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High White Blood Cell Concentration in the Peripheral Blood Stem Cell Product Can Induce Seizures during Infusion of Autologous Peripheral Blood Stem Cells

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Seizures as a complication of the infusion of autologous peripheral blood stem cells (PBSC) are rare. Seizures during infusion of autologous PBSC in 3 of our patients prompted us to review our cell therapy and cytapheresis protocols and procedures. We retrospectively analyzed 159 adult patients collected between January 2006 and July 2009. Patients were collected on either the COBE Spectra (Caridian BCT, Lakewood, CO) cell separator (n = 85) or Fresenius AS (Fresenius Kabi AG, Bad Homburg, Germany) 104 cell separator (n = 74) and mobilized with granulocyte-colony stimulating factor (G-CSF) alone (n = 47), G-CSF and Plerixafor (n = 36), or G-CSF and chemotherapy (n = 76). Patient characteristics (including age, weight, number of collections, volume processed, disease type, and mobilization strategy) did not differ significantly between the COBE and Fresenius cohorts, and adverse effects from infusion were similar except for 3 of 159 patients who experienced seizures upon infusion of PBSC; all 3 were collected on the COBE and had PBSC product white blood cell (WBC) counts of $590 \times 10^3/\mu L$ or above. We prospectively correlated WBC counts mid-collection, with final WBC counts to identify products with high WBC concentration during cytapheresis. Fifty-one patients had 66 cytapheresis procedures using the COBE, with WBC counts midway and at the end of collection of $287 \times 10^3 \pm 150/\mu L$ and $273 \times 10^3 \pm 144/\mu L$, respectively. Mid-WBC therefore correlated with WBC at the end of the collection. Finally, we prospectively collected mid-WBC from 65 patients who underwent 80 PBSC collections between June 2009 and January 2010 to identify products with midcollection WBC concentration $>450 \times 10^3/\mu L$. In those cases, additional autologous plasma was collected at the time of collection to dilute the final product before cryopreservation. Patients who received diluted products experienced no delays in engraftment and no additional seizure episodes occurred.

INTRODUCTION

Autologous hematopoietic stem cell transplantation is a treatment strategy for restoring normal hematopoietic function after myeloablative chemotherapy in patients with hematologic malignancies [1-3]. The procedure includes induction or reinduction chemotherapy, mobilization and collection of CD34+ cells through apheresis, administration of high-dose chemotherapy, and finally, infusion of the cells back into the patient [4,5]. Cryopreservation of autologous stem cells is performed after collection of peripheral blood stem cells (PBSC). Cryopreservation solutions containing dimethyl sulfoxide (DMSO) with or without hydroxyethyl starch are most commonly used to protect cells from damage during the freezing process [6-8]. Complications during infusion of thawed autologous stem cells are mostly related to side effects of DMSO and volume infused at the time of transplantation [9-13]. These include nausea, vomiting, hyper- and hypotension, cardiac arrhythmias, and gastrointestinal side effects, among others. Rarely, tonic-clonic seizures have been described after infusion of cryopreserved PBSC [10,14]. The etiology of seizures in these patients is poorly understood.

Tonic-clonic seizures after infusion of PBSC product occurred in 3 patients between 2006 and 2009. We performed a retrospective review of our cell therapy and cytapheresis protocols and procedures to investigate these adverse events. This retrospective analysis identified high white blood cell (WBC) concentrations in the PBSC product before freezing as a causative factor associated with these adverse events. We

subsequently developed a process to dilute products with autologous plasma based on WBC count during collection of PBSC. We then evaluated infusion-related adverse events and engraftment in subsequent patients including those that required dilution of PBSC products because of high WBC concentration before product freezing. Finally, the use of both Fresenius and COBE apheresis instruments for collection of PBSC allowed us to compare product characteristics using these 2 collection devices.

METHODS

We retrospectively selected sequential patients who underwent PBSC collection at the Texas Transplant Institute between January 2006 and July 2009 in anticipation of autologous transplant. This was the time interval when seizure-related adverse events were identified during infusion of PBSC. Patients that developed seizures during PBSC infusion were included in this analysis. Patients underwent mobilization with granulocyte-colony stimulating factor (G-CSF), either with or without plerixafor, or after G-CSF and chemotherapy. Patients underwent apheresis with either a COBE Spectra (Caridian BCT, Lakewood, CO) or Fresenius AS104 (Fresenius Kabi AG, Bad Homburg, Germany) according to standard procedures. Patients were randomly collected on either device by our cytapheresis department according to machine availability. Apheresis products were cryopreserved in 10% DMSO using controlled rate freezing. PBSC were then stored in liquid nitrogen until the day of transplantation when they were thawed at the bedside and infused. Patients received premedication 15 to 30 minutes before PBSC infusion consisting of methylprednisolone 20 mg and diphenhydramine 25 mg both given orally. Data from patients who failed to mobilize were not included in this analysis. Clinical data from 3 patients who developed seizures during infusion of PBSC included imaging studies of the brain, electroencephalogram, cerebral spinal fluid analysis, electrolyte analysis, arterial blood gases, and evaluation of other medications and past history to exclude conditions predisposing to seizures (including prior history of seizures, head trauma, and leptomeningeal carcinomatosis).

We identified high WBC concentration in PBSC product after collection as the only laboratory parameter associated with seizures. We then evaluated the possibility of predicting WBC concentration at the end of the collection by measuring WBC in the collection bag at the halfway volume of cytapheresis. All patients underwent an 18- to 20-liter blood volume collection and mid-WBC counts were obtained at 9 to 10 liters into their PBSC collection. This information allowed us to collect autologous plasma during PBSC collection in patients with high WBC concentration and dilute these products before freezing.

Finally, in a subsequent cohort, we prospectively collected data from patients who underwent PBSC collections in anticipation of an autologous or allogeneic stem cell transplant between June 2009 and January 2010. WBC counts were obtained halfway through their PBSC collections. Products with WBC counts $>450 \times 10^3/\mu L$ were identified, additional plasma was collected, and PBSC was diluted before cryopreservation. Infusion-related adverse events and engraftment were then evaluated in these patients. All data was statistically analyzed, and all *P* values were calculated using a 2-tailed, heteroscedastic Student *t* test; statistical significance was defined as $P < .05$.

RESULTS

One hundred fifty-nine patients were selected for the retrospective review of PBSC infusion-related adverse events. Patients were mobilized with G-CSF alone (n = 47), G-CSF + plerixafor (n = 36), or G-CSF + chemotherapy (n = 76). Patients were collected using either the COBE Spectra (n = 85) or the Fresenius AS104 (n = 74). Patients underwent transplantation for multiple myeloma (n = 89), non-Hodgkin lymphoma (n = 42), Hodgkin disease (n = 20), testicular cancer (n = 5), neuroblastoma (n = 1), acute lymphocytic leukemia (n = 1), or acute promyelocytic leukemia (n = 1) (Table 1).

Parameters measured during collection (Table 2) included age, weight at collection, number of collections per patient, patient's blood volume processed during each collection, volume of product at the end of the collection, and total time per collection. Volume of product at the end of collection was larger in the Fresenius group than the COBE group (402 ± 72.0 mL versus 241 ± 56.8 mL, respectively, $P < .0001$) and required a longer time of collection (5.20 ± 0.88 hours versus 4.25 ± 0.64 hours, respectively, $P < .0001$).

Differences in PBSC product are described in Table 3. Patients collected on the COBE separator

Table 1. Patient Characteristics

Disease	
Multiple myeloma	89
Non-Hodgkin lymphoma	42
Hodgkin's disease	20
Testicular carcinoma	5
Neuroblastoma	1
Acute lymphocytic leukemia	1
Acute promyelocytic leukemia	1
Mobilization strategy	
G-CSF alone	47
G-CSF + chemotherapy	76
G-CSF + plerixafor	36
Mobilization device	
COBE spectra	85
Fresenius ASC04	74
Age at collection	
	56 ± 14

Table 2. PBSC Collection

	COBE (±SD)	Fresenius (±SD)
Number of products	165	180
Number of patients	85	74
Age at collection	56 ± 14	56 ± 15
Weight at collection (kg)	82.7 ± 17.9	79.5 ± 15.9
Collections/patient	2 ± 1	2 ± 1
Blood volume processed at end of collection (L)	18.0 ± 2.4	18.1 ± 2.7
*Product volume (mL)	241 ± 56.8	402 ± 72.0
*Collection time (hours)	4.25 ± 0.65	5.20 ± 0.88

*$P < .05$.

had higher WBC, higher total nucleated count, and lower cell viability than patients collected with the Fresenius. Patients collected on the COBE had a higher number of total nucleated count but a lower percentage of mononuclear cells than patients collected on the Fresenius.

Absolute $CD34^+$ cells in the PBSC product, $CD34^+$ cells/kg, and total $CD34^+$ cells/kg infused at transplantation were not significantly different (Table 4). $CD34^+$ yields were calculated as the ratio of $CD34^+$ cells/μL of the PBSC product to the patient's peripheral blood $CD34^+$ cells/μL taken on the day of collection and before start of cytapheresis. $CD34^+$ cell yields (Figure 1A and B) were significantly higher on the COBE than the Fresenius. $CD34^+$ cell yields were higher across all disease types and mobilization strategies (data not shown).

Seizures are included among the category of serious adverse events during infusion of PBSC at our center. PBSC infusion-related serious adverse events undergo internal review through our quality management plan. No serious adverse events occurred during PBSC infusion except that 3 of 159 patients experienced tonic-clonic seizures during infusion of PBSC; all 3 were collected on the COBE and all 3 had product WBC of 590×10^3/μL or above (compared with a median of 163.3×10^3/μL for all other products) (Figure 2). All 3 patients with infusion-related seizures had multiple myeloma with small paraprotein levels at the time of PBSC collection (Table 5). Evaluation of patients developing seizures did not identify

Table 3. PBSC Product

	COBE (±SD)	Fresenius (±SD)
Peripheral WBC ($\times 10^3$/μL)	36.6 ± 18.9	33.3 ± 24.5
*Product WBC ($\times 10^3$/μL)	163.3 ± 136.0	55.8 ± 29.3
HCT (%)	3.1 ± 3.3	3.2 ± 1.7
*TNC (10^{10})	3.51 ± 1.86	1.95 ± 1.19
*MNC (10^{10})	2.36 ± 1.19	1.60 ± 0.094
*MNC (%)	75.0 ± 23.3	85.0 ± 10.8
Volume before freezing (mL)	100 ± 54	100 ± 32
*Postfreeze viability (%)	70 ± 14	75 ± 10
DMSO (mL)	10 ± 5.4	10 ± 3.2

TNC indicates total nucleated count; MNC, mononuclear cells.
*$P < .05$.

Table 4. CD34 Analysis

|	COBE (±SD)	Fresenius (±SD)
Peripheral $CD34^+$/μL	24.0 ± 43.8	25.3 ± 79.1
*Product $CD34^+$/μL	726.7 ± 1325.9	264.63 ± 781.0
*Product/peripheral $CD34^+$	24.8 ± 10.9	10.9 ± 6.6
Absolute product $CD34^+$ cells (10^8)	1.77 ± 3.52	1.14 ± 3.35
Product $CD34^+$/kg (10^6)	2.02 ± 4.67	1.39 ± 4.15
Total $CD34^+$ cells infused (10^6/kg)	3.85 ± 3.20	3.85 ± 2.24

*$P < .05$.

abnormalities in imaging studies, cerebrospinal fluid analysis, electrolytes, or past history, which might explain the etiology of seizures. Electroencephalogram performed within 24 hours of seizures only demonstrated slow waves. Seizures lasted 5 to 10 minutes and were not associated with significant changes in blood pressure, desaturation, or documented cardiac arrhythmias. All patients fully recovered after a postictal state with no further neurologic sequel.

We then correlated WBC counts midway and at the end of PBSC collections. Fourteen patients had a total of 15 apheresis procedures using the Fresenius cell separator. Mid- and post-WBC concentrations were $64 \pm 23 \times 10^3$/μL and $69 \pm 20 \times 10^3$/μL, respectively. Fifty-one patients had 66 cytapheresis procedures using COBE, with WBC counts obtained midway and at the end of collection of $287 \pm 150 \times 10^3$/μL and $273 \pm 144 \times 10^3$/μL, respectively.
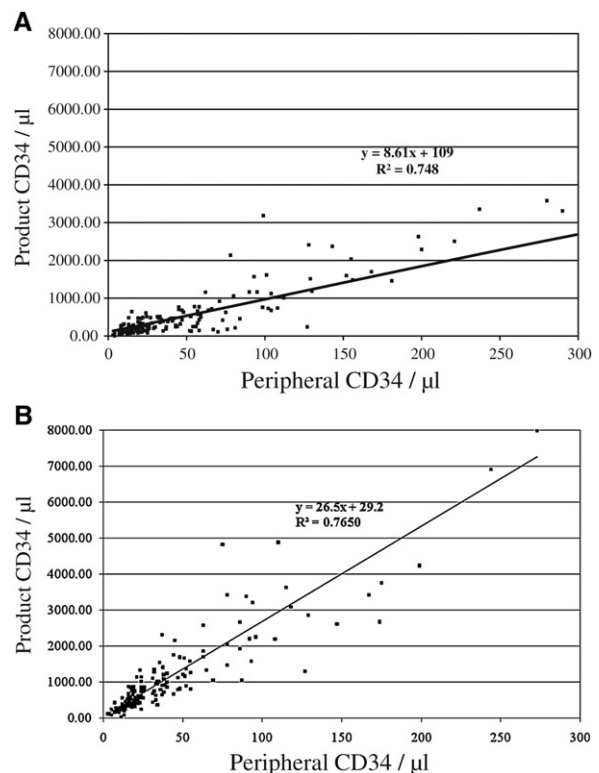
Figure 1. (A) Fresenius peripheral CD34 versus product CD34. (B) COBE peripheral CD34 versus product CD34.
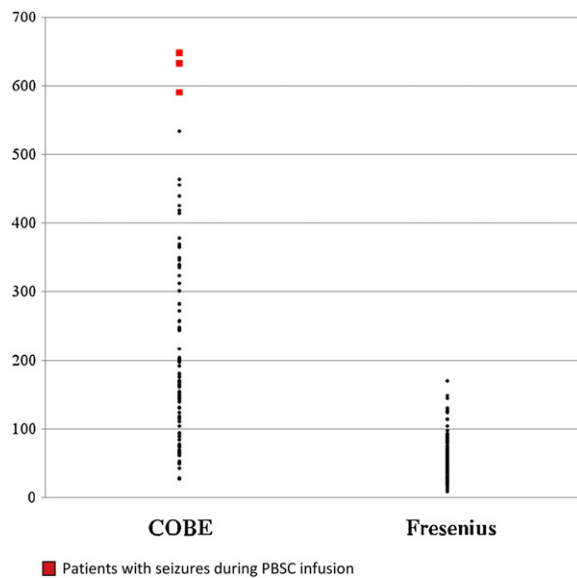
Figure 2. COBE versus Fresenius product WBC concetration ($\times 10^3$/ μL).

Mid-WBC accurately correlated with WBC at the end of the collection in both the COBE and Fresenius cohorts ($r^2 = .940$ and $r^2 = .904$, respectively) (Figure 3A and B).

Using this information, we prospectively collected mid-WBC in order to identify high WBC in 65 patients who underwent 80 hematopoietic stem cell transplant collections in anticipation of an autologous (n = 58) or allogeneic (n = 7) stem cell transplantation between June 2009 and January 2010. Collections for these patients were performed using the COBE Spectra (n = 65) or the Fresenius AS104 (n = 15). WBC concentrations were obtained halfway through their collections. Products with mid-collection WBC concentration greater than 450×10^3/μL were identified, and additional autologous plasma was collected at the time of collection so that the final product could be diluted before cryopreservation. We collected enough autologous plasma to dilute PBSC products to a concentration $<450 \times 10^3$/μL. Patient weight, volume of PBSC product, and number of CD34 cells/kg infused during transplantation did not differ significantly between the patients who received diluted PBSC product and those who did not (Table 6). Importantly, there was also no difference observed in days to engraftment between the 2 groups, in either ANC engraftment (12 ± 1.3 days versus 11.5 ± 1.3 days, dilution versus nondilution, P = .760) or in platelet engraftment (18 ± 3.7 days versus 16 ± 2.7 days, dilution

versus nondilution, P = .561). Similarly, the precryopreservation cell viability and product mononuclear cells percentage did not differ significantly between the 2 cohorts; the only significant difference was found in postthaw viability, where the undiluted products had a viability nearly 10% greater than the diluted products (63 ± 13% versus 54 ± 13%, respectively, P = .008). No serious adverse infusion effects were observed in either group.

DISCUSSION

Infusion of thawed PBSC can be associated with side effects that are usually mild and reversible [9-13]. These include nausea, vomiting, abdominal cramping, diarrhea, shortness of breath, chest pressure, changes in blood pressure, and cardiac arrhythmias, among others. Some of these side effects are thought to be related to DMSO used as a cryopreservant, red cells in the product, volume of product infused, and vagal responses. Epileptogenic seizures associated with infusion of thawed PBSC are rare and only described in case reports [10,14]. The etiology of these seizures is therefore poorly understood. In our retrospective review, an elevated WBC concentration before PBSC freezing correlated with seizures during PBSC infusion.

Several studies have described the effect of cell concentration on viability and the side effects of infused cryopreserved autologous PBSC [15-17]. Rowley et al. [18] established that median concentration 370 ± 190 × 10^3 (range: 40-800) cells/μL were not associated with loss of engraftment or unexpected infusion-related toxicities. Infusion-related seizures in our cohort occurred only in patients with high WBC concentrations in PBSC products and were only seen in patients collected on the COBE Spectra. Other clinical factors associated with seizures were excluded. It is unclear how a high WBC concentration could lead to PBSC infusion-related seizures in our patients. Increased thickness and occasional clumping of PBSC products with high WBC concentration were occasionally seen at the time of thawing and before infusion. It is therefore possible that clumping of the PBSC product contributed to seizure episodes in our patients.

We first identified PBSC products with high WBC concentration by measuring WBC during apheresis. Our data showed a strong correlation between

Table 5. Characteristics of Patients with PBSC Infusion Seizures

	Age (years)	Weight (kg)	Diagnosis	M-Spike (g/dL)	Mobilization	Volume of PBSC Frozen (mL)
Patient #1	60	80	Multiple myeloma	0.8	G-CSF	200
Patient #2	53	68	Multiple myeloma	0.5	G-CSF	160
Patient #3	45	63	Mutliple myeloma	1.1	G-CSF	200
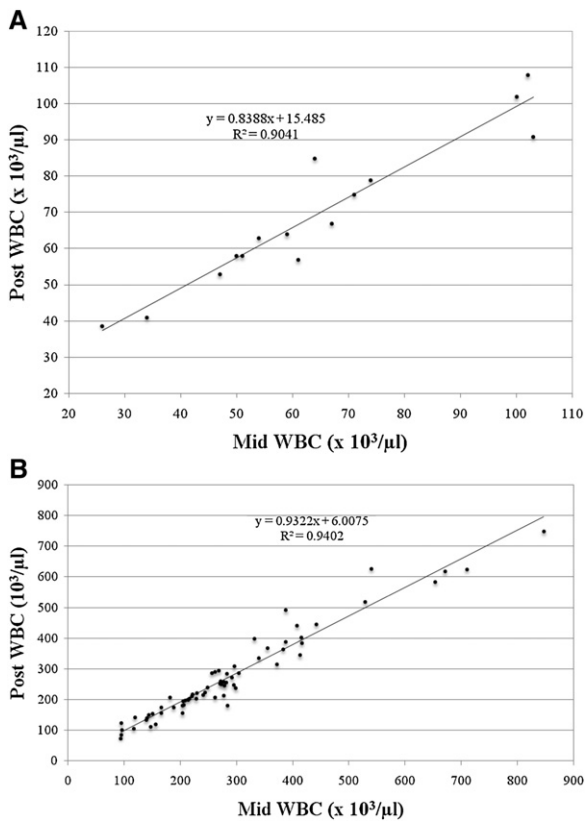
Figure 3. (A) Correlation of WBC at collection midpoint with WBC in PBSC product—Fresenius. (B) Correlation of WBC at collection midpoint with WBC in PBSC product—COBE.

mid-WBC and final WBC counts, making it feasible to accurately predict products with high WBC that can potentially be associated with seizures. Identification of PBSC collection with high WBC concentration during apheresis allowed us to collect additional autologous plasma to dilute these products before cryopreservation. Identification of PBSC products with high WBC concentration can obviate the unnecessary dilution of all products and therefore decrease the cost associated with cryopreservation and storage of PBSC. We used WBC concentrations

$>450 \times 10^3/\mu L$ as the cutoff to use autologous plasma for dilution of PBSC products. This concentration is both within the average concentration used to cryopreserve PBSC as described by others [15-17] and a cell concentration not associated with seizures in our study. Subsequent evaluation of patients identified no adverse effects after dilution with autologous plasma in PBSC products with high WBC concentration. Finally, we also added 10% of anticoagulant citrate dextrose solution at the time of thawing in patients requiring dilution of PBSC products because of high WBC concentration. It is therefore possible that absence of seizures in patients with diluted PBSC products is also related to addition of Acid-citrate-dextrose (ACD) before infusion.

Use of both Fresenius AS104 and COBE Spectra cell separators gave us the opportunity to determine differences in product characteristics collected with these 2 devices. Cytapheresis on the Fresenius AS104 cell separator took more time and produced fewer total cells and lower CD34$^+$ cell yields than the COBE Spectra. Absolute CD34$^+$ cells per collection were not significantly different because the total volume of the PBSC product per collection was higher with the Fresenius AS 104. Only patients collected with the COBE Spectra developed seizures during PBSC infusion. PBSC products collected with the COBE spectra had higher WBC and a lower percentage of mononuclear cells.

In conclusion, a higher number of WBC including mature granulocytes in COBE Spectra collections is a possible cause of PBSC infusion-related seizures. Dilution of PBSC with high WBC concentration with autologous plasma and addition of ACD during thawing prevented additional seizure episodes. Since making these changes, we continue to monitor patients for PBSC infusion-related seizures, other infusion-related serious adverse events, and engraftment.

Table 6. Dilution of PBSC with High WBC

	Plasma Added (Diluted) (N = 29)	Plasma Not Added (Undiluted) (N = 51)
*WBC ($\times 10^3/\mu L$)	525 ± 164	357 ± 93.2
Final WBC ($\times 10^3/\mu L$)	351 ± 60.8	335 ± 65.1
*TNC (10^{10})	12.4 ± 3.8	7.35 ± 2.3
*Total volume frozen (mL)	400 ± 88.5	200 ± 51.2
Pre cryo viab. (%)	98 ± 6.1	98 ± 1.4
*Postthaw viab. (%)	54 ± 13.2	63 ± 12.7
% MNC	60 ± 21.9	65 ± 21.9
CD34/kg infused ($\times 10^6$/kg)	3.85 ± 1.48	4.66 ± 1.68
ANC engraftment (days)	12.0 ± 1.3	11.5 ± 1.3
PLT engraftment (days)	18.0 ± 3.7	16.0 ± 2.7

MNC indicates mononuclear cells.

*$P < .05$.

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