Bedside Filtration of Blood Products in the Prevention of HLA Alloimmunization—A Prospective Randomized Study

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To test the efficacy of poststorage bedside leucodepletion of blood products in the prevention of primary HLA alloimmunization and its clinical sequelae, 172 patients with hematologic malignancy requiring intensive red blood cell and platelet support were randomized to receive either standard or filtered red blood cells and platelets. Quality control of bedside filtration was explored by sequential sampling downstream of the filter, but this did not predict the total number of leucocytes transfused. After exclusions, 123 evaluable patients were assessed every two weeks until the end of therapy. HLA antibodies developed in 21 of 56 (37.5%) nonfilter (NF) and 15 of 67 (22%) filter (F) patients (risk ratio estimate, 0.60 [95% confidence interval, 0.34 to 1.05]; P = .07). Patients with acute myeloid leukemia (AML; n = 53) had higher alloimmunization rates in both arms of the study, with a greater effect of filtration (62.5% NF and 31.0% F; P = .025). Bedside filtration did not affect the overall incidence of febrile transfusion reactions (FTRs; 37% NF and 34% F; P = .71) or of platelet refractoriness assessed in 50 patients (30% NF and 26% F), despite an association between broad HLA reactivity and both FTRs and refractoriness. However, FTRs were also seen in 28 patients without HLA antibodies. Five alloimmunized refractory patients (2 F and 3 NF) required HLA-selected platelets. This report, the first prospective study of bedside filtration, has failed to show clear clinical benefit. Methodological limitations may account in part for this failure, notably the difficulties in accurately assessing the number of leucocytes transfused.

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MODERN TREATMENT regimens for hematologic malignancy result in prolonged periods of pancytopenia requiring blood product support. Exposure to multiple red blood cell (RBC) and platelet transfusions leads to HLA alloimmunization in 30% to 70% of patients. Alloimmunization may be associated with febrile transfusion reactions (FTRs) and failure to achieve clinically useful increments with random donor platelets. The alloimmunized patient with platelet refractoriness may need costly and logistically difficult platelet support from HLA-matched donors; therefore, much emphasis has been placed on the prevention of alloimmunization.

Early experimental and clinical studies showed that alloimmunization is much more efficient in the presence of residual allogeneic leucocytes in cellular blood components and that platelets alone, lacking class II HLA antigens on their surface, are less immunogenic. When used correctly, the latest generation of 3-log leucodepleting filters can achieve the reduction in leucocyte count (residual count, <5 x 10^9/L) associated with a decrease in the incidence of primary HLA alloimmunization. Clinical studies with these filters have shown significant reductions in HLA alloimmunization and improved platelet increments. However, in these studies leucodepletion was performed on components less than 48 hours old under laboratory conditions. The current trend is for leucodepletion by filtration to be performed at the bedside on stored RBCs and platelets, but animal data suggest that poststorage filtration does not match fresh filtration in preventing refractoriness.

We report the first multicenter, randomized, controlled trial of bedside leucodepletion in patients requiring intensive blood product support for hematologic malignancy. HLA alloimmunization, FTRs to blood products, and platelet refractoriness were monitored in patients receiving either RBCs and platelets leucodepleted at the bedside or standard blood products.

MATERIALS AND METHODS

Patients. Adult patients presenting to participating centers with hematologic malignancies likely to require intensive blood product support were eligible for entry to the study. Patients with aplastic anemia and any patient who had received RBCs or platelets from more than 10 donors were excluded, as were patients found to have anti-HLA antibodies at entry. Transfusions from 10 donors or less and all previous pregnancies were noted but were not reasons for exclusion. The protocol was approved by local Ethical Committees at participating hospitals, and all patients gave informed written consent.

Treatment protocols. Of the 57 acute myeloid leukemia (AML) patients, 27 were treated on the Medical Research Council AML 10 protocol involving induction with daunorubicin, cytosine arabinoside, and 6-thioguanine, followed by consolidation with the same agents plus etoposide, amascrine, and mitozantrone. The remaining patients also received regimes including an anthracycline and cytosine arabinoside.

Of 14 patients with acute lymphoblastic leukemia (ALL), 1 received induction with vincristine, high-dose prednisolone, and daunorubicin, followed by L-asparaginase, etoposide, and cytosine arabinoside.

Of the 15 patients with Hodgkin’s Disease (HD) and of the 28 patients with non-Hodgkin’s lymphoma (NHL)/chronic lymphocytic leukemia (CLL), 12 and 23 patients, respectively, were treated in one participating center (Cambridge). All HD patients and 11 NHL/CLL patients received Carmustine, cytosine arabinoside, etoposide, and melphalan, followed by bone marrow (BM) autografting.
whereas the remaining 17 were treated with a combination of cyclophosphamide, etoposide, vincristine, bleomycin, methotrexate, and doxorubicin. Of the 10 chronic myeloid leukemia (CML) patients, 9 were treated in one center (Hammersmith, London, UK). All 10 received BM allografting (conditioning with cyclophosphamide and total body irradiation) or autografting (conditioning with busulfan and cyclophosphamide). All 5 myeloma patients were autografted after high-dose melphalan or cyclophosphamide.

**Patient monitoring.** Patients were randomized separately within each of five participating centers to receive either standard or filtered RBC and platelet concentrates from the outset of therapy, and unless withdrawn, remained on study until the end of intensive blood product support. Blood samples were taken at entry, at 2-week intervals during the period of blood product support to minimize the risk of missing transient HLA antibodies, and at 3 weeks after the last transfusion to detect late seroconversions.

Platelet increments (uncorrected for surface area) were measured 1 hour after transfusion; alloimmunized patients could be withdrawn from the study if they could no longer be safely supported with random donor platelets. Patients who became refractory (1-hour uncorrected platelet increment of <10 x 10^9/L on two consecutive measurements) without the development of HLA antibodies were kept on study, and equally, the development of anti-HLA antibodies alone, without clinical sequelae, was not a reason for withdrawal.

All FTRs and other reactions to RBC and platelet transfusions were noted by ward staff on study documentation, and the trial coordinator was notified directly. An FTR was defined as an increase in temperature of more than 1°C during or within 4 hours of a transfusion, associated with chills or rigors and not, in the opinion of the ward staff, caused by infection or drug reaction. Febrile episodes thought to be caused by these causes were not noted. Prophylactic antipyretics were not permitted unless an FTR had already been recorded for that patient. Patients were withdrawn if they had two consecutive FTRs not prevented by antipyretics.

**Blood products.** RBCs and platelets were produced to national specifications11 and stored at Regional Transfusion Centres (Bristol, Cambridge, Leeds, Manchester, and North London, UK). The transfusion policy was defined at local centers but was consistent in that prophylactic platelets were administered every 1 to 2 days while the platelet count was less than 20 x 10^9/L (higher if the patient was bleeding or undergoing invasive procedures).

**Standard products.** RBC transfusions were either of concentration of RBCs or RBCs in optimal additive solution. The buffy coat was not removed; therefore, RBCs contained approximately 5 x 10^9 leucocytes/U. Storage was at 4°C for up to 35 days. Platelet transfusions were either processed from whole blood by the platelet-rich plasma method or collected from random donors by apheresis on Haemonetics PCS+ (Leeds, UK) or Baxter Autopheresis C machines (Compton, UK). These products were used interchangeably, according to availability. Quality control procedures were performed on 1% of products. As required by national specifications, 80% of single platelet concentrates prepared from whole blood contained greater than 5.5 x 10^10 platelets, less than 1.2 x 10^10 leucocytes, and less than 1.2 x 10^8 RBCs/ pack. Apheresis donations were equivalent to either four or five single platelet concentrates in terms of both platelet numbers and leucocyte contamination. Platelets were stored at 22°C with agitation (either flat-bed shaker or end-over-end rotator) for up to 5 days and were ABO-compatible as far as possible.

**Leucocyte-depleted products.** RBCs and platelets of any age were filtered during infusion into the patient using Pall RC50 and RC100 RBC and PL50 and PL100 platelet filters according to the manufacturer’s instructions (Pall Biomedical, Portsmouth, UK). RC50 and RC100 filters were used for 1 and 2 U of RBCs, respectively, whereas PL50 and PL100 filters were used for up to 5 and for 5 to 10 single platelet concentrates or apheresis equivalents respectively. Staff were formally trained and assessed in filter usage, and, as recommended, no bedside pumps were used. Manufacturers’ data showed 99.9% leucocyte removal from RBCs and platelets with 6% to 10% RBC and 5% platelet loss.

Quality control of bedside leucodepletion is limited by the fact that the entire postfiltration unit of RBCs or platelets is not available for counting. To establish whether meaningful quality control samples could be taken during or at the end of bedside filtration, a laboratory simulation was performed. Fourteen-day-old RBC units of 300 mL volume in citrate-phosphate-dextrose-adenine were filtered in the laboratory over 60 minutes through a Pall RC50 filter into a transfer pack. Samples were taken from the blood pack before filtration, downstream of the filter at different time points during filtration, and from the transfer pack at the end of filtration. Leucocyte counts in samples taken during and after filtration were performed in a Nageotte counting chamber (Karl Hecht Gmbh and Co, KG, Sondheim, Germany).

**Detection of HLA antibodies.** All laboratory investigations were performed in one laboratory. HLA antibodies were detected using a standard lymphocytotoxicity assay12 on a cryopreserved panel of 18 preselected cells; positive results were confirmed against a second panel of 18 selected cells. Results were considered positive if cytotoxicity was observed with greater than 10% (4 cells) of the total of 36 cells.

**Statistical analysis.** To compare the rates of alloimmunization in the trial arms, the chi-square test for contingency tables was applied.13 Where appropriate, risk ratio estimates were calculated as the ratio of the proportions of HLA alloimmunized patients in the two arms.13 To take account of patients being on study for varying lengths of time, the product-limit method of Kaplan and Meier14 was used to estimate distributions of the time free from HLA alloimmunization. Pointwise confidence intervals for the estimates were based on Greenwood’s formula.15 In the analysis of time to HLA alloimmunization, reported statistics and P values for the differences between the distributions were based on the log rank test.16 An analysis of covariance was performed to adjust the comparison of alloimmunization rates in the trial arms for any chance imbalance in factors thought to affect outcome. Covariate-adjusted risk ratio estimates and confidence interval (CI) were calculated along with associated significance levels from the Cochran-Mantel-Haenszel test. Allowing for the time to HLA alloimmunization, the analysis of the effect of filtration was undertaken with adjustment for covariates via the variable-adjusted log rank test.15 To be evaluable, patients had to have had at least one sample taken after randomization. The date of seroconversion was doubly censored, and the method of midpoint imputation was used,12 whereby this date was estimated to be midway between the last negative and first positive tests. This time to seroconversion was taken from randomization. It was assumed that no transient HLA alloimmunization occurred between two consecutive negative tests. If only negative results were obtained, the data were censored at the date of the last sample taken, regardless of the reason for withdrawal from the study. Statistical analysis was performed using SAS statistical program (Statistical Application Software, Inc, Cary, NC).18 From previous studies of laboratory leucodepletion, it was estimated that the HLA alloimmunization rates in the control and filter arms might be 50% and 10%, respectively. To detect this difference at the two-sided 5%-significance level with 90% power, at least 30 patients in each arm were required. To increase the power of the study, it was decided to enrol 80 to 100 evaluable patients. An interim analysis of the control arm only, after 27 patients had completed treatment, suggested that the alloimmunization rate in this...
group was closer to 40%. A target of 100 evaluable patients still provided high power to detect a decrease to 10% in the filter arm.

RESULTS

Patients. Between October 1989 and June 1992, 172 patients from five centers were entered into the study and randomized to receive either standard (n = 88) or filtered (n = 84) blood products. Of these, 15 (8.7%) patients were found to have HLA antibodies at entry; 7 patients had ineligibility diagnoses, HLA-antibody status at entry was not tested in 11, and 10 patients were found to have previously received greater than 10 donor units. Therefore, 129 patients (68 filtered [F] and 61 nonfiltered [NF]) were eligible for analysis, and their characteristics are shown in Table 1. The trial arms were evenly balanced with respect to age, sex, previous pregnancies, and the number and timing of previous transfusions, but there were differences in the distribution of patients undergoing BM transplantation (allogeneic or autologous) and in the distribution of A and O blood groups.

Patient follow-up data. Of the 129 eligible patients, 19 died during the trial period (see Fig 1). Only 2 deaths related to hemorrhage, both in the NF arm, but in neither case was the patient alloimmunized. A further 17 patients were withdrawn during the study but were not excluded from analysis; 5 were withdrawn because of clinical refractoriness in combination with HLA alloimmunization, and 12 were withdrawn because of uncontrollable FTRs. Two patients were not followed up until the end of treatment. The remaining 91 patients were followed up for a median of 67 days (range, 13 to 237 days) to the completion of treatment, and there was no difference between trial arms in the percentage of patients successfully reaching the end of blood product support.

Postfiltration leucocyte counts. As shown in Table 2, absolute leucocyte counts in samples taken downstream of the filter increased over 100-fold during the duration of the procedure. To establish whether sampling at any postfiltration timepoint was predictive of the total postfiltration leucocyte count, ratios of each timepoint sample:total leucocytes were calculated for each pack (Table 2). The wide interpack variation indicated that timepoint sampling during or at the end of bedside leucodepletion was not predictive of the total number of leucocytes in the entire unit after filtration. Importantly, it was also noted that all four units had total leucocyte counts of greater than 10^8/pack, one of which was greater than 5 \times 10^8/pack.

HLA alloimmunization. No blood was taken after the initial sample in 6 patients, and they are, thus, excluded from further analysis. For the remaining 123 patients, the median sampling interval was 14 days, and 75% of all samples were taken less than 20 days apart. To ensure that the difference in alloimmunization rates between trial arms was not because of clinically unimportant HLA antibodies of restricted specificity, the groups were compared according to the number of cells with which they reacted in a 36-cell panel. As shown in Table 3, there was no difference in the pattern of HLA reactivity between the two arms of the study. All analyses of HLA alloimmunization reported below were thus performed using a 10% cut-off for positivity, unless otherwise stated.

Details of HLA alloimmunization are shown in Table 3. Of 67 patients who had received filtered blood products, 15 developed HLA antibodies (22.4%) compared with 21 of 56 (37.5%) NF-arm patients. The estimate of the ratio of the risk of HLA alloimmunization from receiving F to NF blood products was 0.6 (95% confidence interval, 0.34 to 1.05). The chi-squared test statistic was 3.37, with one degree of freedom and an associated P value of .07. Of the 36 patients who developed HLA antibodies, 24 had the result confirmed on a further sample. In 6 patients, the next sample was negative, but, meanwhile, 2 of these became refractory and were withdrawn; 6 other patients had no further samples taken; in 3 cases, this was because of withdrawal from the study because of severe FTRs.

Using the Kaplan-Meier method and midpoint imputation, HLA alloimmunization as a function of time exposed to blood products is shown in Fig 2. There was no difference in the median time to alloimmunization between seroconverters in the two arms of the study. Both arms appeared to enter a plateau phase at around 45 days, when just over one third of patients in the study remained at risk. Seronegatives in both arms were comparable with respect to the average
duration of transfusion support, with an overall median of 44 days. The log-rank test statistic was 3.22, with one degree of freedom and an associated $P$ value of .07. The median interval between last negative and first positive tests was similar for seroconverters in both arms of the study with an overall median of 14 days (75% of intervals <23 days), thereby indicating the effect of midpoint imputation to be roughly equal across the two arms.

The effect of filtration and the level of significance of the variable-adjusted test statistic were found to be unchanged after adjustment for previous pregnancy, transfusion, or ABO blood group. Although the effect of treatment regime (ablative v nonablative) on alloimmunization did not reach statistical significance ($P = .09$), the HLA alloimmunization rate in the ablative group was 7 of 41 (17%) compared with 28 of 82 (34%) in the nonablative group. Owing to the slightly higher proportion of patients undergoing ablative treatment in the F group, the level of significance of the regime-adjusted test statistic was reduced to .09, with a common relative risk estimate of 0.62 (95% CI, 0.36 to 1.08).

Patients with AML had higher rates of HLA alloimmunization than the study group as a whole and showed a significant difference in alloimmunization between the two arms of the study. Of 29 AML patients receiving filtered blood products, 9 developed HLA antibodies (31.0%) compared with 15 of 24 (62.5%) control patients. The estimate of the risk ratio from receiving F to NF products was 0.50 (95% CI, 0.27 to 0.93), and the log-rank test statistic was 5.01, with one degree of freedom; $P = .025$.

**Blood product usage.** Details of the amount of blood product support received by patients in each trial arm are shown in Table 4, excluding 5 patients for whom detailed information was not available. The number of RBC and platelet donor units transfused was similar in both arms of the study; therefore, the difference in seroconversion rates between the trial arms cannot be attributed to inadequate donor exposure. However, alloimmunized patients in both arms received approximately 50% more units of RBCs and platelets per unit time than did seronegative patients, a difference which was apparent even before seroconversion.

**FTRs.** Information on FTRs was unavailable for 13 patients, including 1 in each arm known to have been alloimmunized. FTRs developed in 20 of 59 (34%) patients in the F arm and in 19 of 51 (37%) patients in the NF arm (the chi-square statistic was 0.135 with one degree of freedom; $P = .714$). There was no association between FTRs and overall HLA alloimmunization, with FTRs observed in 32% and 37% of alloimmunized and nonalloimmunized patients, respectively. However, FTRs were more likely in patients.

**Table 2. Leucocyte Counts in Units of RBC Before, During, and After Passage Through Pall RC50 Filters (n = 4)**

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>WCC ($\times 10^6$/L)</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefiltration</td>
<td>1.39</td>
<td>0.99-1.70</td>
<td></td>
</tr>
<tr>
<td>Postfiltration</td>
<td>4.54</td>
<td>1.39-7.78</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Details of HLA Alloimmunization by Trial Arm (n = 123)**

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>NF</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>67</td>
<td>56</td>
<td>123</td>
</tr>
<tr>
<td>HLA antibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (&lt;10%)</td>
<td>52</td>
<td>35</td>
<td>87</td>
</tr>
<tr>
<td>11%-50%</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>51%-100%</td>
<td>11</td>
<td>14</td>
<td>25*</td>
</tr>
<tr>
<td>Total no. of alloimmunized patients (%)</td>
<td>15 (22.4)</td>
<td>21 (37.5)</td>
<td>36 (29.3)†</td>
</tr>
</tbody>
</table>

**Table 3 Details of HLA Alloimmunization by Trial Arm (n = 123)**

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>0.03</td>
<td>&lt;0.004-0.08</td>
</tr>
<tr>
<td>15 min</td>
<td>0.09</td>
<td>0.02-0.19</td>
</tr>
<tr>
<td>30 min</td>
<td>0.26</td>
<td>0.17-0.37</td>
</tr>
<tr>
<td>Final min</td>
<td>3.54</td>
<td>1.21-5.40</td>
</tr>
</tbody>
</table>

**Abbreviations:** WCC, white cell count.
with broad rather than restricted alloimmunization (Table 5). In the 34 alloimmunized patients for whom FTR data were available, 4 of 14 (29%) F and 7 of 20 (35%) NF recorded FTRs. FTRs were severe enough to cause withdrawal from the trial in 12 patients, 6 in each arm. Of these, only 5 had HLA antibodies (1 F and 4 NF), and 3 were having FTRs before the detection of HLA antibodies.

Platelet refractoriness. A total of 50 patients (23 F and 27 NF) had at least two 1-hour posttransfusion platelet increments performed. In 14 of 50 patients (28%), the 1-hour uncorrected increment was less than $10 \times 10^9/L$ on two consecutive measurements, and there was a highly significant correlation between HLA alloimmunization and refractoriness (Table 6). However, refractoriness in association with HLA alloimmunization was observed equally in both arms of the study (7 of 13 NF and 4 of 6 F). Only 4 of 14 refractory patients had had BM transplantation. Alloimmunized patients could be withdrawn if they became clinically unsupportable with random donor platelets, but this occurred in only 5 patients, 2 in the F and 3 in the NF arm.

### DISCUSSION

This is the first prospective, randomized study to examine the efficacy of bedside filtration of cellular blood products in the prevention of HLA alloimmunization and its clinical consequences in intensively transfused patients. As with all studies of this type, 25% of initially enrolled patients proved ineligible, but the 123 patients comprise the largest study of blood product filtration thus far (albeit with multiple diagnoses). We defined HLA alloimmunization using a greater than 10% cut-off in panel reactivity (1) to allow comparison of the results with other studies and (2) because even a limited range of alloantibodies can interact with a high percentage of random donor platelet units if they recognize common HLA specificities. The HLA alloimmunization rate in the control arm of this study was 37.5% overall and 62% for AML, which is consistent with other reports. The

### Table 4. Blood Product Support Received During Time on Study (n = 118)

<table>
<thead>
<tr>
<th>No. of Donor Units Received</th>
<th>F</th>
<th>NF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloimmunized patients before first positive test</td>
<td>n = 15</td>
<td>n = 21</td>
</tr>
<tr>
<td>RBCs</td>
<td>10 (0-23)</td>
<td>9 (2-44)</td>
</tr>
<tr>
<td>Platelets</td>
<td>40 (1-91)</td>
<td>45 (0-99)</td>
</tr>
<tr>
<td>Sero negatives before last negative test</td>
<td>n = 51</td>
<td>n = 31</td>
</tr>
<tr>
<td>RBCs</td>
<td>9 (2-64)</td>
<td>10 (2-26)</td>
</tr>
<tr>
<td>Platelets</td>
<td>43 (0-242)</td>
<td>39 (0-178)</td>
</tr>
<tr>
<td>Alloimmunized patients before withdrawal from study</td>
<td>RBCs</td>
<td>16 (4-36)</td>
</tr>
<tr>
<td>Platelets</td>
<td>64 (1-277)</td>
<td>64 (0-174)</td>
</tr>
</tbody>
</table>

Results are shown as the median with the range in parentheses.

### Table 5. FTR and Relationship to HLA Alloimmunization (n = 110)

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>NF</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>59</td>
<td>51</td>
<td>110</td>
</tr>
<tr>
<td>No. experiencing FTRs (%)</td>
<td>20 (33.9)</td>
<td>19 (37.3)</td>
<td>39 (36.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of alloimmunized patients</th>
<th>FTRs</th>
<th>No FTRs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA antibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (&lt;10%)</td>
<td>28</td>
<td>48</td>
<td>76</td>
</tr>
<tr>
<td>11%-50%</td>
<td>1</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>51%-100%</td>
<td>10</td>
<td>13</td>
<td>23*</td>
</tr>
<tr>
<td>Total no. of alloimmunized patients</td>
<td>11</td>
<td>23</td>
<td>34</td>
</tr>
</tbody>
</table>

* Mantel-Haenszel $\chi^2$ statistic equals 0.04, with 1 degree of freedom; $P = .85$. 

**Fig 2 Cumulative risk of HLA alloimmunization is shown. (—) filter; (---), nonfilter; log-rank test, $P = .073$. Patients with only negative results were censored at the time of the last negative sample, irrespective of their reason for withdrawal from the study. Error bars represent 95% confidence limits.**
higher rate in AML may have been caused by either more prolonged exposure to blood products, or the more immunosuppressive regimes received by non-AML patients. The alloimmunization rate in the F arm was 22.4% overall and 31% in AML patients, with both groups showing a 40% to 50% reduction when compared with controls. In drawing conclusions about the benefits of bedside leucodepletion in the prevention of HLA alloimmunization and its clinical consequences, a number of methodological factors relating to the study must be considered.

First, the trial included 42 BM transplant patients who had a lower alloimmunization rate than nontransplant patients. The overall alloimmunization rate of 38% in the control arm meant that the statistical power of the study was maintained, but the imbalance in the trial arms with respect to BM transplantation slightly favored the F arm. Theoretically, the imbalance in ABO groups could also have favored the F arm, because of the suggestion that patients receiving large numbers of ABO-incompatible platelets have higher rates of HLA alloimmunization. However, adjusting for blood group did not affect the estimate of the filter effect.

Secondly, filter failure or operator error may have gone undetected, or filtration of RBCs at the bedside may have been adversely affected by the high ambient temperature. We do not know how many units transfused in this study were not truly leucodepleted. Ideally, bedside filtration should have been quality controlled, but the logistics of this remain difficult. Because the entire postfiltration unit is not available for sampling, performance can be assessed only by collecting an aliquot of blood at a predetermined point in the transfusion. As shown previously and by our laboratory data, filter performance deteriorates during the procedure, so that snapshot sampling is a poor predictor of leucocyte numbers transfused to the patient. Even under laboratory conditions, some units fail to reach acceptable levels of leucocyte removal. Therefore, to assess clinical efficacy of the procedure as performed in practice, analysis of this study was performed on an intention-to-treat basis.

There appeared to be no reduction in the rate of FTRs by bedside filtration, with FTRs observed in 37% and 34% of NF and F arms, respectively. These figures represent a greater number of patients than those alloimmunized to HLA, suggesting that either many reported febrile episodes were not caused by transfusions or that transfusion-related FTRs have other major causes. The true cause of a febrile episode in an immunosuppressed patient may only be apparent retrospectively or sometimes not at all, and the inclusion of such episodes in leucodepletion studies renders the true value of leucocyte depletion difficult to ascertain. This may explain why this study, as well as two others, has failed to find any overall benefit from bedside leucodepletion with respect to FTRs. The first study showed a 14% FTR rate associated with transfusion of platelets filtered at the bedside and found that, of 36 patients who had previously experienced FTRs to unfiltered platelets, 28 continued to have FTRs when filtered platelets were introduced. The second study found no reduction in FTRs to platelets, discontinuation of platelet transfusion, or the need for premedication for platelet transfusion after bedside filtration was introduced on an oncology ward.

Other transfusion-related factors, such as noncytotoxic HLA antibodies and antigranulocyte antibodies, were not examined in this study. Some proposed causes of FTR are not amenable to bedside leucocyte depletion, including antibodies to plasma proteins, immune complex-mediated release of recipient interleukin-1β from macrophages, and the generation of pyrogenic cytokines such as interleukin-6 or tumour necrosis factor-α during storage of platelets. This latter effect may explain the success of laboratory, as opposed to bedside, filtration in the prevention of FTRs.

Refractoriness in this study was defined by two 1-hour uncorrected platelet increments of less than 10 × 10⁹/L on two consecutive measurements. No overall benefit of filtration on refractoriness was established by this study; however, increment data were available for only half the patients in the study. Because refractoriness was seen in only 30% of the control patients studied, an examination of 50 patients has limited statistical power to detect an effect of filtration. In addition, refractoriness has many nonimmunological causes, including BM transplantation itself, which are not amenable to filtration. Although we found a strong association between broad HLA alloimmunization and refractoriness, only a minority of patients required support with HLA-matched platelets.

No reduction in overall blood product support was conferred by leucodepletion, although alloimmunized patients used more RBCs and platelets per unit time than did seronegative patients. After alloimmunization, this could be explained by shortened platelet survival combined with occult hemorrhage, but patients destined to become alloimmunized received more intensive blood product support than did others, even before HLA antibodies were detected. Whether this was a contributory factor to HLA alloimmunization remains unknown. A recent study has shown an apparent reduction in infection risk and a greater relapse-free survival in AML patients receiving leucodepleted products. These interest-
ing observations require confirmation, and AML patients in this study will be followed up to relapse.

Apart from difficulties in monitoring filter performance, the efficacy of bedside filtration may also have biologic limitations because of storage changes in transfused products. Supernatants of stored blood have long been known to be immunogenic in rabbits, and platelet refractoriness has been prevented in animals by prestorage but not by poststorage filtration. Leucocyte fragments have been shown downstream of leucodepleting filters, but their immunogenicity, and that of soluble HLA antigens, is not known in humans. These experimental data and the findings from this study pave the way for a clinical comparison of prestorage and poststorage filtration. Such a study would involve a great many patients and would require very carefully defined clinical and laboratory endpoints but would be useful in justifying the resource implications of a laboratory-based leucodepletion program. In addition, the relevance of leucodepletion remains to be established in settings where pancytopenia is short, such as after peripheral blood progenitor-cell infusion or in combination with granulocyte colony-stimulating factor, as shown in mice.

A recent International Forum debated whether all platelet concentrates should be leucocyte depleted, and participants were divided in their views. This study has not been prevented in animals by prestorage but not by poststorage filtration. Such a study would involve a great many patients and would require very carefully defined clinical and laboratory endpoints but would be useful in justifying the resource implications of a laboratory-based leucodepletion program. In addition, the relevance of leucodepletion remains to be established in settings where pancytopenia is short, such as after peripheral blood progenitor-cell infusion or in combination with granulocyte colony-stimulating factor, as shown in mice.

ACKNOWLEDGMENT

The authors are indebted to the many nursing, medical, and blood bank staff who helped with this study, and to Mrs Carol Holmes for secretarial help.

APPENDIX


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