

# Use of Leukocyte-Depleted Platelet Concentrates for the Prevention of Refractoriness and Primary HLA Alloimmunization: A Prospective, Randomized Trial

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Compared with conventional transfusion regimes a strong reduction in HLA alloimmunization and refractoriness to platelet transfusions is obtained when both red blood cell concentrates (RBCs) and platelet concentrates (PCs) are depleted of leukocytes by filtration. Because most of the leukocyte contamination is introduced by transfusion of RBCs, filtration of RBCs appears rational, but uncertainty exists regarding the degree of leukocyte-depletion of PCs needed for the prevention of HLA alloimmunization and refractoriness. We conducted a prospective trial and randomized patients with acute leukemia to receive leukocyte-depleted PCs prepared either by centrifugation (mean leukocyte count  $35 \times 10^6/\text{PC}$  of 6 U) or by filtration (mean leukocyte count  $< 5 \times 10^6/\text{PC}$  of 6 U). Both groups received RBCs that were filtered after prior removal of the buffy coat. Clinical

refractoriness occurred in 46% (12 of 26) of the evaluable patients that were transfused with centrifuged PCs and only in 11% (3 of 27) in the filtered group ( $P < .005$ ). De novo anti-HLA antibodies were detected in 42% (11 of 26) patients in the centrifuged group and only in 7% (2 of 27) of the patients receiving filtered PCs ( $P < .004$ ). In 8 of 11 alloimmunized patients in the centrifuged group antibodies were detected in the first 4 weeks of transfusion therapy while none of the patients in the filtered group became immunized against HLA antigens during that period. We conclude that for the prevention of HLA alloimmunization and refractoriness to platelet transfusions from random donors, both RBCs and PCs have to be leukocyte-depleted by filtration.  
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**D**URING THE last decade the use of prophylactic platelet transfusions for thrombocytopenic patients has increased exponentially. The major problem associated with long-term platelet support is the induction of HLA antibodies that occurs in about 40% to 50% of these patients and results in refractoriness to random donor platelet concentrates (PCs).<sup>1-4</sup> The management of these alloimmunized patients is accompanied with substantial logistic and financial problems. Because studies in rats<sup>5</sup> and mice<sup>6</sup> have demonstrated that the leukocytes contaminating PCs are responsible for the induction of anti-HLA antibodies, the removal of leukocytes from blood products has been advocated to prevent HLA alloimmunization. Recent clinical studies indeed demonstrated that the combined use of leukocyte-poor red blood cell concentrates (RBCs) and PCs prepared by filtration significantly reduced the incidence of HLA alloimmunization and refractoriness to random platelet transfusions.<sup>7-10</sup> However, these studies compared the use of nondepleted blood products to a transfusion regime where patients received RBCs and PCs that were both leukocyte-depleted by filtration. Filtration of RBCs is a simple and reproducible method that results in a three-log removal of leukocytes and is now generally accepted for the prevention of HLA immunization. However, white blood cell (WBC) contamination in PCs is about 10 times lower than in RBCs, and to date it remains uncertain to what extent PCs have to be depleted of leukocytes to prevent HLA alloimmunization. Filtration resulting in a two- to three-log reduction in leukocytes has proved to be effective for this purpose, but substantially raises the costs of platelet transfusion therapy.

We performed a prospective randomized study to determine whether there is a difference in the rate of primary HLA alloimmunization and refractoriness to random platelets between patients with acute leukemia receiving leukocyte-poor PCs either prepared by centrifugation or by filtration while both groups received filtered RBCs.

## MATERIALS AND METHODS

**Patients.** Seventy-seven patients with untreated acute leukemia who were referred to the University Hospital Utrecht (The Netherlands) from April 1987 to February 1990 were eligible for the study. To focus the study on the primary immune response to HLA antigens, 13 females with a history of pregnancies, 3 patients with HLA antibodies at presentation, and 2 other patients who had received transfusions in the 6 weeks before the study were excluded. The remaining 59 patients were randomized using a computer-generated randomization scheme and received leukocyte-poor PCs prepared by centrifugation (centrifuged group, 29 patients) or by filtration (filtered group, 30 patients). Both groups received filtered RBCs. Patients who received less than four PCs or with a follow-up period of less than 3 weeks after the first platelet transfusion, major protocol violation, or inadequate anti-HLA-antibody screening were considered nonevaluable.

During the period of the protocol patients remained on study until refractoriness occurred. Therefore, patients who did not become refractory during remission/induction or postremission/induction therapy remained on study and were followed when a bone marrow transplantation (BMT) was performed or during reinduction therapy for a relapse. To correct for the long intervals without transfusion therapy, only the weeks in which patients received transfusions were used to calculate the time of exposure.

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The protocol was conducted in accordance with local policies regarding research in humans, including an informed consent of the patients.

**Treatment protocols.** Patients with acute myeloid leukemia (AML)  $\leq 60$  years were treated according to the protocol of the Dutch Hemato-Oncology Working Party (HOVON) and received remission/induction and postremission/induction therapy, which consisted of three courses of chemotherapy containing high-dose cytosine arabinoside, daunorubicin, AMSA, mitoxantrone, and etoposide. Eligible patients  $\leq 45$  years with an HLA-identical sibling received an allogeneic BMT after conditioning with high-dose cyclophosphamide and busulfan while the remaining patients were randomized between no further treatment and autologous BMT after conditioning with high-dose cyclophosphamide and busulfan.

Patients with AML greater than 60 years were treated according to the protocol of the European Organization for Research and Treatment of Cancer (EORTC) with two courses of chemotherapy containing daunorubicin (or mitoxantrone) and cytosine arabinoside.

All patients with acute lymphocytic leukemia (ALL) were treated according to the protocol of HOVON. Induction therapy consisting of prednisone, daunorubicin, asparaginase, and vincristine was followed by three courses of intensive postinduction chemotherapy containing high-dose cytosine arabinoside, AMSA, mitoxantrone, and etoposide. Eligible patients  $\leq 45$  years with an HLA-identical sibling received an allogeneic BMT after conditioning with high-dose cyclophosphamide and total body irradiation.

**Preparation of blood components and transfusion policy.** The blood components were prepared and stored by the Red Cross Blood Center in Utrecht. Blood (450 mL) was collected in citrate-phosphate-dextrose solution and centrifuged (1,100g, 4 minutes, 22°C) to separate platelet-rich plasma (PRP) and RBCs. RBCs underwent a second high-spin centrifugation (4,000g, 10 minutes, 22°C) to remove the buffy coat and were then stored in saline-adenine-glucose-mannitol solution. RBCs were filtered through a cellulose acetate filter (Cellselect; NPBI, Emmer-Compasuum, The Netherlands). Filtration was performed within 48 hours after collection to prevent the possibility of immunization by leukocyte fragments that arise during storage and are not removed by filtration. The platelets in the PRP were sedimented by centrifugation (3,000g, 10 minutes, 22°C) and stored in about 50 mL of autologous plasma for a maximum of 48 hours. After pooling 6 U of platelets the PCs were leukocyte-depleted either by centrifugation (defined as centrifuged PCs) or by filtration (defined as filtered PCs). Centrifuged PCs were prepared by an additional centrifugation step (220g, 5 minutes, 22°C) while filtered platelets were prepared by passing the PCs through a Cellselect filter after transient inactivation of the platelets with 12.5  $\mu$ g prostacyclin per PC as described previously.<sup>11</sup> No adverse effects due to the use of prostacyclin were observed among patients who received filtered PCs. Platelets were counted automatically (Platelet analyzer 810; Baker, Allentown, PA) while leukocytes were counted manually in both segments of a Bürker chamber. The characteristics of the PCs with regard to volume, platelet, and leukocyte counts are shown in Table 1. Leukocyte counts in RBCs after filtration were consistently less than  $5 \times 10^6$  leukocytes/RBC, which is in concert with previously reported results using this filtration technique.<sup>12</sup>

ABO-compatible platelets were transfused prophylactically when the platelet count dropped below  $20 \times 10^9/L$ , when the Ivy bleeding time needed correction for invasive procedures or when major hemorrhagic problems occurred. All patients received filtered RBCs (usually 2 U) with a hemoglobin concentration below 6 mmol/L. None of the patients received granulocyte transfusions.

**Table 1. Preparation of Platelet Concentrates**

Characteristics	Platelet Concentrate*		
	Standard	Centrifuged	Filtered
Volume (mL)	339 $\pm$ 51	308 $\pm$ 48	434 $\pm$ 54
Platelet count ( $\times 10^9$ )	422 $\pm$ 79	338 $\pm$ 69	357 $\pm$ 67
Leukocyte count ( $\times 10^6$ )	334 $\pm$ 180	35 $\pm$ 34	<5†

Results are expressed as means  $\pm$  SD.

\*Pool of 6 U from randomly selected donors.

†All filtered PCs contained  $< 10 \times 10^6$  leukocytes and 96% contained  $< 5 \times 10^6$  leukocytes.

**Assessment of platelet recovery and definition of refractoriness.** One hour and 20 hours following a platelet transfusion, platelet counts were performed on the recipient. The platelet recovery was calculated according to the following formula:

$$\text{Recovery (\%)} = 100 \times \frac{\text{Absolute Increment} \times \text{Blood Volume}}{\text{No. of Transfused Platelets} (\times 10^{11})}$$

Blood volume was calculated as body surface area ( $m^2$ )  $\times 2.5$ .

Patients were considered refractory to random donor platelets when two consecutive platelet transfusions resulted in a 1-hour recovery of less than 20% in the absence of other clinical factors that are known to adversely affect platelet recovery, such as disseminated intravascular coagulation (DIC), fever (temperature  $> 38.5^\circ\text{C}$ ), septicemia, or splenomegaly ( $> 4$  cm below the left costal margin).

**Anti-HLA-antibody screening.** Sera from all patients were collected at entry of the study and weekly during a transfusion period. Also, a serum sample was obtained 2 to 4 weeks after the last transfusion. Sera were stored at  $-80^\circ\text{C}$  until testing. Each serum was tested in the complement-dependent lymphocytotoxicity test against a comprehensive panel of 30 selected donors covering most of the defined HLA-A and -B specificities.<sup>13</sup> The screening test was considered positive when at least 10% of the panel showed reproducible cytotoxicity ( $> 25\%$ ).

**Statistical analysis.** The incidence of clinical refractoriness and HLA alloimmunization as the percentage of patients achieving complete remission in both groups were compared by using the Fisher's exact test. The probabilities of clinical refractoriness, HLA alloimmunization, and of remaining in remission as a function of time were estimated with the Kaplan-Meier method<sup>14</sup>; comparison between the two groups was performed using the log-rank test. For other statistical analysis the Wilcoxon rank sum test was used.

## RESULTS

**Patients.** Six patients were excluded from evaluation. Two patients in the centrifuged group and one patient in the filtered group suffered an early death. One patient in both groups received less than four PCs, and one patient in the filtered group who received multiple centrifuged PCs was excluded because of protocol violation. Fifty-three patients remained evaluable. As shown in Table 2, there were no major differences between the two groups with regard to age, sex, underlying disease, or prior exposure to blood transfusions.

The characteristics of the transfusion regime of the evaluable patients is shown in Table 3. The median time of exposure in which patients received transfusions was shorter in the group receiving centrifuged PCs than in the group receiving filtered PCs. This difference can be explained by the high number of patients in the centrifuged group who

**Table 2. Patient Characteristics**

	Patient Group	
	Centrifuged	Filtered
No. patients	26	27
Age (y)		
Mean $\pm$ SD	46 $\pm$ 19	41 $\pm$ 18
Range	12-74	16-74
Sex ratio		
F	5	3
M	21	24
Diagnosis		
AML	20	18
ALL	6	9
Prior transfusions	6	5

became refractory during remission/induction or postremission/induction therapy and were taken off study. Platelet recoveries after transfusion were significantly lower in the centrifuged group, which explains the relative high number of PCs transfused in this group.

**Refractoriness and HLA alloimmunization.** In this study 847 PCs were transfused. For the determination of platelet recoveries, 1-hour counts were obtained after 745 (88%) of these transfusions and 24-hour counts were obtained in 703 (83%) of these events. A poor 1-hour recovery (20%) was observed in 113 of the 745 evaluable transfusions.

Poor increments coincided with fever ( $>38.5^{\circ}\text{C}$ ) or septicemia (positive blood cultures) in 47 episodes; DIC was found in 4 such episodes; 5 platelet transfusions with poor increments coincided with major bleeding and in 4 episodes with splenomegaly. In another 23 of these events a subsequent transfusion resulted in a satisfactory 1-hour recovery and, therefore, were considered to be a random happening. In the remaining 30 events two consecutive platelet transfusions gave poor increments, which was taken as evidence for refractoriness. The incidence of refractoriness dropped from 47% (12 of 26) in the centrifuged group to 11% (3 of 27) in the group of patients receiving filtered platelets while HLA alloimmunization occurred in 42% (11 of 27) and 7% (2 of 27), respectively, as shown in Table 4. The overall incidence of HLA alloimmunization in the group of patients with prior transfusion therapy (3 of 11, 27%) was not significantly different from the group of patients who had not received prior transfusions (10 of 42, 24%).

**Table 3. Transfusion Regimens of the Evaluable Patients**

	Patient Group		P Value
	Centrifuged Median (range)	Filtered Median (range)	
Time of exposure*	8 (2-16)	13 (5-31)	$<.001$
Units of blood products			
RBCs	20 (6-48)	28 (8-82)	$<.05$
PCs	72 (18-206)	72 (24-276)	NS
Platelet recovery (%)			
At 1 h	41 (1-128)	48 (1-135)	$<.001$
At 20 h	25 (1-95)	35 (1-99)	$<.001$

Abbreviation: NS, not significant.

\*Expressed as weeks in which patients were transfused.

**Table 4. Incidence of Clinical Refractoriness and HLA Alloimmunization**

	Patient Group		P Value
	Centrifuged	Filtered	
Clinical refractory patients	12/26 (46%)	3/27 (11%)	$<.005$
Time of exposure until refractoriness*	4 (2-12)	18 (6-23)	$<.001$
Units of blood products until refractoriness			
RBCs	15 (6-27)	33 (12-38)	NS
PCs	52 (18-128)	43 (24-112)	NS
Patients with HLA antibodies	11/26 (42%)	2/27 (7%)	$<.004$
Time of exposure until HLA alloimmunization*	4 (2-12)	12 (6-18)	$<.03$
Units of blood products until HLA alloimmunization			
RBCs	10 (8-20)	21 (12-30)	NS
PCs	56 (12-130)	38 (24-52)	NS

Abbreviation: NS, not significant.

\*Expressed as weeks in which patients were transfused.

This finding clearly suggests that patients with transfusion therapy in the past are not at risk for a secondary immune response to HLA antigens. Also, no significant difference in the rate of HLA alloimmunization was found between patients with ALL (3 of 15, 20%) or AML (10 of 38, 26%).

To evaluate whether we could detect a "threshold level" for the degree of leukocyte contamination in PCs above which patients are at risk for HLA alloimmunization, patients in the centrifuged group were divided into a group who had received only PCs with a maximum of  $50 \times 10^6$  leukocytes/PC and those who had also received PCs with greater than  $50 \times 10^6$  leukocytes/PC. In the first group only 1 of 9 (11%) became immunized, whereas 10 of 17 (59%) patients in the second group developed anti-HLA antibodies ( $P = .09$ ). Thus, for the entire group of patients (patients of the filtered group included) who had only received PCs with a maximum of  $50 \times 10^6$  leukocytes/PC, the rate of HLA alloimmunization (3 of 36, 8%) was significantly lower than among patients who also received PCs with a higher number of leukocytes ( $P < .003$ ).

The time before refractoriness developed as defined by the number of weeks with transfusions increased with the use of filtered PCs. Refractoriness occurred during the first 4 weeks in 8 of 12 patients (67%) in the centrifuged group, while none of the patients in the filtered group showed signs of refractoriness during that time period. The probability of remaining responsive to platelet transfusions of random donors as a function of weeks with transfusion events is shown in Fig 1. Using the log-rank test the difference between the two groups was statistically significant ( $P < .0006$ ).

Refractoriness coincided with the detection of multispecific anti-HLA antibodies in 8 of 12 patients in the centrifuged group. Three patients in this group developed anti-HLA antibodies with limited specificities that did not result in refractoriness. In the filtered group anti-HLA antibodies

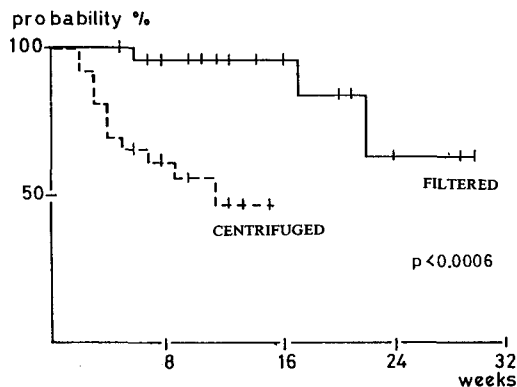


Fig 1. Probability of not becoming refractory to random donor platelets as a function of weeks in which patients were transfused (Kaplan-Meier plot).

were found in the serum of only two patients who were both clinically refractory. Thus, in both groups there was one refractory patient who did not develop lymphocytotoxic antibodies. Tests to detect platelet-specific antibodies were not performed. The use of filtered PCs not only decreased the incidence of HLA alloimmunization but also delayed the time before immunization occurred. Eight of the 11 immunized patients in the centrifuged group had developed anti-HLA antibodies within 4 weeks of transfusions. In the same period no antibodies were detected in the serum of the patients in the filtered group. The probability of not becoming immunized as a function of weeks with transfusion events is shown in Fig 2. The difference between the groups was statistically significant according to the log-rank test ( $P < .008$ ).

To investigate whether the transfusion policy influenced the prognosis, a Kaplan-Meier analysis for the probability of remaining in remission was made. In the centrifuged group 65% (17 of 26) of the patients achieved complete remission as compared with 74% (20 of 27) of the patients in the filtered group ( $P =$  not significant). The probability of remaining in remission as a function of time is shown in Fig 3. Again no statistically significant difference between the two groups was observed.

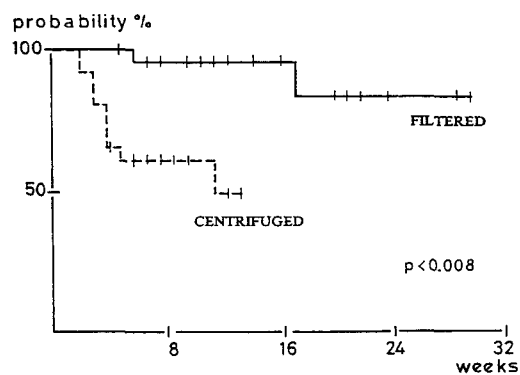


Fig 2. Probability of not becoming immunized to HLA antigens as a function of weeks in which patients were transfused (Kaplan-Meier plot).

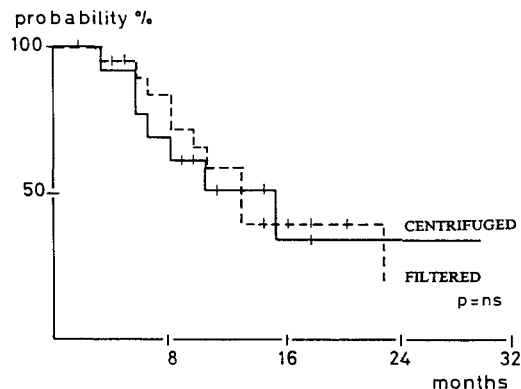


Fig 3. Probability of remaining in remission for patients achieving first complete remission as a function of time (months) (Kaplan-Meier plot).

## DISCUSSION

Previous studies on the use of leukocyte-depleted PCs have reported different results with regard to the incidence of HLA alloimmunization. Schiffer et al<sup>15</sup> randomized patients with acute leukemia into two groups receiving either standard PCs or PCs made leukocyte-poor by centrifugation, while both groups received leukocyte-poor frozen RBCs. Between the patients who had not been previously pregnant or transfused, there was no significant difference in the incidence of alloimmunization between the control group receiving standard PCs (33%) and the group who received leukocyte-poor PCs (27%). In contrast, Brand et al<sup>16</sup> transfused patients with filtered RBCs and leukocyte-poor PCs prepared by centrifugation in a large, nonrandomized, prospective study and reported that only 19% of 264 non-presensitized patients developed HLA antibodies and 6% became clinically refractory, requiring HLA-matched platelet transfusions. The discrepancy between these two studies can be explained by the difference in the number of contaminating leukocytes in the PCs. Brand et al used an open system and achieved a leukocyte count of less than  $20 \times 10^6/PC$  ( $> 80\%$  less than  $10 \times 10^6/PC$ ), while Schiffer et al used a closed-bag system for centrifugation and found  $72 \times 10^6$  leukocytes/PC.

In another study Andreu et al<sup>9</sup> reported HLA alloimmunization in 12% of the patients transfused with filtered RBCs and PCs containing a mean leukocyte count of  $36 \times 10^6/PC$  after filtration. Although the mean leukocyte contamination of centrifuged PCs ( $35 \times 10^6/PC$ ) in the present study is comparable with that of the filtered PCs in the study by Andreu et al, we observed a much higher incidence of refractoriness (46%) and HLA alloimmunization (42%) among patients receiving centrifuged PCs. However, centrifugation shows a much wider range of postfiltration leukocyte counts compared with filtration, which probably explains these differences.

The present study confirms the beneficiary effects of leukocyte-depletion of PCs as demonstrated by the low incidence of HLA alloimmunization in patients transfused with filtered PCs (11%) and in patients who received centrifuged PCs with a maximum of  $50 \times 10^6$  (11%).

We conclude that for the prevention of HLA alloimmunization and clinical refractoriness filtration of RBCs alone is not sufficient. Also, PCs have to be leukocyte-depleted using a method that guarantees a low level of leukocyte contamination ( $< 10$  to  $50 \times 10^6$ ) with optimal reproducibility. At present, filtration is the best method available to achieve this goal, as centrifugation is difficult to standardize and results in a wide range of leukocyte contamination. Recently, third-generation filters have become available that are highly effective, easy to handle, and do not require transient inactivation of platelets before filtration.<sup>17</sup> Therefore, the use of filtered PCs in combination with filtered RBCs should be recommended for all patients requiring long-term platelet support.

A matter of concern is the observation made by Tucker et al<sup>18</sup> that patients treated with leukocyte-poor blood products show a decreased disease-free survival as compared with those receiving standard blood components. This

difference was explained by a possible graft-versus-leukemia effect of the leukocytes that contaminate the blood products. A comparable mechanism has also been suggested for patients with graft-versus-host disease following allogeneic BMT who show an improved survival.<sup>19</sup> In the present study Kaplan-Meier analysis of the probability of remaining in remission failed to demonstrate a significant difference between the two groups. Obviously, the number of patients is too small to draw definite conclusions and further clinical trials are required to reveal a possible transfusion-induced graft-versus-leukemia effect.

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