

Clinical Toxicity of Cryopreserved Bone Marrow Graft Infusion

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We prospectively evaluated infusion-related toxicities in 82 recipients of autologous bone marrow grafts. The grafts were cryopreserved in 10% dimethylsulfoxide and stored in liquid nitrogen. All grafts were concentrated and buffy-coat cells were collected. Forty-seven grafts were treated *ex vivo* with 4-hydroperoxycyclophosphamide (4-HC) at 100 $\mu\text{g}/\text{mL}$; 26 grafts were further processed using density-gradient separation and treated with 4-HC at 60 $\mu\text{g}/\text{mL}$. Nine buffy-coat concentrates were frozen without drug treatment. Before infusion, patients were medicated with mannitol, hydrocortisone, and diphenhydramine. Grafts were rapidly thawed and immediately infused without further manipulation. During the infusions, 33 (70%) recipients of treated buffy-coat, 5 (56%) recipients of untreated buffy-coat, and 6 (23%) recipients of density-gradient separated grafts experienced varying symptoms including nausea, abdominal cramping, and flushing. Forced vital capacities for 83% of the recipients of treated buffy-coat concentrates decreased after the graft infusion; six of these patients complained of dyspnea and one patient experienced an acute episode of respiratory decompensation. Decreased heart rates were observed in 98% of the recipients of treated buffy-coat cells with asymptomatic bradycardia occurring in 45%. Forty-five patients (96%) in

this group experienced transient hypertension, with 18 (38%) requiring additional medications within 6 hours after the infusion for control of blood pressure. Similar cardiovascular changes were observed in the recipients of untreated buffy-coat concentrates. One recipient of an untreated buffy-coat concentrate had 2° heart block after the graft infusion. Twenty-three (88%) recipients of density-gradient separated grafts had decreased heart rates and 21 (81%) had increased blood pressure. However, the degrees of change were less than those experienced by the recipients of treated buffy-coat cells ($P < .01$). Forced vital capacities were not affected by the infusion of the density-gradient separated grafts. No renal failure or obvious hemolytic episodes occurred for any patient group. Minor to moderate toxicities were associated with cryopreserved graft infusions. Recipients of buffy-coat separated grafts, both treated and untreated, experienced more complications than the recipients of density-gradient separated grafts. These toxicities may relate to the volumes of cryoprotectant and cell lysis products infused, which were less for the more highly purified density-gradient separated grafts.

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AUTOLOGOUS bone marrow transplantation (ABMT) after high-dose cytotoxic therapy has been used as a treatment for acute leukemias, lymphomas, and solid tumors.¹⁻⁶ Current treatment regimens require the cryopreservation and storage of bone marrow grafts for variable periods of time. Published bone marrow cryopreservation techniques generally use dimethylsulfoxide (DMSO) alone,^{2,3,6} or in combination with hydroxyethyl starch⁷ as the cryoprotectant. These cryopreservation techniques adequately preserve hematopoietic progenitor cells but poorly preserve the mature granulocytes and erythrocytes that may be present in the graft. The quantity of mature blood cells remaining in the graft varies with the method of graft concentration. However, the infusions of both buffy-coat and light-density (density-gradient separated) cells have successfully reconstituted the hematologic system of autograft recipients.^{2,8}

Toxicities have been associated with the infusion of bone marrow grafts preserved with DMSO. The degree of reported complications has ranged from mild nausea and vomiting to acute renal failure⁹ and cardiac and pulmonary arrest.^{10,11} At this center we observed oliguric renal failure and second degree heart block after cryopreserved graft infusions. Nevertheless, these cryopreservation procedures do not require graft washing before infusion to remove the cryoprotectant or the products of cell lysis.^{1-7,12}

We prospectively monitored 82 autologous graft recipients to evaluate the occurrence of toxicities associated with the infusion of cryopreserved bone marrow. Although generally mild, we found that most patients experienced transient symptomatic or physiologic changes associated with their graft infusion. The degree and incidence of these changes were less for recipients of density-gradient separated grafts.

MATERIALS AND METHODS

Patient Selection and Transplant Procedures

Recipients of 4-hydroperoxycyclophosphamide (4-HC)-treated grafts. Infusion data were collected on 47 patients who received treated buffy-coat concentrates while undergoing ABMT between March 5, 1987 and October 6, 1988 at The Johns Hopkins Oncology Center, Baltimore, MD. Twenty of the patients were transplanted for the diagnosis of acute nonlymphoblastic leukemia (ANLL) and 17 for the diagnosis of refractory Hodgkin's disease (HD, 10) or non-Hodgkin's lymphoma (NHL, 7). Also transplanted were 6 patients with metastatic breast cancer, 3 patients with pediatric solid tumors, and 1 patient with acute lymphoblastic leukemia. The age range for these patients was 1 to 56 years (mean, 30 years). Twenty-four of the patients were male. Similar data were collected on 26 patients who received density-gradient separated grafts between October 21, 1988 and May 19, 1989. Eight of the patients were transplanted for the diagnosis of ANLL, 15 for NHL, 2 for HD, and 1 for multiple myeloma. The age range for these patients was 10 to 53 years (mean, 35 years), and 16 were male. All patients

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had their bone marrow harvested within 14 days of starting their regimen for transplantation.

Forty-three patients with ANLL, HD, or NHL with bulky disease, and Wilm's tumor (one of the pediatric solid tumors) received busulfan (Bu, 4 mg/kg orally \times 4) and cyclophosphamide (Cy, 50 mg/kg intravenously [IV] \times 4)¹³ for induction. Two patients with drug-resistant lymphoma received Bu (4 mg/kg orally \times 4): one patient then received etoposide (VP-16, 50 mg/kg IV \times 1) and Cy (60 mg/kg IV \times 3), the other received VP-16 (60 mg/kg IV \times 1) and Cy (60 mg/kg IV \times 2). Two patients with neuroblastoma received melphalan (60 mg/m² IV \times 3) and total body irradiation (TBI, to 1,150 rad). The induction regimen for the breast cancer protocol was thiotepa (200 mg/m² IV \times 4) concomitantly with Cy (1.5 gm/m² IV \times 4) followed by an interval of 4 days before marrow infusion. The remaining patients received a regimen consisting of Cy (50 mg/kg IV \times 4) and TBI (to 1,200 rad).¹⁴

Recipients of untreated grafts. Data were collected on nine patients who received untreated buffy-coat concentrates between August 17, 1987 and April 11, 1989. Six of the patients were treated for pediatric solid tumors, two for refractory HD, and one for NHL. The age range for this group was 2 to 38 years (mean, 17 years), and 5 were male. Five of the six pediatric patients were prepared for transplant on a phase I protocol consisting of carboplatin (300 or 450 mg/m²/d \times 2), VP-16 (600 mg/m²/d \times 3), and Cy (60 mg/kg/d \times 2). The remaining pediatric patient was prepared with melphalan and TBI as described above. One patient with HD received Bu and Cy, and the other two patients received Cy followed by TBI at the same doses used for the recipients of treated autografts.^{13,14}

Approval for the therapeutic application of ABMT at this center was obtained from the Joint Committee on Clinical Investigation of the Johns Hopkins Hospital and the Johns Hopkins University, and informed consent was obtained for all patients before bone marrow harvesting.

Bone marrow processing. Forty-seven of the 82 autologous bone marrow grafts were processed as previously described.² Briefly, buffy-coat cells were obtained by centrifugation of the graft. The cells were then incubated with 100 μ g/mL of freshly dissolved 4-HC and 20% autologous plasma at a nucleated cell concentration of 2×10^7 cells/mL for 30 minutes at 37°C. Twenty-six buffy-coat concentrates were further processed (after buffy-coat isolation) by density-gradient separation using the COBE 2991 Cell Washer (COBE Laboratories, Lakewood, CO).¹⁵ These light-density (specific gravity ≤ 1.077 g/mL) cells were incubated with 60 μ g/mL of 4-HC under the same conditions described above. After drug incubation, all grafts were cooled to 4°C, pelleted, and then resuspended for cryopreservation with 10% DMSO (Cryoserv, Research Industries, Salt Lake City, UT) and 5% autologous irradiated (1,500 rad) plasma, and frozen as 50-mL aliquots at 4×10^7 cells/mL in liquid nitrogen. The remaining nine grafts had buffy-coat cells collected and were frozen, but not 4-HC treated. For infusion, aliquots were thawed in a 37°C water bath and infused consecutively without manipulation.

Bone marrow infusion. Patients were prepared for autologous marrow infusions in the following manner: 12 hours before infusion, hydration with 0.45% saline supplemented with sodium bicarbonate was initiated to ensure a urine output of 100 mL/min and a urine pH > 7 . Fifteen minutes before infusion, the patients were medicated IV with 50 mL of 25% mannitol, 250 mg of hydrocortisone, and 50 mg of diphenhydramine (doses were adjusted for pediatric patients). Antiemetics were administered occasionally. Marrows were infused unfiltered through a central IV catheter. Each 50-mL aliquot was infused over 3 to 5 minutes. No macroscopic clumping was noted in any bone marrow aliquot.

Patient monitoring. All patients were monitored by telemetry for 24 hours after infusion. Electrocardiograms were obtained on 35 recipients of buffy-coat isolated grafts and on 23 recipients of density-gradient separated grafts before and immediately after infusion. Additionally, 6 to 12 hours after the graft infusion, 28 of the 35 recipients of buffy-coat cells had another electrocardiogram performed. Pulse and blood pressure were recorded every 15 minutes during infusion, every 30 minutes for 2 hours after infusion, and every hour for the subsequent 4 hours. Pulmonary function was evaluated using bedside spirometry to obtain forced vital capacities before and within 4 hours after infusion. Serum creatinine, potassium, and sodium levels (Hitachi 737; Boehringer Mannheim, Indianapolis, IN) were obtained before and 24 hours after infusion. Total bilirubin, aspartate amino transferase (AST), and alanine amino transferase (ALT) levels were measured (Hitachi 737) within 24 hours before infusion and again within 48 hours after infusion. Packed red blood cell (RBC) volumes (PCV; ELT 800, Ortho Diagnostics Systems, Inc, Westwood, MA) were measured every morning on the day before, the day of, and the day after graft infusion. RBC transfusion histories were obtained for this period. Symptoms occurring during the infusions were recorded by the staff nurse assisting in the reinfusion.

Statistical evaluation. The significance of the changes within patient groups was tested by paired Student's *t* test. Comparisons between two patient groups were tested by nonpaired Student's *t* test. One way analysis of variance was used when all groups were compared. The relationship of the occurrence of symptoms between the groups was tested by chi-square analysis.

RESULTS

The average 4-HC treated buffy-coat graft volume infused was 6.4 ± 1.7 mL/kg (mean \pm SD; range, 1.7 to 11.4) containing 0.7 ± 0.2 g/kg (mean \pm SD; range, 0.2 to 1.3) of DMSO. Infusion rates ranged from 10 to 17 mL/min, with the average infusion procedure lasting 38 minutes. When necessary, short (5-minute) breaks were taken between aliquot infusions if the patient experienced somatic complaints. The unpurged grafts contained similar volumes (5.9 ± 2.2 mL/kg, $P = .43$) and contained similar amounts of DMSO (0.6 ± 0.2 g/kg, $P = .53$) when compared with the 4-HC-treated buffy-coat grafts. Recipients of density-gradient separated grafts received less volume (2.7 ± 1.2 mL/kg, mean \pm SD; range, 1.5 to 5.9) and less DMSO (0.2 ± 0.1 g/kg, mean \pm SD; range 0.1 to 0.5) than the buffy-coat isolated graft recipients (volume and DMSO: $P < .01$). The average duration of these infusions was 25 minutes.

The symptoms experienced by these patients are shown in Table 1. Nausea, frequently with vomiting, was the most common complaint occurring during graft infusion. Recipients of 4-HC-treated buffy-coat cells complained of abdominal cramping, which could be relieved by a decrease in the infusion rate. We observed flushing of the skin, most often involving the head and neck, in these patients. Six (13%) became dyspneic, with one patient experiencing an acute episode of respiratory decompensation accompanied by tachycardia and severe, but transient, hypotension. Sixteen (34%) recipients of treated buffy-coat cells experienced more than one symptom. Headache was the only symptom other than nausea associated with the infusion of density-gradient separated grafts. The incidence of symptoms was different between these groups (70% *v* 23%; $P < .01$). Four of the nine

Table 1. Symptoms Associated With Autologous Bone Marrow Infusions

	Graft Processing		
	Buffy Coat*		Density Gradient
	4-HC Treated (n = 47)	Untreated (n = 9)	4-HC Treated (n = 26)
Nausea and vomiting	19	4	5
Flushing	13	0	0
Abdominal cramping	10	1	0
Dyspnea and chest tightness	6	2	0
Diarrhea	4	0	0
Headache	0	0	1

*Multiple symptoms were experienced by 16 4-HC-treated and 2 untreated graft recipients, while 14 4-HC-treated and 4 untreated graft recipients tolerated the bone marrow infusion without complaint.

recipients of untreated buffy-coat cells complained of nausea. The incidence of symptoms in this patient group did not differ from the treated buffy-coat graft recipients ($P = .45$), suggesting the symptoms were not related to the infusion of any 4-HC remaining in the graft after the incubation.

Cardiovascular changes associated with the graft infusions are shown in Table 2. Heart rates decreased in 46 of 47 (98%) recipients of 4-HC-treated buffy-coat cells with asymptomatic bradycardia (rate ≤ 60 beats per minute) occurring in 21 (45%). The average time from the start of the procedure to the maximum change in heart rate was 2.1 ± 1.4 hours (mean \pm SD; range, 0.1 to 6.5 hours). Over a similar time course, 45 (96%) patients in this group developed an increase in blood pressure. Decreases in heart rates were observed in 22 (85%) recipients of light-density cells, with only one patient having asymptomatic bradycardia. Twenty-one (81%) of these patients had increased blood pressures. The degrees of change in heart rate and blood pressure were greater for the recipients of treated buffy-coat cells ($P < .01$). All recipients of untreated buffy-coat grafts had decreased heart rates (bradycardia in two patients) and increased blood pressures. These changes were similar in degree to those experienced by the recipients of treated buffy-coat grafts. Heart rates and blood pressures for all patients returned to baseline levels 12 to 24 hours after graft infusion. Eighteen recipients (38%) of treated buffy-coat cells, eight (89%) recipients of untreated buffy-coat cells,

and four (15%) recipients of light-density cells required additional diuresis for control of their hypertension.

Electrocardiograms before and after graft infusions were obtained for 35 recipients of treated buffy-coat isolated grafts. We found that voltage decreased in either lead I or lead II in 27 (77%) of these patients (mean decrease in lead I: -1.9 ± 1.4 mm [\pm SD]; lead II: -1.4 ± 0.8 mm). The voltage returned to baseline 6 to 12 hours after infusion (data available for 28 patients). Eleven of 23 (48%) recipients of density-gradient separated grafts had a voltage decrease in either lead I or II (lead I: -1.2 ± 0.4 mm [mean \pm SD]; lead II -1.2 ± 0.4 mm). Occasional ventricular extrasystoles were noted by telemetry in the recipients of buffy-coat cells only. Transient second degree heart block occurred in one patient who received an untreated buffy-coat concentrate.

The forced vital capacity (FVC) for 30 of 36 (83%) recipients of treated buffy-coat isolated grafts (measurements were not obtained for the younger pediatric patients) decreased after the graft infusion ($P = .0001$). The average decrease was $-15.4\% \pm 15.6\%$ (mean \pm SD; range, -3% to 83%). Measurements were available for 4 of the 6 patients who complained of dyspnea; three had decreased vital capacities (-51% average; range, -29% to 83%). Recipients of light-density cells did not have a significant change in FVC ($P = .79$).

Serum chemistry results are shown in Table 3. After the infusion of treated buffy-coat cells, mild increases in ALT, AST, and total bilirubin were observed. Similar increases were not found in the recipients of light-density cells. The groups did not differ in their preinfusion laboratory values (ALT, $P = .82$; AST, $P = .47$; bilirubin, $P = .84$). Recipients of untreated buffy-coat cells had ALT and AST levels decrease after their graft infusions. However, the preinfusion values for these patients were greater than those of the treated buffy-coat recipients (ALT, $P < .01$; AST, $P < .01$). All patients had increased sodium and decreased potassium levels after the graft infusions. The alkaline diuresis appeared to protect renal function as shown by the stable creatinine levels.

To evaluate if hemolysis was associated with the infusion procedure, comparisons were made between the peripheral blood packed cell volumes (PCVs) measured the day before transplant (D-1), the day of transplant (D0), and the day

Table 2. Cardiovascular Changes Associated With Autologous Bone Marrow Infusions

	Graft Processing				
	Buffy Coat			Density Gradient	
	4-HC Treated*	Untreated	P Value†	4-HC Treated	P Value‡
Decrease in heart rate	32 \pm 12	32 \pm 11	.91	16 \pm 9	<.01
Lowest heart rate	63 \pm 12	70 \pm 12	.09	79 \pm 16	<.01
Increase in systolic pressure	26 \pm 13	31 \pm 20	.37	10 \pm 7	<.01
Increase in diastolic pressure	23 \pm 10	24 \pm 13	.71	9 \pm 6	<.01
Maximum systolic pressure	146 \pm 19	136 \pm 17	.17	131 \pm 15	<.01
Maximum diastolic pressure	99 \pm 13	91 \pm 12	.16	86 \pm 10	<.01

*Heart rates are measured in beats per minute; blood pressures measured in millimeters of mercury. Shown are mean \pm SD for those patients who showed decreased heart rate or increased blood pressure (see text for numbers of patients).

†Significance of comparison between recipients of 4-HC-treated and untreated buffy-coat concentrated graft infusions.

‡Significance of comparison between recipients of 4-HC-treated buffy-coat concentrated and 4-HC-treated density-gradient separated graft infusions.

Table 3. Serum Chemistry Values Before and After Autologous Bone Marrow Infusions

Test	Buffy-Coat Recipients, 4-HC Treated (n = 47)			Buffy-Coat RRipients, Untreated (n = 9)			Density-Gradient Recipients (n = 27)		
	Before	After	P Value*	Before	After	P Value	Before	After	P Value
Sodium (mEq/L)	137.8 ± 3.2†	139.2 ± 2.4	<.01	136.0 ± 3.4	138.4 ± 3.9	<.01	137.4 ± 2.5	138.8 ± 2.5	<.01
Potassium (mEq/L)	3.7 ± 0.4	3.5 ± 0.5	<.01	3.6 ± 0.4	3.3 ± 0.5	.08	3.9 ± 0.4	3.6 ± 0.5	<.01
Creatinine (mg/dL)	0.7 ± 0.3	0.7 ± 0.3	.61	0.8 ± 0.1	0.8 ± 0.2	.17	0.8 ± 0.2	0.8 ± 0.2	.75
Total bilirubin (mg/dL)	0.5 ± 0.2	0.7 ± 0.3	<.01	0.5 ± 0.3	0.7 ± 0.4	.09	0.5 ± 0.3	0.4 ± 0.3	.06
AST (IU/L)	31.4 ± 15.1	52.0 ± 32.9	<.01	58.4 ± 38.7	45.4 ± 28.1	.05	35.3 ± 22.1	33.0 ± 37.9	.67
ALT (IU/L)	37.5 ± 30.1	51.1 ± 39.5	.02	95.1 ± 84.9	65.6 ± 57.2	.02	40.1 ± 34.5	48.1 ± 70.0	.38

*Significance of comparisons between levels before and after infusion for each particular group.

†Shown are mean ± SD.

after transplant (D+1). During this period 34 recipients of treated buffy-coat cells were not transfused RBCs. The decrease in PCVs from D-1 to D0 and from D0 to D+1 was $-1.2\% \pm 2.0\%$ and $-0.9\% \pm 1.8\%$, respectively (mean ± SD, $P = .4$). RBC transfusions were administered to four patients on the day before transplant. For this group of patients the decrease in PCVs from D0 to D+1 was $-0.3\% \pm 2.0\%$ (mean ± SD), which did not differ from the untransfused patients ($P = .55$). Nine patients were transfused on the day of their transplant because of low morning PCVs and could not be evaluated for infusion-related hemolysis. The decrease in PCVs for 21 untransfused recipients of density-gradient separated grafts from D-1 to D0 and from D0 to D+1 was $-0.4\% \pm 1.6\%$ and $-2.0\% \pm 2.0\%$, respectively (mean ± SD, $P = .02$). Four patients in this group were transfused on D-1. The change in PCVs from D0 to D+1 was $-2.6\% \pm 2.3\%$ (mean ± SD), which did not differ from the untransfused patients ($P = .62$). Six patients who received untreated buffy-coat cells were not transfused. The change in their PCVs from D-1 to D0 and from D0 to D+1 was $-0.3\% \pm 1.8\%$ and $-1.4\% \pm 1.5\%$, respectively (mean ± SD, $P = .38$). There were no differences between the groups in regard to PCV changes from D0 to D+1 ($P = .10$). No severe hemolysis occurred.

Within 6 hours after the graft infusions, additional diuretics were administered to 17 (36%) treated buffy-coat cell, 8 (89%) untreated buffy-coat cell, and 4 (15%) light-density cell recipients, and nitropaste to 1 treated buffy-coat cell recipient for control of hypertension. Three recipients of treated buffy-coat cells received additional diphenhydramine, and two received epinephrine.

DISCUSSION

ABMT is associated with a variety of complications, mostly resulting from the profound bone marrow aplasia or the intensive cytotoxic preparative regimen. Patients usually receive several days of chemotherapy before marrow reinfusion requiring techniques to preserve the function of the previously harvested bone marrow graft. Most centers cryopreserve the grafts using DMSO alone or in combination with other cryoprotectants. The processing and infusion of these products have become routine, but case reports of complications associated with graft infusions have been published.⁹⁻¹¹ In this study we found that the infusion of cryopreserved grafts caused minor to moderate toxicities for

most recipients. Many of our patients became ill during the infusion, complaining of nausea, abdominal cramping, and chest tightness. One patient experienced an anaphylactic reaction. Another developed 2^o heart block. Virtually all patients receiving treated buffy-coat concentrated autografts lost pulmonary capacity and electrocardiographic voltage, and developed hypertension and bradycardia. Mild elevations in serum hepatic enzyme levels occurred for this group. These complications were transient, resolving within several hours to a few days after completion of the infusion. These complications also appeared dose-related, with patients who received the more purified density-gradient separated grafts having a lower incidence or degree of toxicity.

Cryopreserved bone marrow is a complex mixture. Possible causes for these complications include the infusion of large graft volumes, damaged cells, products of cell lysis, or DMSO. DMSO has been administered IV to treat spinal cord injuries,¹⁶ arthritis,¹⁷ and cerebral edema.¹⁸ The toxicity of DMSO infusion in humans has not been adequately evaluated, and the incidence of side effects is not well-described.¹⁹ However, adverse effects (intravascular hemolysis, hyperosmolality, and increased serum transaminase levels) have been reported with the IV administration of DMSO.^{17,18,20}

All of the somatic complaints experienced by our patients may have derived from the use of DMSO as a cryoprotectant. DMSO induces histamine release,²¹ and the occurrence of flushing and pulmonary and abdominal complaints during infusion can be attributed to this agent. Our use of diphenhydramine premedication may have ameliorated more severe toxicities resulting from histamine release. Also, in murine models, DMSO has been shown to have a vagaltonic effect on the heart resulting in bradycardia.^{22,23} The occurrence of hypertension may result from cardiovascular or smooth muscle effects of DMSO, or a combination of DMSO with hydration and mannitol administration. Both hypertension and hypotension have been reported after IV administration of DMSO in animal models.^{24,25} Hypotension also has been observed during the infusion of DMSO cryopreserved grafts in patients not prepared with antihistamines,^{8,11} and may have resulted from histamine release. In animal studies, histologic lesions of the liver²⁶ and elevated serum transaminase levels²⁷ have occurred after DMSO infusion.

The symptoms experienced by our patients were temporally related to the bone marrow infusion, and therefore

probably did not result from previous chemotherapy. The mild increase for most recipients of treated buffy-coat cells in serum transaminases also cannot be attributed to the cytoreductive regimen because similar changes were not found in the recipients of light-density cells (or in 50 matched allogeneic graft recipients, data not shown). Although there was a decrease after autograft reinfusion in serum transaminases in the recipients of the untreated grafts, this may have reflected the high preinfusion values for this small group of patients. The minimal decrease in serum potassium and increase in sodium may have resulted from the hydration and diuresis of the patients. We also do not believe these toxicities were related to any 4-HC present in the grafts. Similar complications were observed for patients who received untreated buffy-coat cells. The amount of toxicity observed was significantly lower for those patients who received density-gradient separated, 4-HC-treated grafts.

Although we can attribute most of our observations to the infusion of DMSO, we cannot exclude concomitant effects from the infusion of cell lysis products or the administration of premedications. Density-gradient separated grafts contain fewer mature blood cells.^{8,15} Therefore, this separation technique reduces the total quantity of cells frozen and results in a decrease in the infusion of both DMSO and poorly preserved erythrocytes and granulocytes. However, in another clinical series infusing lower quantities of DMSO but using no premedications, the reported incidence of somatic complaints was 50%.⁸ This was greater than the 23% rate experienced by recipients of density-gradient separated grafts in this series possibly because of the antihistamine given to our patients, suggesting that DMSO contributes at least partly to the observed toxicities. Also, the degree of hypertension in our recipients of the more purified grafts was less than for the buffy-coat concentrate recipients, suggesting that mannitol administration could not totally account for the

cardiovascular changes observed. The slow development of bradycardia and hypertension suggest a pharmacologic, not a volume-related cause for these changes.

Regardless, processing of grafts to reduce the volumes of both DMSO and cell lysis products appears to decrease infusion-related complications. One approach is to more completely isolate the bone marrow progenitor cells, as with density-gradient separation techniques. Another approach would be to wash the grafts before infusion.²⁸ However, the removal of DMSO or lysed cells by washing may reduce the number of hematopoietic progenitor cells infused.²⁹ Because *ex vivo* tumor-cell purging may also decrease the engraftment potential of the autologous bone marrow,³⁰ additional post-thaw manipulation may increase the risk of engraftment failure and must be attempted with caution.

One patient in our series experienced life-threatening complications during the infusion of cryopreserved buffy-coat cells. Moreover, we cannot exclude the possibility that acute toxicities during the autologous graft infusion may affect the patient's ability to tolerate the subsequent transplant course. The implementation of density-gradient separation techniques to process bone marrow grafts before cryopreservation appears to reduce infusion-related toxicities. As the use of ABMT is expanded, the development of other cryopreservation and hematopoietic progenitor enrichment techniques may decrease the risk of infusion-related toxicity.

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