Universal prestorage leukoreduction in Canada decreases platelet alloimmunization and refractoriness

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Randomized controlled trials have shown a reduction in platelet alloimmunization and refractoriness in patients with acute leukemia (AL) with the use of poststorage leukoreduction of blood products. Universal prestorage leukoreduction (ULR) of red cell and platelet products has been performed in Canada since August 1999. We conducted a retrospective analysis of 13 902 platelet transfusions in 617 patients undergoing chemotherapy (CT) for AL or stem cell transplantation (SCT) before (n = 315) and after (n = 302) the introduction of ULR. Alloimmunization was significantly reduced (19% to 7%, *P* < .001) in the post-ULR group. Alloimmune platelet refractoriness was similarly reduced (14% to 4%, *P* < .001). Fewer patients in the post-ULR group received HLA-matched platelets (14% vs 5%, *P* < .001). Alloimmunization and alloimmune refractoriness in the 318 patients who were previously pregnant and/or transfused were also reduced after ULR (*P* = .023 and *P* = .005, respectively). In a Cox regression model, the 3 independent factors that predicted for alloimmune refractoriness were nonleukoreduced blood products (relative risk [RR], 2.2 [95% CI, 1.2-4.3]), a history of pregnancy and/or transfusion (RR, 2.3 [95% CI, 1.3-4.2]), and receipt of 13 or more platelet transfusions (RR, 6.0 [95% CI, 2.4-15.3]). In conclusion, ULR reduces alloimmunization, refractoriness, and requirements for HLA-matched platelets when applied as routine transfusion practice to patients receiving CT or SCT. (Blood. 2004;103: 333-339)

Introduction

Clinical trials have suggested that leukoreduction of cellular blood products reduces posttransfusion alloimmunization,[1-10] which is a major factor contributing to reduced efficacy of platelet transfusions as measured by posttransfusion platelet count increments.[1,3,10-12] Although alloimmune destruction of platelets is mediated by expression of foreign class I HLA antigens on these cells, the presence of contaminating leukocytes enhances the immunogenicity of platelet products in transfusion recipients.[5,13] Effective management of platelet transfusion requirements in alloimmunized patients requires the harvest of platelet components from HLA-compatible donors. Procurement of these HLA-matched platelets is logistically complicated, costly, and often ineffective at circumventing refractoriness.[14] Prevention of the alloimmunized state is clearly a more attractive alternative. The possibility that the removal of leukocytes from cellular blood products might achieve that goal has been confirmed to some extent in a variety of clinical studies. However, most studies have used poststorage, bedside filtration methods, rather than prestorage techniques, which may be more effective in preventing this complication,[7,15-18] and have focused on patients receiving chemotherapy for acute leukemia.[1-3,5,7,8] Prestorage leukoreduction is performed in the blood collection center using standard operating procedures and quality controls, which should result in less failure of leukoreduction, detected in 5% or more of blood components filtered at the bedside during clinical trials.[17,19,20] In addition, prestorage leukoreduction will minimize the transfusion of potentially immunogenic white blood cell (wbc) fragments, which accumulate during storage and may not be removed by filtration.[18]

Universal prestorage leukoreduction (ULR) of red blood cell and platelet products was instituted by the Canadian Blood Services between November 1997 and August 1999 with the goal of preventing the complications of transfusion associated with "passenger leukocytes." In addition to allosensitization these include febrile nonhemolytic transfusion reactions (FNHTRs) and transmission of infectious disease.[21,22] There is controversy as to whether the theoretical benefit of prestorage leukoreduction will be realized in clinical practice and whether the cost of this procedure is justified.[23] Prior to the current study, the effect of ULR on reducing the incidence of platelet alloimmunization in Canada was unknown. Furthermore, it was uncertain whether those patients who were already potentially sensitized to foreign HLA antigens by prior pregnancy or transfusion would derive any benefit from prestorage leukoreduction in their response to subsequent platelet transfusions. Another area that has not been studied in detail was the effect of prestorage leukoreduction in the heavily immunosuppressed population of stem cell transplant recipients.[6] Thus, the purpose of this retrospective analysis was to analyze the effect of ULR on alloimmunization and platelet refractoriness in heavily transfused patients undergoing chemotherapy for acute leukemia or stem cell transplantation (for any diagnosis) in the setting of routine clinical practice in a tertiary care referral center.

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Submitted March 28, 2003; accepted August 28, 2003. Prepublished online as *Blood* First Edition Paper, September 4, 2003; DOI 10.1182/blood-2003-03-0940.

Supported by The Bennett Fund.

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Patients, materials, and methods

Patients

Consecutive patients treated on the Leukemia/Bone Marrow Transplantation (BMT) service in Vancouver after the implementation of prestorage leukoreduction (post-ULR group) were compared retrospectively with a similar consecutive group of patients treated prior to prestorage leukoreduction (pre-ULR group). There were 1105 patients evaluated for inclusion in this study, ranging in age from 14 to 79 years (median, 46 years) who began platelet transfusion therapy following chemotherapy for acute leukemia or myeloablative conditioning for a stem cell transplantation (SCT) procedure between January 1, 1994, and December 31, 2001, and on whom clinical characteristics, postplatelet transfusion increments, and lymphocytotoxic antibody (LCTAb) screening were available. Also included were 21 patients (12 in the pre-ULR and 9 in the post-ULR groups) who had a diagnosis of severe aplastic anemia (n = 5) or myelodysplasia (n = 16) and were treated with supportive care only (primarily red blood cell and platelet transfusion support). The patients' medical records were reviewed to ensure accuracy of diagnostic and clinical information, including prior history of pregnancy or blood transfusion and the incidence and severity of hemorrhagic events during the study period. Approval was obtained from the University of British Columbia institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki. Prestorage leukoreduction was gradually implemented in British Columbia between November 11, 1997, and August 7, 1999. Thus, leukoreduction of blood products was incomplete during this period, and patients who began to receive platelets and/or red blood cell transfusions between these dates were excluded from the study. In addition, patients who had begun to receive transfusions before November 11, 1997, but had had fewer than 8 weeks of follow-up by November 11 were also excluded, resulting in a total of 341 of the initial 1105 patients being excluded from analysis due to the aforementioned time criteria. Also excluded from analysis were patients who received the following: antithymocyte globulin, which may give rise to falsely positive LCTAb assays (n = 28); granulocyte transfusions (n = 2); or fewer than 2 platelet transfusions (n = 87). Of 647 remaining patients, 30 patients (18 in the control group and 12 in the treatment group) had detectable LCTAb at study entry consistent with prior allosensitization. These patients were analyzed separately leaving a total of 617 patients available for analysis of primary study end points—315 patients in the pre-ULR group and 302 in the post-ULR group (Table 1). Follow-up was stopped on November 11, 1997, for patients in the pre-ULR group who continued to receive transfusions after that date. Patients in the post-ULR group who continued to receive transfusions after December 31, 2001, were followed until their last platelet transfusion or until the date of last analysis (July 31, 2002). All other patients were followed for the entire duration of their requirement for platelet transfusions. Follow-up time was defined as the elapsed time from first to last platelet transfusion.

Table 1. Patient characteristics at study entry (n = 617)

	Pre-ULR group, n = 315	Post-ULR group, n = 302	*P*
Age, y, mean ± SD	45 ± 15	47 ± 14	.16‡
Female, no. (%)	147 (47)	111 (37)	.016§
Nulliparous/nontransfused (%)*	144 (48)	158 (53)	.27§
Disease (%)			
AML	124 (39)	135 (45)	
ALL	37 (12)	19 (6)	
CML	31 (10)	21 (7)	
MM	28 (9)	41 (14)	
MDS	22 (7)	19 (6)	
AA	7 (2)	2 (1)	
HD	27 (9)	15 (5)	
NHL	32 (10)	47 (16)	
GCT	7 (2)	3 (1)	
Treatment (%)			
Chemotherapy/supportive care†	116 (37)	117 (39)	.68§
SCT	199 (63)	185 (61)	
Allogeneic sibling	75 (38)	72 (39)	
Allogeneic VUD	39 (19)	32 (17)	
Autologous	84 (42)	80 (43)	
Syngeneic	1 (1)	1 (1)	

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; MDS, myelodysplastic syndrome; AA, aplastic anemia; HD, Hodgkin disease; NHL, non-Hodgkin lymphoma; GCT, germ cell tumor; SCT, stem cell transplantation; and VUD, volunteer unrelated donor.

*Nulliparous patients and no transfusion more than 2 weeks before study entry; data were not obtained in 22 patients (17 in the before group and 5 in the after group).

†Patients receiving chemotherapy for AML (n = 181) or ALL (n = 31) or supportive care only for AA (n = 5) or MDS (n = 16).

‡*T* test.

§Chi-square test.

Preparation and leukoreduction of blood components

Units of random donor platelet (RDP) concentrate were prepared at the Canadian Blood Services Vancouver Centre (CBS) from whole-blood, platelet-rich plasma. Prestorage leukoreduction was carried out using Pall integral in-line filters (Pall, East Hills, NY). Whole blood was leukoreduced using the WBF-2 in-line filter prior to preparation of packed red blood cells (pRBCs). When platelets and pRBCs were manufactured from the same whole blood unit, the RCM-1 filter was used in-line to leukoreduce pRBCs, and the ATS-LPL filter was used in-line to leukoreduce platelets. The maximum storage time prior to leukodepletion was 8 hours at room temperature for platelets and 72 hours for whole blood placed in cold storage. Apheresis platelets were collected using either the Fenwal CS3000 (Baxter, Deerfield, IL) or Cobe Spectra (Gambro BCT, Lakewood, CO) blood cell separator machines. Apheresis platelets were leukoreduced using the Cobe Spectra LRS system or using CS3000 closed-system dual-needle apheresis kits with LRS, which contains an integral leukocyte filter for postcollection leukoreduction. Effectively leukoreduced RBCs and apheresis platelets contained less than 5×10^6 white cells. For units of RDP concentrate, the upper limit of residual leukocytes was 0.83×10^6 wbc/unit. As part of routine quality-control procedures, the leukocyte content of 1% of all blood products was determined by Nageotte chamber counting (apheresis platelets) or by flow cytometry (other products). The platelet yields were also determined by automated cell counting in 1% of units of RDP concentrate after processing at the CBS. The mean ± SD platelet content of a unit of RDP concentrate as determined from this quality control data is $0.7 \pm 0.06 \times 10^{11}$ platelets (personal oral communication, K. O'Shea, Quality Control Laboratory, Canadian Blood Services Vancouver Center, March 2003). The number of platelets in a transfusion of pooled RDP concentrate was thus calculated as the number of units transfused multiplied by 0.7×10^{11}. Platelet counts were performed on all units of apheresis single-donor platelets (SDPs) using an automated cell counter (Gen.S System 2; Coulter Electronics, Hialeah, FL). To determine the platelet content of the apheresis unit, the bag of concentrate was weighed to determine the volume of its contents and the volume thus calculated multiplied by the automated platelet count.

Prior to November 1997, bedside leukoreduction of blood products was used for individual patients to prevent FNHTRs (in patients who had a history of severe FNHTR to unfiltered products). The proportion of platelets and pRBCs that were leukoreduced for that indication in the pre-ULR group was 10% or less. These patients were not excluded from this analysis. Following the introduction of ULR, bedside filtration of blood products was not performed.

Transfusions

Daily morning complete blood counts were performed on all patients. All red blood cell and platelet products received 1500 cGy of gamma irradiation prior to transfusion. Packed red blood cell transfusions were administered for a hemoglobin level of less than 90 g/L. Platelets were transfused prophylactically when the daily platelet count was less than 20×10^9/L prior to 1998. In May 1998, the platelet transfusion threshold was reduced to 10×10^9/L in the absence of clinical indications for a more aggressive transfusion policy for individual patients.[24,25] Most platelet transfusions given to nonalloimmunized recipients consisted of 5 units of pooled RDP concentrate. However, some random SDPs were available and

Table 2. Platelet transfusions

	Pre-ULR group	Post-ULR group	*P*
No. of platelet transfusions/pt*			
All patients	28 ± 31	17 ± 20	< .001†
Chemotherapy or supportive care patients			
ALL	19 ± 18	15 ± 9	.398†
AML	27 ± 23	20 ± 19	.044†
Supportive care	33 ± 45	9 ± 9	.100†
Stem cell transplantation patients			
ALLO RD	25 ± 28	17 ± 17	.051†
ALLO VUD	51 ± 40	34 ± 32	.044†
AUTO BM	36 ± 41	18 ± 19	.023†
AUTO PB	10 ± 10	5 ± 4	< .001†
No. of donor exposures/pt*	107 ± 119	61 ± 69	< .001†
No. of platelet transfusions (%)			
Total	8698	5204	
Pooled RDP	6002 (69)	3422 (66)	
Random SDP	2169 (25)	1603 (31)	
HLA-matched SDP	527 (7)	147 (3)	< .001‡
Platelets per transfusion,* × 10^{11}	3.9 ± 1.8	3.9 ± 1.8	.873†
CCI*	12.6 ± 8.6	11.7 ± 7.0	.20†
Pts receiving HLA transfusions, no.(%)	44 (16)	16 (5)	< .001‡
Duration of follow-up, d			
Median (range)	48 (2-1083)	79 (2-1025)	.004†

Pt indicates patient; ALLO, allogeneic; RD, related donor; VUD, volunteer unrelated donor; AUTO, autologous; PB, peripheral blood progenitors; RDP, random donor platelets; SDP, single-donor platelets; and CCI, corrected count increment.

*Mean ± SD.

†Student unpaired *t* test.

‡Chi-square test.

comprised 25% and 31% of the transfusions administered to the pre-ULR and post-ULR patient groups, respectively (Table 2). Cytomegalovirus (CMV)–seronegative red cell and platelet products were transfused to seronegative patients who were undergoing or were potential candidates for an SCT procedure. If possible, platelets would also be selected that were ABO compatible with the recipient. However, ABO-incompatible platelets would be given if they were the only CMV-negative cells available for a CMV-seronegative recipient. Patients not requiring CMV-negative products would receive ABO-compatible platelets if they were available. Over the course of this study this policy did not change, and the proportion of platelet transfusions that were ABO-compatible with the recipient was 50% or less. When possible, HLA-matched platelets were obtained for patients who were alloimmunized and refractory to random donor transfusions.[12] HLA-matched platelets were as closely matched as possible at the A and B class I loci. Only grade A or B (usually B1 or B2) HLA-matched donors[26] whose cells failed to react with patient serum in a lymphocytotoxicity cross-match[27] were used. If a patient's serum showed specific reactivity against only 1 or 2 HLA antigens that were identifiable in the lymphocytotoxicity (LCTAb) screen, donors carrying that antigen were avoided.

Calculation of the corrected count increment (CCI) and refractoriness to platelet transfusions

The Cell Separator Unit of the Vancouver General Hospital maintains a database containing the platelet transfusion records of patients on the Leukemia/BMT service. This database includes pre- and posttransfusion platelets counts, the results of LCTAb screening, the patients' body surface area (BSA), and cell counts from platelet transfusion products. These data were used to determine a patient's alloimmunization status and to calculate CCIs for each transfusion. The CCI was calculated using the platelet count within an hour after transfusion and the platelet count before transfusion and correcting for the patient's BSA and the number of platelets transfused using the following formula: CCI = (postplatelet count − preplatelet count × 10^9/L) × BSA (m^2)/platelets transfused × 10^{11}. Refractoriness was defined as two, 1-hour posttransfusion CCIs of less than 5 after sequential platelet transfusions.

Lymphocytotoxic antibody (LCTAb) testing

Serum samples for LCTAb testing were obtained in the first week after entry into the study and weekly thereafter until the patient either reached independence from platelet transfusion or died. Samples were tested against a panel of lymphocytes from 10 donors selected to include as many common HLA class I antigens as possible in the Immunology laboratory of the Vancouver General Hospital using an antiglobulin-augmented, complement-dependent lymphocytotoxicity assay.[28] In some cases, usually when weak or low-level reactivity was detected, the serum was tested against a larger panel of 30 lymphocytes, which encompassed a greater number of rare HLA class I antigens. The results were reported as the number of lymphocytes on the panel against which the serum had reactivity out of the total number tested. Serum samples were considered positive if they reproducibly caused cytotoxicity against 3 or more cells in the panel of 10, or 9 or more cells from the panel of 30 cells.[29] A patient was considered alloimmunized if the lymphocytotoxicity assay was positive on 2 or more consecutive tests. Alloimmune refractoriness was defined as a 1-hour posttransfusion CCI of less than 5 after 2 sequential platelet transfusions in patients with alloimmunization (as defined by detection of LCTAbs as discussed above) detected within 2 weeks before or after these transfusions.

Hemorrhage

Hemorrhagic events were defined as one or more clinically apparent, severe or life-threatening bleeds during the study period. These included intracranial, pulmonary, gastrointestinal, and tumor-site bleeding, as well as macroscopic hemorrhagic cystitis. Excluded were petechiae, ecchymoses, minor epistaxis, or mild bleeding in areas of mucositis.

Study end points

The primary end point was refractoriness to platelet transfusions in patients with lymphocytotoxic antibodies that were detected within 2 weeks before or after the diagnosis of refractoriness (alloimmune-mediated platelet refractoriness). The secondary end points were as follows: alloimmunization as reflected by persistent lymphocytotoxic antibodies (LCTAbs); overall refractoriness to platelet transfusions; the proportion of patients receiving HLA-matched platelet support; and the incidence of patients with hemorrhagic complications.

Statistical analysis

The baseline characteristics and absolute event rates were compared by *t* tests or chi-square tests where appropriate. Cox regression analyses[30] were used to examine the effects of covariates on the rates of overall and alloimmune refractoriness, and development of lymphocytotoxic antibodies. For these analyses, patients were censored at the time of last transfusion, which occurred either because of platelet recovery, death, or the end of the follow-up period. The factors considered in the multivariate analysis to identify predictors of study end points were treatment group (pre-ULR vs post-ULR), number of platelet transfusions received (< or ≥ 13 transfusions [the median number of transfusions per patient received by the entire group]), year of transfusion therapy, age (≤ or > 46 years), sex, prior pregnancy and/or blood transfusion more than 2 weeks before entry into the study, and type of therapy (SCT vs chemotherapy or supportive care). A parsimonious model was determined for each end point by using forward and backward stepwise procedures in which covariates with *P* values of less than .05 were retained in the final model. The relative risks (RRs) with 95% confidence intervals (CIs) are reported for risk factors with *P* values of less than .05. All results are reported according to the group to which the patient was initially assigned.

Results

Patients

There were no statistically significant differences between groups in terms of age, proportion of patients without previous HLA antigen exposure through pregnancy and/or blood transfusion, and type of treatment (SCT vs chemotherapy or supportive care) (Table 1).

Among the 293 patients who had been pregnant or had a previous transfusion history, 105 had a history of pregnancy only, 128 a history of transfusion only, 51 a history of both pregnancy and transfusion, while the remaining 9 patients had been transfused but their parity was unknown. There was a significantly higher proportion of women in the pre-ULR group ($P = .016$). There were 5 children between the ages of 14 and 16 included, of which 4 were in the pre-ULR group.

Platelet transfusions

The median duration from first to last transfusion was 48 days (range, 2-1083 days) for the pre-ULR group and 79 days (range, 2-1025 days) for the post-ULR group (Table 2). The median (range) number of transfusions per patient for the entire group was 13 (2-167) while it was 16 (2-167) and 11 (2-116) for the pre- and post-ULR groups, respectively. Similar differences for the mean number of transfusions are reported in Table 2, confirming the smaller number of platelet transfusions and transfused units per patient after ULR compared with before ULR. The proportion of platelet transfusions that were HLA-matched for the recipient was higher in the pre-ULR group ($P < .001$), as was the percentage of patients who received these transfusions (14% vs 5%, $P < .001$). Platelet yields per transfusion and mean CCI calculations were similar in both groups.

Alloimmunization

The absolute incidence of alloimmunization was higher in the pre-ULR than the post-ULR group (19% vs 7%, $P < .001$) (Table 3). A subgroup analysis showed that this difference remained evident in the SCT (18% vs 5%, $P = .004$) and chemotherapy (22% vs 9%, $P = .011$) patients. This reduction was evident in patients with a history of pregnancy and/or blood transfusion (25% vs 11%, $P = .023$) as well as those without such a history (11% vs 4%, $P = .026$). However, the benefit of ULR among the parous/transfused group was seen predominantly among those with a history of prior transfusion only ($P = < .001$).

Platelet refractoriness: overall and alloimmune

The overall rate of refractoriness was reduced in the post-ULR group from 40% to 23% ($P < .001$) (Table 4). The proportion of patients with alloimmune platelet refractoriness was also reduced in the post-ULR group from 14% to 4% ($P < .001$). The reduction in this end point was seen in patients treated after ULR regardless of whether they had received a stem cell transplant or chemotherapy and regardless of whether they had been previously pregnant or transfused. However, in the latter group the reduction in alloimmune refractoriness was largely confined to those with a prior history of transfusion only ($P < .001$).

Table 3. Incidence of platelet alloimmunization

	Pre-ULR group* (%)	Post-ULR group* (%)	P
Overall	61/315 (19)	21/302 (7)	< .001§
Chemotherapy or supportive care	26/116 (22)	11/117 (9)	.011§
ALL	2/20 (10)	1/11 (9)	.99‖
AML	19/84 (23)	10/97 (10)	.027‖
Supportive care	5/12 (42)	0/9 (0)	.045‖
SCT	35/199 (18)	10/185 (5)	.004‖
ALLO RD	10/75 (13)	3/72 (4)	.050§
ALLO VUD	11/39 (28)	6/32 (19)	.35§
AUTO BM	7/39 (18)	1/24 (4)	.14‖
AUTO PB	7/46 (15)	0/57 (0)	.003‖
Nulliparous/nontransfused†	16/144 (11)	6/152 (4)	.026§
Parous/transfused‡	39/154 (25)	15/139 (11)	.023§
Parous only	7/46 (15)	6/59 (10)	.44‖
Transfused only	22/75 (29)	1/53 (2)	< .001‖
Both	6/26 (23)	7/25 (28)	.75‖

Abbreviations are explained in Table 2.
*Number of patients with positive lymphocytotoxic antibody screen on 2 or more consecutive occasions/total number of patients in the group.
†No history of pregnancy and/or blood transfusion.
‡History of pregnancy and/or blood transfusion. The parity of 9 transfused patients could not be determined.
§Chi-square test.
‖Fisher exact test.

Table 4. Platelet refractoriness

	Pre-ULR group* (%)	Post-ULR group* (%)	P
Overall refractoriness†	27/315 (40)	68/302 (23)	< .001§
Overall alloimmune refractoriness‡	44/315 (14)	12/302 (4)	< .001§
Chemotherapy or supportive care patients	19/116 (16)	8/117 (7)	.038§
ALL	1/20 (5)	1/11 (9)	.99‖
AML	13/84 (16)	7/97 (7)	.097‖
Supportive care	5/12 (42)	0/9 (0)	.045‖
SCT patients	25/199 (13)	4/185 (2)	.003§
ALLO RD	7/75 (9)	1/72 (1)	.063‖
ALLO VUD	8/39 (21)	2/32 (6)	.102‖
AUTO BM	5/39 (13)	1/24 (4)	.394‖
AUTO PB	5/46 (11)	0/57 (0)	.016‖
Nulliparous/nontransfused	12/144 (8)	3/152 (2)	.021§
Parous/transfused	28/154 (18)	9/139 (7)	.005§
Parous only	5/46 (11)	5/59 (9)	.75‖
Transfused only	15/75 (20)	0/53 (0)	< .001‖
Both	6/26 (23)	3/22 (12)	.47‖

Abbreviations are explained in Table 2.
*Number of refractory patients/total number of patients in the group.
†There were 2 consecutive corrected count increments of less than 5.
‡Refractoriness within 2 weeks of 2 or more consecutive positive lymphocytotoxicity screens.
§Chi-square test.
‖Fisher exact test.

Univariate and multivariate analysis

In the Cox logistic regression analysis, the independent variables predictive of alloimmunization were nonleukoreduced blood products (RR, 1.9 [95% CI, 1.1-3.1]; $P = .014$), a history of previous pregnancy and/or blood transfusion (RR, 2.5 [95% CI, 1.5-4.1]; $P = < .001$), and the number of platelet transfusions received (RR, 4.7 [95% CI, 2.4-9.6]; $P < .001$) (Table 5). These same 3 factors were also found to independently predict for overall refractoriness and alloimmune refractoriness (Table 5). Although female sex predicted for alloimmunization and alloimmune refractoriness in univariate analysis, this variable was no longer significant on multivariate analysis. Age and type of therapy (SCT or chemotherapy) were not predictive of these end points. The year in which transfusion therapy was given was entered into the Cox regression analysis as a variable both for the entire group of patients and for the pre-ULR and post-ULR patients considered independently. When the pre- and post-ULR groups were considered separately there was no significant association between the year and the incidence of alloimmunization ($P = .49$ for the pre-ULR group and $P = .82$ for the post-ULR group). When considering the entire group of patients and adjusting for the effect of before versus after ULR there was also no significant effect of the year in which platelet transfusions were given on the incidence of alloimmunization ($P = .61$).

Hemorrhagic events

The overall incidence of hemorrhagic events was similar in the post- and pre-ULR groups (14% vs 13%, $P = .70$) (Table 6). The incidence of these events was not statistically different when analyzing the alloimmunized, refractory, or alloimmune refractory groups separately.

Table 5. Univariate and multivariate analysis of risk factors for alloimmunization and platelet refractoriness

	Univariate		Multivariate	
	RR (95% CI)	*P*	RR (95% CI)	*P*
Alloimmunization*				
Nonleukoreduced blood	2.3 (1.4-3.7)	.001	1.9 (1.1-3.1)	.014
Female sex	1.9 (1.2-3.0)	.005	—	—
Parous/transfused	2.6 (1.6-4.2)	< .001	2.5 (1.5-4.1)	< .001
No. of platelet transfusions†	5.3 (2.6-10.5)	< .001	4.7 (2.4-9.6)	< .001
Platelet refractoriness‡				
Nonleukoreduced blood	1.7 (1.3-2.3)	< .001	1.4 (1.0-1.9)	.034
Parous/transfused	1.5 (1.1-2.0)	.001	1.4 (1.1-2.0)	.002
No. of platelet transfusions	3.9 (2.7-5.7)	< .001	3.7 (2.6-5.4)	< .001
Alloimmune platelet refractoriness§				
Nonleukoreduced blood	2.8 (1.4-5.3)	.002	2.2 (1.2-4.3)	.016
Female sex	2.4 (1.4-4.1)	< .001	—	—
Parous/transfused	2.4 (1.3-4.4)	.004	2.3 (1.3-4.2)	.007
No. of platelet transfusions	6.8 (2.7-17.1)	< .001	6.0 (2.4-15.3)	.001

—indicates not significant.

*Positive lymphocytotoxic antibody screen on 2 or more consecutive occasions.

†Receipt of 13 or more platelet transfusions (the median number of platelet transfusions for entire patient group).

‡There were 2 consecutive corrected count increments of less than 5.

§Refractoriness within 2 weeks of 2 or more consecutive positive LCTAb screens.

Patients alloimmunized at presentation

Of the patients, 18 in the pre-ULR and 12 in the post-ULR group were alloimmunized at the time of referral for SCT or chemotherapy. The baseline characteristics were similar for both groups. Disappearance of LCTAbs occurred in 9 pre-ULR and 6 post-ULR patients. Responsiveness to RDP after an initial requirement for HLA-matched platelets occurred in 8 (44%) pre-ULR and 6 (50%) post-ULR patients. Neither difference was statistically significant.

Aplastic anemia and supportive care patients

Of the 9 patients with aplastic anemia, 2 of 7 in the pre-ULR group and neither of the 2 patients in the post-ULR group became alloimmunized. Of these patients, 4 received allogeneic stem cell transplants and 5 were treated with supportive care alone. None of these aplastic anemia patients demonstrated alloimmune refractoriness. There were 16 myelodysplasia (MDS) patients treated with supportive care only; 4 of the 8 who received nonleukoreduced blood and none of the 8 who received leukoreduced cells became both alloimmunized and refractory to platelet transfusion ($P = .08$).

Discussion

Alloimmunization is a side effect of cellular blood component therapy, which can lead to significant patient morbidity. This is particularly so in those who require prolonged blood product support such as the leukemia and SCT patients analyzed in this study. The provision of HLA-matched platelets to such individuals adds cost and inconvenience to their management. A variety of studies have demonstrated an apparent reduction in the incidence of alloimmunization and platelet refractoriness in leukemia patients receiving leukoreduced blood products.[15] However, this effect has not been uniformly observed, particularly in studies that enrolled relatively small patient numbers. Even in studies with the most convincing results, the protection from allosensitization with leukoreduced transfusions was incomplete[1-8] (Table 7). Nevertheless, it is unlikely that another randomized controlled trial will be conducted to examine the effect of leukoreduction, as the transfusion of "passenger" leukocytes is now considered by many to be a potentially harmful and thus ethically unacceptable manuver.[22] In 1997 a decision to introduce universal prestorage leukoreduction of cellular blood products into the Canadian blood system was made and implemented over the following year and a half. The expectation was that this would reduce the incidence of alloimmunization.[21] The current study was conducted to determine if this effect has been realized in practice in the group of patients who would benefit most from a reduction in alloimmunization, that is, those requiring prolonged blood product support during aggressive chemotherapy for acute leukemia or SCT. At our center, at least 80% of random donor platelet transfusions and almost 100% of the HLA-matched transfusions are administered to acute leukemia or SCT patients. Thus, this patient group is not only at the highest risk for the development of alloimmunization but is also the group in which the most resources are invested to treat its consequences. It remains controversial whether the benefits of leukoreduction will extend beyond this intensively transfused group to patients requiring fewer blood products, usually in a surgical setting.

In this study of consecutively treated and largely unselected patients the group receiving nonleukoreduced blood products was well matched with the group receiving leukoreduced cells for age, disease, treatment type, and proportion of patients with prior foreign HLA antigen exposure through pregnancy and/or blood transfusion. One major difference between the pre- and post-ULR groups was the shorter median follow-up in the pre-ULR group, which might have been expected to reduce the detection of alloimmunization and refractoriness. The second major difference was the smaller number of platelet transfusions received by patients in the post-ULR era. In 1998, the prophylactic platelet transfusion threshold was reduced at our institution from 20×10^9/L to 10×10^9/L since randomized trials and earlier prospective studies had demonstrated that this was both safe, from the standpoint of prevention of bleeding, and effective in reducing the number of platelet transfusions patients receive.[24,25,31] The 30% or more reduction in numbers of platelet transfusions per patient in the post-ULR group is likely due, at least in part, to the lowering of the platelet transfusion threshold, which was temporally associated with the introduction of ULR (Table 2).[24,25,31] In addition, although there were only minor changes in chemotherapy administered to leukemia patients, or conditioning or graft-versus-host disease prophylaxis for SCT patients over the study period, the cumulative effect of subtle changes of this kind may also have affected platelet utilization. Thus, the significant reduction in platelet transfusions that patients received after ULR cannot be attributed to their receipt of leukoreduced blood. In addition, as shown by the univariate and multivariate analysis in Table 5, a reduction in platelet transfusions is associated with a reduced incidence in both alloimmunization and platelet refractoriness. Thus, the reduction in alloimmunization and refractoriness shown in Tables 3 and 4 may not be completely attributed to ULR. Nevertheless, as also shown in Table 5, when controlling for the number of platelets transfused in multivariate analysis, the use of leukoreduced blood was still associated with a

Table 6. Hemorrhagic events

	Pre-ULR group* (%)	Post-ULR group* (%)	*P*†
Overall‡	44/315 (14)	38/302 (13)	.70
Platelet refractory	27/127 (21)	18/68 (27)	.52
Alloimmunized	14/61 (23)	3/21 (14)	.59
Alloimmune refractory	11/44 (25)	2/12 (17)	.83

*No. of refractory and/or alloimmunized pts/total pts in the group.

†Chi-square test.

‡More than one hemorrhagic event occurred in 25 of these patients.

Table 7. Comparison of the current study with randomized controlled trials of leukoreduction and alloimmunization

Study	Sample size	Prestorage LR	Chemotherapy patients %* (*P*)		SCT patients %* (*P*)		Parous/transfused patients %* (*P* value)	
			Allo	Ref	Allo	Ref	Allo	Ref
Current study	716	Yes	13 (.01)	9 (.04)	13 (.004)	11 (.003)	14 (.02)	12 (.005)
TRAP[7]	400	No	28 (< .0001)	9 (.03)	—	—	29 (.02)	—
Sintnicolaas et al[8]	46	No	NS	NS	—	—	NS	NS
Williamson et al[6]	123	No	31 (.025)	NS	NS	NS	—	—
van Marwijk Kooy et al[5]	53	Yes	35 (< .04)	35 (< .05)	—	—	NS	NS
Oksanen et al[4]	31	No	NS	NS	—	†	NS	NS
Andreu et al[2]	69	No	20 (< .05)	26 (< .02)	—	—	—	—
Sniecinski et al[3]	40	No	35 (.01)	35 (.01)	—	—	NS	—
Schiffer et al[1]	98	No	NS	NS	—	—	37 (.05)	—

LR indicates leukoreduction; SCT, stem cell transplantation; —, not done; and NS, not significant.

*Absolute percent reduction in incidence of HLA alloimmunization (Allo) or alloimmune refractoriness (Ref). Differences are listed only for statistically significant changes; other results are listed as NS.

†Only 3 patients underwent SCT in this study.

significant reduction in the incidence of both alloimmunization and platelet refractoriness.

In order to exclude the possibility that a gradual reduction in platelet alloimmunization and refractoriness occurred over the 8-year study period that could not be attributed to ULR, the year in which transfusion therapy was received was entered into the Cox regression analysis. No significant association between the year and the incidence of alloimmunization was demonstrated. The incidence of alloimmunization did not change significantly during the pre-ULR period (19% overall) and was consistently lower (between 6% and 7%) for each of the post-ULR years (1999, 2000, and 2001).

Most studies that have investigated allosensitization in patients receiving leukoreduced blood products used poststorage filtration of variable effectiveness.[1-8] Prestorage leukoreduction has a number of theoretical and practical advantages over poststorage or bedside filtration.[17,18,32,33] Only one previous randomized study has examined the effect of prestorage leukoreduction on alloimmunization.[5] Interestingly, in spite of the small patient sample size studied, both alloimmunization and alloimmunized refractoriness were reduced among the chemotherapy patients who received leukoreduced blood products (Table 7).

Prior to the Trial to Reduce Alloimmunization to Platelets (TRAP) study, no investigation was able to show a statistically significant reduction in alloimmunization among patients with a history of previous exposure to foreign HLA antigens through pregnancy and/or blood transfusion[15] (Table 7). Such patients comprised approximately half of the total leukemia and SCT patients who presented to our institution over the study period. The current analysis shows a clear reduction in the incidence of alloimmunization and platelet refractoriness with the use of prestorage leukoreduction in patients with a prior transfusion history, while this benefit is less clear for patients with a history of previous pregnancy.

All previous studies excluded patients with serologic evidence of alloimmunization at baseline.[7] It is possible that continued exposure to foreign HLA antigens on leukocytes will encourage sustained antibody production in such patients, while leukoreduction might prevent this process and allow larger numbers of these patients to receive random donor rather than HLA-matched transfusions. In the small group of such patients included in this study, transfusion of leuko-poor blood products did not lead to a significant reduction in either the incidence of detectable LCTAbs or the need for HLA-matched platelets. Nevertheless, it is possible that if larger numbers of such patients had been available for analysis significant differences would have emerged.

Many studies of platelet alloimmunization have restricted their analysis to acute leukemia patients receiving chemotherapy where the duration of transfusion requirements is long but the complicating issues of prolonged immunosuppression and donor/host reactivity seen in the SCT setting can be avoided.[1-3,5,7,8] However, the data from Williamson et al in a study that included both SCT and chemotherapy patients suggested that the incidence of alloimmunization may be reduced in SCT patients who receive leukoreduced blood products.[6] The current study demonstrates that the benefit of ULR, in significantly reducing alloimmunization and platelet refractoriness, extends to both the allogeneic and autologous SCT group.

We were unable to demonstrate a statistically significant reduction in the incidence of severe hemorrhagic events following the introduction of ULR. These findings are similar to the results of the TRAP study, where deaths due to hemorrhage were equal in patients receiving filtered and unfiltered platelets.[7] Although rates of bleeding in alloimmune refractory patients may be higher than in nonalloimmunized patients,[34] improvements in supportive care have brought about reductions in overall hemorrhagic events such that it is likely that much larger sample sizes than those so far studied will be required in order to detect a statistically significant improvement in this complication.[35] Additionally, almost half of the hemorrhagic events in our study occurred in patients who were not platelet refractory. This reinforces the conclusion that a variety of factors in addition to thrombocytopenia contribute to abnormal bleeding in this patient population.[35]

In the current investigation it was not possible to study another important potential benefit of prestorage leukoreduction (ie, a reduction in FNHTRs) since the method of recording these events at our center changed over the study period.[36-40] Similarly, the other predicted advantages of prestorage leukoreduction, such as a reduction in transfusion-associated infections and posttransfusion immune suppression, require further study.[41-44] If these benefits are confirmed they will add support to the results of this study and the growing body of literature in favor of prestorage leukoreduction.

The data presented here help justify the implementation of ULR by the Canadian blood supplier and may influence similar decision-making in other jurisdictions. Success in reducing alloimmunization and HLA-matched platelet utilization should result in reduced morbidity and mortality when platelet support is difficult or impossible. However, the cost of ULR is high and was not offset by the money saved by an overall reduction of 380 HLA-matched platelet collections in the ULR group of transfusion recipients seen here.[21] In addition, ULR did not completely overcome the need for HLA-matched platelets to support a small group of patients. Thus, the need for maintaining a large database of HLA-typed platelet donors has not been eliminated.

In conclusion, a policy of universal prestorage leukoreduction reduces platelet alloimmunization and refractoriness in multiply

transfused patients. This effect extends to patients undergoing stem cell transplantation as well as to acute leukemia patients receiving only aggressive chemotherapy. It also benefits patients who are potentially sensitized by previous blood transfusion. However, no clinical reduction in severe hemorrhagic events has been demonstrated. Further studies are required to determine whether this policy is effective in terms of other relevant outcomes and whether the cost of ULR can be justified by the overall benefit obtained.

Acknowledgments

We are indebted to the nursing and support staff of the Cell Separator Unit and the Leukemia/BMT nursing wards at the Vancouver General Hospital and British Columbia Cancer Agency. We also thank Janice Fujisawa (Canadian Blood Services), Daphne Brockington, Janet Nitta, and Daryl Armstrong (Leukemia/BMT program of BC) for their assistance in data management.

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2004 103: 333-339
doi:10.1182/blood-2003-03-0940 originally published online September 4, 2003

Universal prestorage leukoreduction in Canada decreases platelet alloimmunization and refractoriness

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.