal transplant recipients. This, however, is unlikely to occur. Many blood distribution organizations have in recent years adopted a policy of universal leukoreduction of blood products, and although not without controversy, this practice is now widespread (37, 38). In Canada and most of Europe, universal leukoreduction has been in place for several years, whereas approximately 70% of U.S. RBC units are currently leukoreduced before distribution. Organizations that monitor transfusion standards are unlikely to permit a change to previous blood-handling procedures; thus, retrospective studies such as this are necessary to investigate the effects of leukoreduction in patient populations other than those with hematologic malignancies (39).

Several reasons may underlie why leukoreduction fails to diminish allosensitization rates in patients with ESRD. Individuals with ESRD are likely more immunocompetent than those who have hematologic malignancies and undergo treatment with myeloablative chemotherapy. This is supported by the similar sensitization rates in the two populations, despite the considerably greater overall exposure to allogeneic blood products in the latter. The mean number of RBC units transfused in the current study was 3 ± 0.3, whereas patients who received leukoreduced products in the TRAP trial, in which approximately 20% became allosensitized, received fivefold

Table 3. Risk factors for transfusion-associated allosensitization

<table>
<thead>
<tr>
<th>Risk Factors (RR, 95% CI)</th>
<th>Univariate</th>
<th>P</th>
<th>Multivariate</th>
<th>P</th>
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<tbody>
<tr>
<td>FlowPRA +ve pretransfusion</td>
<td>4.5 (1.9–11)</td>
<td>&lt;0.001</td>
<td>2.4 (0.8–7.1)</td>
<td>0.10</td>
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<tr>
<td>Pregnancy</td>
<td>7.8 (3.1–19.5)</td>
<td>&lt;0.001</td>
<td>8.2 (2.8–24)</td>
<td>0.0001</td>
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<tr>
<td>Previous transplant</td>
<td>2.8 (0.9–8.7)</td>
<td>0.08</td>
<td>2.4 (0.6–9.9)</td>
<td>NS</td>
</tr>
<tr>
<td>≥5 Previous transfusions</td>
<td>5.1 (2.0–13.1)</td>
<td>&lt;0.001</td>
<td>2.6 (0.8–8.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Leukoreduction</td>
<td>0.5 (0.3–1.7)</td>
<td>NS</td>
<td>2.0 (0.7–6.0)</td>
<td>NS</td>
</tr>
<tr>
<td>RBC units given (per unit)</td>
<td>1.1 (1.0–1.3)</td>
<td>0.10</td>
<td>1.1 (0.9–1.3)</td>
<td>0.10</td>
</tr>
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</table>

*RR, relative risk; CI, confidence interval.
this amount of platelet and RBC transfusions (27). Similarly, the degree of leukoreduction achieved with current techniques may be inadequate to prevent allosensitization in ESRD patients. Quality control assessments performed for Canadian Blood Services reveal reliable three to four logfold reductions in RBC unit leukocyte content. However, it may be that the residual leukocyte content (approximately $3 \times 10^5$/unit) represents a sufficient residual exposure to allogeneic HLA to induce an alloimmune response. Current leukoreduction techniques are similar in the degree of leukoreduction achieved, and there are no clinical data to favor current cutoff standards of approximately 1 to 5 $\times 10^6$/unit for minimizing allosensitization (9, 38, 40–42). It is interesting that rodent models suggest that too great a degree of leukoreduction may in fact promote allosensitization, although the clinical relevance of this is unknown (43). Finally, leukocytes are not the sole source of allogeneic HLA in transfusions as soluble HLA and even RBC-bound HLA are present as well, and these are not diminished by leukoreduction (44–46).

If standard leukoreduction fails to diminish allosensitization in potential renal transplant candidates, then alternative approaches must be sought. One approach that is occasionally used is the prescription of a brief course of immunosuppression beginning at the time of transfusion (e.g., with azathioprine or cyclosporine). This strategy has not been evaluated adequately, and its safety and generalizability are questionable (47–49). Many patients who require transfusions are likely too acutely ill to be prescribed such therapy, which would presumably be necessary for a number of weeks peritransfusion. HLA-matched transfusions have in the past been successful in preventing allosensitization, and this strategy would be preferable but is limited by logistic concerns (50, 51). Existing blood distribution centers may have sizable numbers of HLA-matched donors on file (e.g., in bone marrow donor registries), but blood from HLA-typed individuals may not necessarily be on hand and may not be available in the time frame required for transfusion. Last, there is hope that less allogeneic blood substitutes or modified blood products will become available; however, this seems unlikely in the near future (52, 53).

In summary, transfusions continue to be an important cause of allosensitization for potential kidney transplant recipients. Leukoreduction of RBC transfusions does not seem to reduce allosensitization rates in this patient population. The need for periodic transfusions persists for many patients on transplant waiting lists, and alternative transfusion strategies to prevent allosensitization must be found.

Acknowledgments
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