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LEUKOCYTE REDUCTION AND ULTRAVIOLET B IRRADIATION OF PLATELETS TO PREVENT ALLOIMMUNIZATION AND REFRACTORINESS TO PLATELET TRANSFUSIONS

THE TRIAL TO REDUCE ALLOIMMUNIZATION TO PLATELETS STUDY GROUP*

ABSTRACT

Background We conducted a multi-institutional, randomized, blinded trial to determine whether the use of platelets from which leukocytes had been removed by a filter or that had been treated with ultraviolet B irradiation would prevent the formation of antiplatelet alloantibodies and refractoriness to platelet transfusions.

Methods Patients who were receiving induction chemotherapy for acute myeloid leukemia were randomly assigned to receive one of four types of platelet transfusions: unmodified, pooled platelet concentrates from random donors (control); filtered, pooled platelet concentrates from random donors (F-PC); ultraviolet B-irradiated, pooled platelet concentrates from random donors (UVB-PC); or filtered platelets obtained by apheresis from single random donors (F-AP). All patients received transfusions of filtered, leukocyte-reduced red cells.

Results Of 530 patients with no alloantibodies at base line, 13 percent of those in the control group produced lymphocytotoxic antibodies and their thrombocytopenia became refractory to platelet transfusions, as compared with 3 percent in the F-PC group, 5 percent in the UVB-PC group, and 4 percent in the F-AP group ($P \leq 0.03$ for each treated group as compared with the controls; there were no significant differences among the treated groups). Lymphocytotoxic antibodies were found in 45 percent of the controls, as compared with 17 to 21 percent in the treated groups ($P < 0.001$ for each treated group as compared with the controls; there were no significant differences among the treated groups). Antibodies against platelet glycoproteins developed in 6 to 11 percent of the patients, with no significant differences among the four groups.

Conclusions Reduction of leukocytes by filtration and ultraviolet B irradiation of platelets are equally effective in preventing alloantibody-mediated refractoriness to platelets during chemotherapy for acute myeloid leukemia. Platelets obtained by apheresis from single random donors provided no additional benefit as compared with pooled platelet concentrates from random donors. (N Engl J Med 1997;337:1861-9.)

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ANTIBODIES against alloantigens on donor platelets are a major cause of refractoriness to platelet transfusions in patients with thrombocytopenia. These antibodies usually arise in response to HLA class I antigens on leukocytes and platelets, and they may result from maternal-fetal incompatibility or repeated blood transfusions. Resources to supply HLA-compatible platelets to patients who have refractoriness to platelet transfusions from random donors are often limited. Moreover, up to 60 percent of transfusions of presumably HLA-compatible platelets fail to increase the platelet count in patients whose thrombocytopenia is refractory to transfusions.¹

Previous studies of ways to prevent alloimmunization in patients with thrombocytopenia have been inconclusive.²⁻⁸ In this trial we transfused blood components that were modified by leukocyte reduction or ultraviolet B irradiation (to remove or inactivate cells bearing alloantigens, respectively),⁹⁻¹¹ or used platelets from single donors (to reduce exposure to multiple HLA antigens).^{12,13}

METHODS

Patient Selection

All patients had previously untreated acute myeloid leukemia. The following patients were ineligible for the trial: patients less than 15 years old; patients who were to receive no chemotherapy, low-dose chemotherapy (total doses of less than 90 mg of daunorubicin per square meter of body-surface area, 30 mg of mitoxantrone per square meter, 30 mg of idarubicin per square meter, or 700 mg of cytarabine per square meter), or corticosteroids; recipients of multiple blood transfusions for a hematopoietic disorder two months or more before study entry, or of transfusions

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from more than 10 different donors between two weeks and two months before study entry; and those given chemotherapy or extensive radiation therapy within the past two years.

The study protocol and consent forms were approved by local institutional review boards.

Randomization

Patients were randomly assigned to receive one of four types of platelet transfusion for eight weeks after the first transfusion of study platelets. The four types of platelet transfusions were unmodified, pooled platelet concentrates from random donors (control); filtered, pooled platelet concentrates from random donors (F-PC); ultraviolet B-irradiated, pooled platelet concentrates from random donors (UVB-PC); and filtered platelets obtained by apheresis from single random donors (F-AP). We refer to the last three types as treated platelets and to the groups of patients who received them as treated groups. Randomization was stratified according to trial site and whether or not subjects had a prior pregnancy or transfusion more than two weeks before enrollment. All patients and investigators, except the study coordinators at the clinical sites, were unaware of the patients' assignments.

Preparation of Blood Components for Transfusion

Blood components were prepared in a blood bank at each trial site. Six units of platelet concentrate prepared from whole-blood, platelet-rich plasma¹⁴ were pooled, and if treated, they were filtered or irradiated with ultraviolet B, usually shortly before transfusion. F-AP platelets were collected with a Cobe Spectra apheresis machine (Cobe Laboratories, Lakewood, Colo.) with version 2.6 or 3.6 software.

All patients received filtered red cells to prevent alloimmunization by leukocytes in packed red cells. Filtered blood components were expected to have no more than 5×10^6 white cells per transfusion. PL-100 filters were used for platelets and either an RC-100 or a BPF-4 filter for red cells (Pall, East Hills, N.Y.). Platelet concentrates were irradiated in a Stericell bag holding 420 ml or less (Dupont, Wilmington, Del.) with a total dose of 1480 mJ per square centimeter with an ultraviolet B irradiator (Haemonetics, Braintree, Mass.).

Transfusions

Prophylactic platelet and red-cell transfusions were given when platelet counts were 20,000 per cubic millimeter or less and hematocrits were 0.25 or less. The assigned transfusions were discontinued only when there were severe adverse reactions, defined as a temperature increase of more than 2°C, shaking chills, extensive urticaria, dyspnea, cyanosis, or bronchospasm. If the patient's assigned platelet component was not available, pooled platelet concentrates were substituted for platelets obtained by apheresis, or vice versa; however, these platelets were treated or not treated according to the patient's randomization assignment. Overall, only 3 percent of the transfusions given were not of the assigned type (3 percent in the control group, 3 percent in the UVB-PC group, 2 percent in the F-PC group, and 5 percent in the F-AP group).

Every six months, ultraviolet B-irradiated platelets from each center were tested by mixed lymphocyte culture. None of these samples contained viable lymphocytes. Platelet and white-cell counts of the platelet preparations were determined after processing by automated cell counters, except for the white-cell counts of the filtered platelet preparations, which were done with pridium iodide staining.¹⁵

Calculation of the Corrected Count Increment and Refractoriness to Platelet Transfusions

The corrected count increment was calculated as the difference between the platelet count within an hour after transfusion and the platelet count before transfusion, multiplied by the body-surface area (in square meters) and divided by the number of plate-

lets transfused times 10^{-11} . The mean (\pm SD) expected corrected count increment in patients receiving transfusions with pooled platelet concentrates from random donors that had been stored for three to five days is approximately $13,500 \pm 6000$.¹⁶ Refractoriness was defined as a corrected count increment of less than 5000 after two sequential transfusions of ABO-compatible platelets, at least one of which had been stored for no more than 48 hours.

Antibody Testing

Serum samples were obtained before entry into the study and weekly thereafter for eight weeks. They were tested for lymphocytotoxic antibodies and antibodies against platelet glycoproteins in central laboratories after the completion of the study. Some patients who were entered into the study were later found to have antiplatelet antibodies in base-line samples, but they were excluded from the primary analyses.

Lymphocytotoxic antibodies against HLA class I antigens were detected with an antiglobulin-augmented, complement-dependent lymphocytotoxicity assay.¹⁷ Serum samples were tested against a panel of 30 to 60 HLA-typed frozen cells. Serum samples were considered positive if they reproducibly caused at least 60 percent cytotoxicity in one or more cells or at least 40 percent cytotoxicity in two or more cells in the panel.¹⁸ Panel reactivity was calculated as a percentage: the number of positive cells divided by the number of panel cells times 100.

Serum samples were tested for antiplatelet antibodies by intact-platelet enzyme-linked immunosorbent assay (ELISA)^{19,21} and flow cytometry.²² These assays are sensitive but nonspecific and were used only to screen for antibodies.^{22,23} The first and most reactive samples from each patient were further tested for antibodies against platelet glycoproteins (GPIIb/IIIa, GPIb/IX, and GPIa/IIa) with a modified antigen-capture ELISA method.^{24,25}

Study End Points

The primary end point was refractoriness to platelet transfusions in patients with lymphocytotoxic antibodies or antibodies against platelet glycoproteins that were detected within two weeks before or after the diagnosis of refractoriness (alloimmune-mediated platelet refractoriness). The secondary end points were refractoriness to platelet transfusions and positive tests for lymphocytotoxic antibodies or antibodies against platelet glycoproteins.

Statistical Analysis

The base-line characteristics of the patients were compared by analysis of variance and the chi-square test. Event rates were estimated by Kaplan-Meier methods over the eight-week study and compared by the log-rank statistic beginning with the first platelet transfusion after randomization. Ninety-five percent confidence intervals estimated with Greenwood's standard errors are reported for event rates. Data on patients were censored at the time of death; no deaths occurred before the first platelet transfusion. Cox regression analyses were used to examine the effect of covariates on the rates of refractoriness and development of lymphocytotoxic antibodies. The factors considered in a multivariate analysis to identify predictors of study end points were treatment assignment, trial site, age, sex, prior pregnancy, whether platelet transfusions were given more or less than two weeks before entry into the study, common HLA type, use of hematopoietic growth factors, and gamma irradiation of more than 50 percent of a patient's transfusions. 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 0.05 were retained in the final model. Treatment effects and interaction-with-treatment effects were added to the final model by using dummy variables. We performed analyses using calendar time and using the transfusion number as the time variable. All results are reported according to the group to which the patient was randomly assigned.

TABLE 1. PATIENT CHARACTERISTICS AND PRIOR EXPOSURE TO ALLOANTIGENS.*

CHARACTERISTIC	CONTROL† (N = 131)	UVB-PC (N = 130)	F-PC (N = 137)	F-AP (N = 132)	TOTAL (N = 530)
Age — yr					
Median	50	57	56	55	54
Range	17–77	16–84	17–79	18–88	16–88
Female — no. (%)	63 (48)	53 (41)	64 (47)	64 (48)	244 (46)
Women who had ever been pregnant — no. (%)	48 (76)	45 (85)	53 (83)	52 (81)	198 (81)
Patients who had received prior transfusions — no. (%)					
Any transfusion	102 (78)	106 (82)	112 (82)	103 (78)	423 (80)
Transfusion >2 wk before study entry	19 (15)	25 (19)	29 (21)	22 (17)	95 (18)
Transfusion ≤2 wk before study entry	95 (73)	100 (77)	106 (77)	97 (73)	398 (75)
All transfusions with leukocytes reduced by filtration	15 (11)	17 (13)	22 (16)	25 (19)	79 (15)
Patients with no prior pregnancy or transfusion — no. (%)	19 (15)	15 (12)	13 (9)	18 (14)	65 (12)

*UVB-PC denotes ultraviolet B-irradiated, pooled platelet concentrates from random donors; F-PC filtered, pooled platelet concentrates from random donors; and F-AP filtered platelets obtained by apheresis from single random donors.

†Controls received unmodified, pooled platelet concentrates from random donors.

The power for the study was based on the exponential maximum-likelihood model.²⁶ With 570 randomized patients anticipated, the power to detect a reduction in the rate of alloimmunization from 0.40 to 0.20 at a significance level of 0.05 by two-sided test was 0.92. The trial was sequentially monitored with use of the O'Brien-Fleming method.²⁷

RESULTS

Patients

We screened 1047 patients with acute myeloid leukemia and enrolled 603 of them between January 14, 1991, and February 28, 1995. Patients were excluded because of low-dose chemotherapy or none (36 percent of the screened patients), a prior hematopoietic disorder treated with transfusions (27 percent), prior chemotherapy (20 percent), logistic reasons (14 percent), prior treatment for leukemia (11 percent), refusal to enter the study (11 percent), or administration of corticosteroids (5 percent).

Of the 603 patients who were enrolled, 41 had lymphocytotoxic antibodies at base line, 15 had antibodies against platelet glycoproteins, and 5 had both. Of these 61 patients, 41 (67 percent) had both been previously pregnant and received transfusions. Within this group of 61 patients with antiplatelet antibodies at base line, refractoriness to platelet transfusions was more frequent among patients who had lymphocytotoxic antibodies (55 percent) than among those who did not (10 percent, $P < 0.001$). The presence or absence of antibodies against platelet glycoproteins at base line did not significantly influence the frequency of refractoriness to platelet transfusions (21 percent and 13 percent, respectively; $P = 0.17$). Study assignment had no significant effect on

the development of refractoriness in the antibody-positive patients. These 61 patients were excluded from further analysis, along with 4 patients with no data on base-line antibodies and 8 patients who received fewer than two platelet transfusions.

Table 1 shows the assignments of the remaining 530 patients into the four study groups. Age, number of pregnancies, and number of prior transfusions were similar in these groups. Base-line hematologic values and type of leukemia were also similar (data not shown). Seventy-five percent of the patients had received transfusions in the two weeks before randomization, and 85 percent of these transfusions were not filtered. Only 12 percent of patients had not received transfusions or been pregnant.

Results of Transfusion

Ninety-five percent of the platelet transfusions were ABO-compatible with the recipient. Patients assigned to filtered products received fewer platelets per transfusion because of platelet loss during filtration ($P < 0.001$),²⁸ but the number of platelet transfusions did not differ significantly among the groups ($P = 0.18$) (Table 2).

More platelet transfusions with at least 5×10^6 white cells were given to the F-PC group than to the F-AP group, probably reflecting the lower initial contamination of the apheresis platelets by leukocytes.²⁹ In the UVB-PC group, 1 percent of the platelet transfusions were not irradiated with ultraviolet B. Ninety-eight percent of the red-cell transfusions were filtered, and only 0.5 percent had 5×10^6 or more white cells per transfusion.

TABLE 2. RESULTS OF TRANSFUSION.*

VARIABLE	CONTROL†	UVB-PC	F-PC	F-AP	TOTAL
Platelets‡					
No. of times patient received a transfusion	14±11	16±13	15±11	13±8	14±11
Exposures to donor platelets					
Median	66	72	72	11	54
Range	12–840	12–625	12–366	2–48	2–840
Platelets per transfusion — ×10 ⁻¹¹	4.5±1.2	4.4±1.2	3.7±1.1	3.7±1.3	4.1±1.3
CCI	12,800±4900	11,200±4600	12,700±5700	14,700±5200	12,900±5300
White cells per transfusion — ×10 ⁻⁶	742±607	704±565	2±20	1±14	368±551
Transfusions with ≥5×10 ⁶ white cells — no. (%)	1764 (99)	2027 (99)	79 (4)	26 (2)	3896 (52)
Patients receiving ≥1 transfusion with ≥5×10 ⁶ white cells — no. (%)	131 (100)	130 (100)	41 (30)	14 (11)	316 (60)
Red cells					
Transfusions — no. per patient	15±7	16±8	16±8	15±7	15±7
Transfusions with ≥5×10 ⁶ white cells — no. (%)	22 (2)	30 (2)	26 (1)	32 (2)	110 (1)
Patients receiving ≥1 transfusion with ≥5×10 ⁶ white cells — no. (%)	11 (8)	8 (6)	15 (11)	10 (8)	44 (8)
Platelets or red cells — no. (%)					
Patients receiving ≥1 transfusion of platelets or red cells not meeting target§	28 (21)	27 (21)	57 (42)	29 (22)	141 (27)
Patients receiving ≥2 transfusions of platelets or red cells not meeting target§	15 (11)	15 (12)	32 (23)	16 (12)	78 (15)
Incidence of severe platelet-transfusion reactions¶					
Transfusions	42 (2)	54 (3)	35 (2)	29 (2)	160 (2)
Patients	31 (24)	35 (27)	28 (20)	20 (15)	114 (22)

*Plus-minus values are means ±SD. UVB-PC denotes ultraviolet B-irradiated, pooled platelet concentrates from random donors; F-PC filtered, pooled platelet concentrates from random donors; F-AP filtered platelets obtained by apheresis from single random donors; and CCI corrected count increment.

†Controls received unmodified, pooled platelet concentrates from random donors.

‡Data reported are for all platelet transfusions, including HLA-selected platelet transfusions given to patients with refractoriness to platelet transfusions.

§Patients are included if the platelet or red-cell product did not meet the given target, as follows: control, filtered platelet product or red-cell product that was not filtered or that had at least 5×10⁶ white cells; UVB-PC, platelet product not UVB-irradiated or red-cell product that was not filtered or with at least 5×10⁶ white cells; F-PC, platelet or red-cell product not filtered or with at least 5×10⁶ white cells; F-AP, not single-donor platelets obtained by apheresis or platelet or red-cell product that was not filtered or with at least 5×10⁶ white cells.

¶For severe platelet-transfusion reactions (defined as increase in temperature of more than 2°C, shaking chills, extensive urticaria, dyspnea, cyanosis, or bronchospasm), column assignments refer to preparation of product, not assigned treatment group.

One hundred sixty (2 percent) of the platelet transfusions were associated with a severe adverse reaction, but there were no differences in the frequencies of such reactions among the four groups (P=0.45, Kruskal–Wallis test). Transfusion reactions led to the withdrawal of only five patients from the study.

Refractoriness to Platelet Transfusions

Among the 530 patients in the study, only 51 (10 percent) met the criteria for refractoriness. Among these 51 patients, Kaplan–Meier estimates (with 95 percent confidence intervals) for the development of refractoriness over the eight weeks of the study were 16 percent (10 to 23 percent) for the control group, 10 percent (6 to 16 percent) for the UVB-PC group, 7 percent (4 to 13 percent) for the F-PC group, and 8 percent (4 to 14 percent) for the F-AP group (P=0.17, P=0.03, and P=0.06, respectively, for the comparison with the control group) (Fig. 1).

Among women who had ever been pregnant, the estimates for the development of refractoriness were 32 percent (21 to 47 percent) for controls as com-

pared with 17 percent (8 to 32 percent) for the UVB-PC group, 10 percent (4 to 22 percent) for the F-PC group, and 16 percent (8 to 29 percent) for the F-AP group (P=0.10, P=0.009, and P=0.09, respectively). Among women who had never been pregnant and men, the rates of refractoriness to platelet transfusions among all four groups were similar and ranged from 3 percent to 6 percent (P≥0.26). A rate of 10 percent was noted in patients with and those without a history of transfusions (423 and 107 patients, respectively).

Lymphocytotoxic Antibodies

During the eight weeks of the study, lymphocytotoxic antibodies developed in an estimated 45 percent of the patients in the control group (95 percent confidence interval, 36 to 54 percent), as compared with 21 percent (15 to 30 percent) in the UVB-PC group, 18 percent (12 to 26 percent) in the F-PC group, and 17 percent (12 to 26 percent) in the F-AP group (P<0.001 for each treated group vs. the control group) (Fig. 2). The average (±SD) reactivity of these antibodies with all cells in the test panel

was 55 ± 32 percent, with no significant differences among the four groups ($P=0.29$). Antibodies were also found in one or two later samples in 89 percent and 80 percent of the patients, respectively.

Lymphocytotoxic antibodies developed more frequently in control patients than in patients in any of the treated groups, regardless of whether they had had prior transfusions. Lymphocytotoxic antibodies appeared to develop sooner and at higher rates in women who had ever been pregnant. However, the rate of development of these antibodies was significantly lower among patients receiving treated platelets than among controls, regardless of prior pregnancy. In women who had ever been pregnant, the rate of antibody development was 62 percent in the control group, as compared with 33 percent in the UVB-PC group ($P=0.02$), 32 percent in the F-PC group ($P=0.01$), and 34 percent in the F-AP group ($P=0.02$).

In women who had never been pregnant and in men, the rates of alloimmunization in both the treated groups (10 percent) and the control group (33 percent) were significantly less than in women who had ever been pregnant ($P<0.001$). The rate of positivity was 32 percent in the control group, as compared with 15 percent in the UVB-PC group ($P=0.01$), 9 percent in the F-PC group ($P<0.001$), and 7 percent in the F-AP group ($P<0.001$). There were no significant differences among the treated groups.

Antibodies against Platelet Glycoproteins

Overall, antibodies against platelet glycoproteins developed in only 8 percent of patients, and there were no significant differences among the four groups (antibodies developed in 11 percent of control patients, 7 percent of patients in the UVB-PC group, 6 percent of patients in the F-PC group, and 7 percent of patients in the F-AP group).

Alloimmune Platelet Refractoriness

The development of refractoriness to platelet transfusions with antiplatelet antibodies was the primary end point of the study. The Kaplan–Meier estimates for the development of alloimmune platelet refractoriness over the eight weeks of the study (with 95 percent confidence intervals) were 13 percent (8 to 20 percent) for the control group, 5 percent (2 to 11 percent) for the UVB-PC group, 3 percent (1 to 8 percent) for the F-PC group, and 4 percent (2 to 9 percent) for the F-AP group ($P=0.03$, $P=0.004$, and $P=0.01$, respectively, for the comparison with control) (Fig. 3).

Multivariate Analysis

The only factors that lowered the rates of refractoriness to platelet transfusions were treatment assignment (UVB-PC group, $P=0.19$; F-PC group,

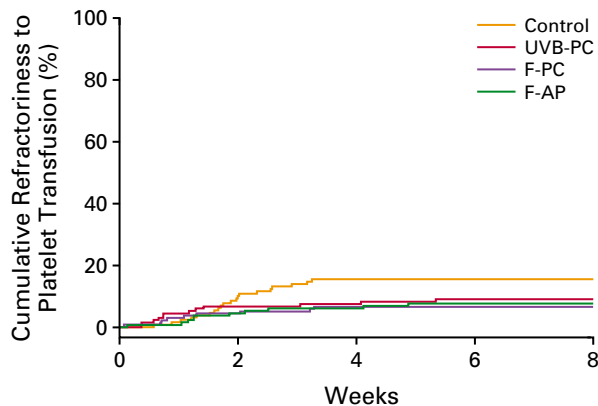


Figure 1. Development of Platelet Refractoriness.

The rates of refractoriness were 16 percent in the control group, 10 percent in the group receiving ultraviolet B-irradiated pooled platelet concentrates from random donors (UVB-PC), 7 percent in the group receiving filtered, pooled platelet concentrates from random donors (F-PC), and 8 percent in the group receiving filtered platelets obtained by apheresis from single random donors (F-AP). There were no significant differences among any of the treated groups (P values ranged between 0.42 and 0.76). The P values for the comparisons between the control group and the treated groups were 0.17 for the UVB-PC group, 0.03 for the F-PC group, and 0.06 for the F-AP group.

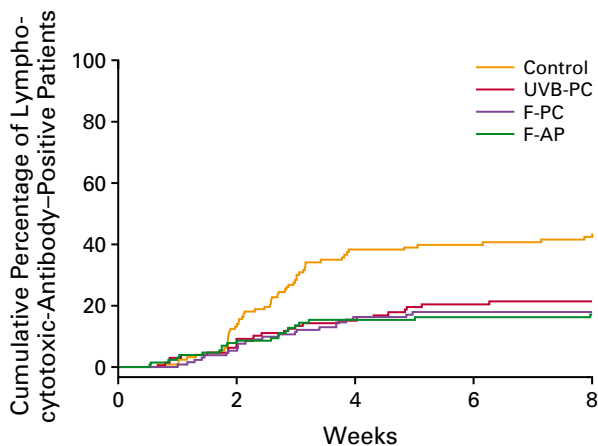


Figure 2. Development of Lymphocytotoxic Antibodies.

Antibodies developed in 45 percent of the patients in the control group, 21 percent of the patients in the group receiving ultraviolet B-irradiated, pooled platelet concentrates from random donors (UVB-PC), 18 percent of the patients in the group receiving filtered, pooled platelet concentrates from random donors (F-PC), and 17 percent of the patients in the group receiving filtered platelets obtained by apheresis from single random donors (F-AP). $P<0.001$ for the comparisons between the control group and each of the treated groups. There were no significant differences among the treated groups (P values ranged from 0.53 to 0.95).

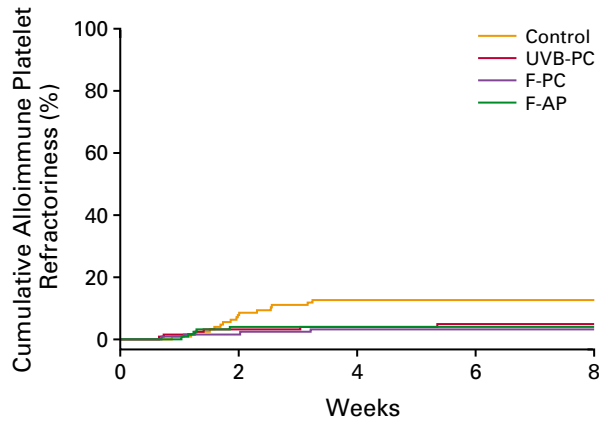


Figure 3. Development of Alloimmune Platelet Refractoriness in Patients Who Had Refractoriness and Also Were Antibody-Positive.

In the control group, 13 percent of the patients had platelet refractoriness and had a positive lymphocytotoxic-antibody test, a positive antibody test against platelet glycoprotein, or both, within two weeks before or after the onset of platelet refractoriness. This percentage is significantly different from the 5 percent of patients in the group receiving ultraviolet B-irradiated, pooled platelet concentrates from random donors (UVB-PC) ($P=0.03$), 3 percent in the group receiving filtered, pooled platelet concentrates from random donors (F-PC) ($P=0.004$), and 4 percent in the group receiving filtered platelets obtained by apheresis from single random donors (F-AP) ($P=0.01$). There were no significant differences among the treated groups.

$P=0.02$; F-AP group, $P=0.05$, for the comparison with control) and absence of prior pregnancy ($P<0.001$). Absence of prior pregnancy ($P<0.001$), transfusions given within two weeks before study entry ($P=0.02$), and treatment assignment ($P<0.001$ as compared with control for each treatment) were associated with a lower incidence of lymphocytotoxic antibodies than in the control group. Absence of prior pregnancy ($P<0.001$) and treatment assignment (UVB-PC group, $P=0.05$; F-PC group, $P=0.01$; F-AP group, $P=0.01$, for the comparison with control) were also associated with lower rates of alloimmune platelet refractoriness.

Similar models were constructed with the transfusion number as the variable. Covariates and P values similar to those in the above analyses were found when calendar time was used.

Clinical Outcomes at Eight Weeks

Seventy-three patients (14 percent) died, and an additional 24 (5 percent) withdrew from the study (Table 3). There were no significant differences among the groups in the frequency and causes of death or withdrawal. Three hundred nineteen patients (60 percent) had a complete remission, and the remission rates were similar in all groups.

DISCUSSION

We evaluated the development of refractoriness to platelet transfusions and of alloantibodies in patients with thrombocytopenia who received transfusions with treated or control platelets. The patients, who were undergoing chemotherapy for acute myeloid leukemia, received platelets obtained by apheresis from single random donors, pooled concentrates from random donors that had been leukocyte-reduced by filtration, or pooled concentrates from random donors whose leukocytes had been inactivated by ultraviolet B irradiation. There were no significant differences among the three treated groups in any study end point. However, all three types of treated platelets significantly reduced the development of lymphocytotoxic antibodies ($P<0.001$) and alloimmune refractoriness to platelet transfusions ($P\leq 0.03$), as compared with untreated, pooled platelet concentrates from random donors. Treated platelets were effective, even though 27 percent of the patients received one or more transfusions of platelets or red cells that did not meet study guidelines, and 88 percent had prior exposure to alloantigens from pregnancy or transfusion. None of the three types of treated platelets affected the development of antibodies against platelet glycoproteins, which, in any case, occurred infrequently, as in other trials.³⁰⁻³² Patients who had no detectable lymphocytotoxic antibodies at entry into the study benefited from transfusions with treated platelets, whereas patients who had such antibodies did not. For this reason, we recommend transfusion with leukocyte-reduced or ultraviolet B-irradiated platelets only for antibody-negative patients.

In patients who received treated platelets, the incidence of lymphocytotoxic antibodies was lower than in controls, whether or not they had ever been pregnant or received a transfusion. Prior studies^{33,34} suggested that leukocyte-reduced platelets helped prevent primary immunization but not an anamnestic response; a recent meta-analysis found that the rates of lymphocytotoxic antibodies in patients who had ever been pregnant or received transfusions were lower in patients given leukocyte-reduced platelets and red cells than in controls who received standard blood components.³⁵ Because of strict entry criteria, only 18 percent of our patients had received transfusions more than two weeks before entering the study. Although our data suggest that patients who had received many transfusions could benefit from transfusions of treated platelets, additional studies will have to address this issue. It was anticipated that F-AP platelets might further reduce the incidence of alloimmunization because of exposure to fewer donors,^{12,13} but there was no evidence of any added benefit as compared with UVB-PC or F-PC platelets.

This study demonstrates that ultraviolet B irradiation and leukocyte reduction give equivalent re-

TABLE 3. STATUS OF PATIENTS DURING INITIAL EIGHT WEEKS.*

STATUS	CONTROL†	UVB-PC	F-PC	F-AP	TOTAL
Deaths	13 (10)	22 (17)	20 (15)	18 (14)	73 (14)
Primary cause of death					
Infection	6 (5)	11 (8)	8 (6)	9 (7)	34 (6)
Hemorrhage	1 (1)	0	0	0	1 (0.2)
Refractory leukemia	3 (2)	5 (4)	5 (4)	5 (4)	18 (3)
Withdrawals	3 (2)	5 (4)	8 (6)	8 (6)	24 (5)
Leukemia in complete remission at 8 wk	85 (65)	74 (57)	85 (62)	75 (57)	319 (60)
G-CSF or GM-CSF administered	12 (9)	6 (5)	6 (4)	2 (2)	26 (5)

*UVB-PC denotes ultraviolet B-irradiated, pooled platelet concentrates from random donors; F-PC filtered, pooled platelet concentrates from random donors; F-AP filtered platelets obtained by apheresis from single random donors; G-CSF granulocyte colony-stimulating factor; and GM-CSF granulocyte-macrophage colony-stimulating factor.

†Controls received unmodified, pooled platelet concentrates from random donors.

sults. A smaller trial showed a trend toward reduction of alloimmunization with ultraviolet B irradiation.³⁶ Since ultraviolet B irradiation is a relatively new method of treating platelets, we attempted to verify the clinical efficacy of ultraviolet B-treated platelets.³⁷⁻⁴⁰ Although the values for the corrected count increment in recipients of ultraviolet B-treated platelets were slightly reduced, the number of platelet transfusions required in these patients was the same as for the other groups, and the clinical outcomes were also similar.

We studied patients with acute myeloid leukemia because they require repeated transfusions of platelets and because alloimmunization can hinder potentially curative therapy. Determining whether our results are applicable to patients who are receiving less intensive chemotherapy or who have a normal immune system, such as patients with aplastic anemia, will require additional studies.^{41,42}

The frequency of refractoriness to platelet transfusions in this study was surprisingly low, perhaps because we excluded patients who were transiently refractory because of causes other than immunization or who were responsive to transfusions of ABO-compatible or fresh platelets.⁴³ In all groups, the incidence of alloantibodies was considerably higher than the incidence of refractoriness to platelet transfusions. For example, in 29 percent of the patients in the control group, lymphocytotoxic antibodies developed without evidence of refractoriness to platelet transfusions; corresponding values were 14 percent in the UVB-PC group, 12 percent in the F-PC group, and 12 percent in the F-AP group. Thus, for some patients, the development of antiplatelet alloantibodies did not necessarily result in refractoriness

to platelet transfusion. Lymphocytotoxic antibodies of restricted specificity may not react against transfused incompatible donor antigens. Moreover, lymphocytotoxic antibodies were often first detected three to four weeks after entry into the study (Fig. 2), when some patients were no longer receiving platelet transfusions. Such patients could not have been evaluated for the effects of the antibody on transfused platelets. Nevertheless, avoidance of lymphocytotoxic antibodies should benefit patients during subsequent chemotherapy, since there is a close relation between these antibodies and refractoriness to platelet transfusions.⁴⁴ Some patients were refractory to platelet transfusions and did not have lymphocytotoxic antibodies. They accounted for 3 percent of the patients in the control group and 4 percent of the patients in each of the treated groups. These patients most likely had adverse clinical factors or drug-related causes of refractoriness.^{45,46}

The clinical outcomes were essentially the same among all four groups of the study. Specifically, overall death rates were similar, and there were no significant differences among these groups in the incidence of death due to hemorrhage, infection, or refractory leukemia. Few patients withdrew from the study, and the overall proportion of patients in complete remission at the end of the eight weeks of observation was 60 percent.

Whether leukocyte reduction or ultraviolet B irradiation should be the primary method of preventing platelet alloimmunization may depend on costs and other considerations. Filtration reduces the risk of infection from viruses harbored by leukocytes, such as cytomegalovirus.⁴⁷ Ultraviolet A irradiation used with photoadditive agents has shown promise in re-

ducing the risk of infection from bacterial and viral contaminants in platelets for transfusion⁴⁸ and may replace gamma irradiation as a method of preventing transfusion-associated graft-versus-host disease.⁴⁹

Whether our results could be improved on by prestorage leukocyte reduction⁵⁰ or a combination of ultraviolet B irradiation and leukocyte reduction remains to be determined.^{51,52} Interestingly, lymphocytotoxic antibodies developed in 19 percent of the 36 patients with no prior alloantigen exposure who received treated transfusions according to all study guidelines. Prevention of alloimmunization in such patients remains a challenge that requires further study.

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APPENDIX

The following persons and institutions participated in the National Heart, Lung, and Blood Institute TRAP study.

Clinical centers — Blood Center of Southeastern Wisconsin: J. McFarland, J. Menitove, and L. Kagen; Johns Hopkins University: H. Braine, T. Kickler, P. Ness, and A. Fuller; Puget Sound Blood Center: S. Slichter, T. Gernsheimer, and D. Townsend-McCall; University of Florida: K.-J. Kao, W. Noyes, R. Weiner, S. Hudson, and A. Waldman-Stone; University of Maryland Cancer Center, University of Maryland School of Medicine: C. Schiffer, E. Lee, and D. Norris; University of Minnesota: J. McCullough, H. Enright, S. Lennon, and M. Clay; University of Wisconsin: R. Woodson, M. Meisch, and P. Noordsij. Coordinating center — University of Washington: K. Davis, M. Mickel, M.J. Gillespie, G. Ng, S. Parker, and S. Corley. Histocompatibility central laboratory — Emory University: G. Rodey. Platelet immunology central laboratory — Blood Center of Southeastern Wisconsin: J. McFarland. Quality control laboratory for platelet and white-cell counting — University of Florida: K.-J. Kao. Chairman of Steering Committee: K. Sell, Emory University. National Heart, Lung, and Blood Institute: G. Nemo, D. Follmann, M. Hernandez, and C. Hollingsworth. Data and Safety Monitoring Committee: H. Klein (chairman), J. Dutcher, J. Frantantoni, M. Kruskal, R. Macklin, R. Strauss, and J. Wittes.

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