

Catastrophic failures of freezing bags for cellular therapy products: description, cause, and consequences

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Background

Container integrity is critical for maintaining sterility of cryopreserved cellular therapy products. We investigated a series of catastrophic bag failures, first noticed in early 2001.

Methods

Process records were reviewed for all PBPC and lymphocyte products cryopreserved in bags from January 2000 through April 2002. Patient charts were also reviewed.

Results

One thousand two hundred and four bags were removed from storage for infusion to 261 patients. All products had been cryopreserved in Cryocyte poly(ethylene co-vinyl acetate) (EVA) bags in either 10% DMSO or 5% DMSO and 6% pentastarch. Product volumes were 25–75 mL, and bags were stored with overwrap bags in a liquid nitrogen tank. From January 2000 to April 2001, failure occurred in 10 of 599 (1.7%) bags. From May 2001 to April 2002, 58 of 605 (9.6%) bags failed, typically with extensive fractures that were visible before

thaw. Of the 58 that failed, 24 were salvaged by aseptic methods and infused to patients under antibiotic coverage; 10 of those 24 (42%) had positive bacterial cultures. Bag failures were not related to product type, cryoprotectant solution, liquid versus vapor storage, or freezer location. Failures were linked to use of four Cryocyte bag lots manufactured in 2000 and 2001. After replacing these lots with a 1999 Cryocyte lot and with KryoSaf^e polyfluoroethylene polyfluoropropylene (FEP) bags, no more failures occurred in 75 and 102 bags, respectively, thawed through April 2002.

Discussion

High rates of bag failure were associated with four Cryocyte bag lots. No serious adverse patient effects occurred, but bag failures led to microbial contamination, increased product preparation time, increased antibiotic use, and increased resource expenditure to replace products.

Keywords

cryopreservation, cellular therapy product, aseptic methods, plastic bags, bacterial contamination

Introduction

Cellular therapy products such as PBPCs and lymphocytes may be stored in the liquid or frozen state before infusion. To avoid the progressive loss of viable cells associated with liquid storage, PBPCs and lymphocytes are cryopreserved in plastic containers, either vials or bags [1]. Unless the cell number has been reduced by specific selection procedures

or aliquot preparation, most PBPCs and lymphocytes are stored in plastic bags. These bags are designed to withstand temperatures as low as -196°C (the temperature of liquid nitrogen), as well as sudden changes in temperature associated with thawing in a 37°C waterbath.

Container integrity is important for maintaining both product quantity and quality. Container failure may lead

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to partial or complete loss of the product, which may be unique and represent the patient's last chance for remission or cure. This is especially true in allogeneic hematopoietic transplantation, where the product is thawed after the patient has already received myeloablative or myelosuppressive conditioning. Although there are many sources of contamination during manufacturing [2], an intact sterile container is a prerequisite for ensuring product sterility throughout cryopreservation, storage, thaw, and infusion. In the spring of 2001, we noticed a change in the rate of catastrophic failures of bags used for cryopreserving cellular therapy products, and undertook an investigation of the possible sources of bag failures, as well as the laboratory and clinical consequences of these occurrences.

Methods

Human subjects

Patients with hematologic malignancies, solid tumors, or genetic diseases, and HLA-matched first-degree related donors were enrolled in clinical trials for autologous or allogeneic hematopoietic transplantation or other cellular therapies. All subjects gave informed consent to procedures described in protocols approved by the Institutional Review Boards of the National Cancer Institute (NCI), the National Heart, Lung, and Blood Institute (NHLBI), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the National Institute of Allergy and Infectious Diseases (NIAID).

Collection of cellular products

PBPC and lymphocyte products were collected by leukapheresis on the CS3000 Plus cell separator (Fenwal Division, Baxter, Deerfield, IL, USA), using peripheral or central venous access. Each procedure consisted of processing 10–25 L of whole blood using ACD-A anticoagulation, with an ACD-A:whole blood ratio of 1:13, and *i.v.* calcium and magnesium replacement as needed to minimize citrate toxicity in the donor.

Processing and cryopreservation of cellular products

Aseptic methods were used, including use of closed systems when available. All open-system processing steps were carried out in a Class 100 environment for airborne particulates. Before October 1, 2000, products were cryopreserved in Plasmalyte A (Baxter, Deerfield, IL, USA)

with 10% DMSO (Research Industries, Salt Lake City, UT, USA), 10–20% human AB serum (manufactured by our facility), 15 U/mL preservative-free porcine heparin (American Pharmaceutical Partners, Los Angeles, CA, USA), and 10 µg/mL DNase (Dornase, Genentech, San Francisco, CA, USA). Beginning on October 1, 2000, the freeze mix was changed to result in final concentrations of 5% DMSO and 6% pentastarch (rather than 10% DMSO) and 4% HSA (Buminate, Baxter Healthcare, Glendale, CA, USA) instead of human AB serum, but without substantial changes in other ingredients. The pentastarch stock solution was prepared by the Pharmacy Development Service at the NIH Clinical Center as follows: 120 g HES (hydroxyethyl starch) pentastarch (B. Braun, Bethlehem, PA, USA) was mixed with 480 mL Plasmalyte A and 1.08 g dextrose, heated to 50°C for 1 h, filtered through a 0.22 µm filter into sterile 30 mL bags, and stored at 4°C for up to 6 months. On the day of cryopreservation, the pentastarch stock solution was mixed, in our facility, with DMSO and 25% HSA to obtain a final freeze mix of 10% DMSO, 8% HSA, and 12% pentastarch; this solution was stored at 2–8°C until use within 24 h in a 1:1 ratio with the cellular product.

Products were cryopreserved in poly(ethylene co-vinyl acetate) (EVA) plastic bags (Cryocyte freezing container, 250 mL with label pocket PL 269 plastic, Baxter, Deerfield, IL, USA) before January 23, 2002, and polyfluoroethylene polyfluoropropylene (FEP) bags (KryoSafe, American Fluorseal Corporation, Gaithersburg, MD, USA) thereafter. Bags contained a maximum WBC concentration of 3×10^8 /mL in a maximum volume of 75 mL. Samples for sterility testing were obtained after addition of the freeze mix, but before splitting the product into multiple bags. Bags containing cells plus freeze mix were placed into aluminum presses and transferred to the pre-chilled chamber (4°C) of a controlled-rate freezing device (CR Freezing System Model 9000, Gordinier Electronics, Inc., Roseville, MI, USA). The automated freezing program had been validated to result in temperature drop of 1°C per minute from start through phase change, until the bag probe reached –40°C, and thereafter 10°C per minute until the bag probe reached –90°C.

After controlled-rate freezing, the bag was sealed inside an overwrap bag (Kapak Heat Sealable Pouch, Kapak Corporation, Minneapolis, MN, USA), and placed into a prechilled aluminum cassette in the top portion (estimated temperature –120°C) of the liquid nitrogen

(LN2) storage freezer (Cryogenics XLC 1200, MVE, Burnsville, MN, USA). Cassettes were placed into a vertically aligned racking system in the LN2 freezer, with four cassettes per rack (a at top, and d at bottom). The LN2 was maintained in the range of 13–17 inches from the bottom, corresponding to the level of the third cassette from the top (level c). Cassettes at levels a and b were therefore consistently in the vapor phase of LN2, while cassettes at level c were mostly in liquid, and those at level d were completely in liquid.

Thawing of products for infusion

Before thawing, the primary container and overwrap bag were laid on top of the racking system within the LN2 storage tank (estimated temperature -120°C), opened and inspected. Overwrap bags were replaced if found to be broken or defective. If defects were noted in the primary bags, containers were kept in the storage tank and the patient's physician was consulted. If a decision was made to thaw, preparations were made for the salvage procedure and the patient was started on antibiotics. To thaw, bags were immersed and gently rocked in a 37°C waterbath. For products cryopreserved in the pentastarch freeze mix, thawed products were rapidly diluted with 6–12 mL of Plasmalyte A. Immediately after thaw, each product was transported to the patient care unit for infusion.

Salvage procedure for products in broken bags

A ring-stand with syringe barrel and attached transfer pack were assembled inside a biologic cabinet. The Cryocyte bag had its tag removed and was sealed inside a new overwrap bag, and a second, 2-quart overwrap bag was applied. The double-overwrapped bag was immersed in a 37°C waterbath, maintaining the product in a position to minimize spillage from the primary bag. After removal of the outer overwrap bag, the product (now with a single overwrap bag) was returned to the biologic cabinet. Using aseptic technique, the product was transferred to the transfer pack with a sterile pipette via the syringe barrel and tubing. A sample was obtained for sterility testing before heat-sealing the tubing and labeling the container for issue.

Microbial assays

Sterility testing was performed on every product prior to cryopreservation, and on every salvaged product. Each

1 mL sample was obtained using aseptic technique and transferred to an aerobic BacT/ALERT culture bottle, and placed in the BacT/ALERT Microbial Detection System (Organon Teknika, Durham, NC, USA) for 7 days. This system is designed to rapidly detect bacteria or fungi in clinical samples. Positive cultures were immediately reported to clinical-care staff.

Record review

Records were reviewed and data were analyzed for all clinical PBPC and lymphocyte products removed from LN2 storage between January 1, 2000 and April 30, 2002 with the intent of thaw for infusion, discard, or transfer to research. Bag failures were classified as minor, if breaks involved the port areas or tubing attached to the bag or a pinhole in the plastic, or major, if they involved more extensive cracks on the faces or edges of the bags. Medical records were reviewed for all patients who received products salvaged from broken bags.

Statistical analysis

Student's unpaired two-sample t test was performed, two-sided at a significance level of 0.05, to determine differences between groups for all data with a presumed normal distribution.

Results

Number of containers, bags, and bag failures

From January 2000 – April 2002 PBPC or lymphocyte products from 1482 product containers, including 1204 bags and 203 vials, were prepared for infusion (Table 1). The 1204 cryopreserved/thawed bags included 1013 bags infused to 218 patients, plus 191 bags removed from LN2 storage for discard. Freshly infused products and all products stored in vials were excluded; there were no failures in these containers. As shown in Table 2, a total of 68 failures occurred in the 1204 cryopreserved product bags. In the 16-month period through April 2001, before we noticed a change in the number and severity of bag failures, there were 10 bag failures, representing a rate of 1.7%. However, in the 12-month period starting in May 2001, there were a total of 58 bag failures, a rate of 9.6%.

Chronology of bag failures and involved lots

As shown in Figure 1, before May 2001, bag failures occurred sporadically at a rate of 0–2 per month, and any failure was cause for investigation. The unusual observation

Table 1. Transplant patients, products, and containers

	Jan 00–Apr 01	May 01–Apr 02	Total (Jan 00–Apr 02)
No. patients infused	156	135	261
No. infusion episodes	271	220	491
No. fresh products	48	27	75
No. cryopreserved/thawed vials	108	95	203
No. cryopreserved/thawed bags ¹	599	605	1204
No. product containers (total) ²	755	727	1482

¹Includes all bags thawed for infusion or discard.

²Total = fresh products + cryopreserved/thawed vials + cryopreserved/thawed bags.

Table 2. Transplant products and bag failures

	Jan 00–Apr 01	May 01–Apr 02	Total (Jan 00–Apr 02)
No. bags thawed	599	605	1204
No. broken bags	10	58	68
Failure rate (%)	1.7	9.6	5.6

of three failures in May 2000 prompted an investigation that revealed a series of breaks at the site of heat-sealed tubing, caused by suboptimal placement of bags in freezing cassettes. This problem was resolved immediately after training staff in optimal bag placement within the cassette. In January 2001 and March 2001, there were two failures and one failure, respectively. These were noted to be major, but a consistent pattern was not yet apparent. However, in May 2001, there was a clear change in the rate of serious bag failures, with four major failures occurring. This pattern continued, and in October and November 2001 failures peaked at 10 per month, with rates of 10/41 (24%) and 10/47 (21%), respectively. Figure 2 shows a typical example of a major failure, with a starburst fracture pattern across the face of the bag, present even before product thaw.

Bags thawed during the 28-month study period were from 19 different manufacturing lots. Table 3 shows the chronology of lot use; there was some overlap in dates of lot usage. Overall failure rates for the eight lots of bags with one or more failures (coded in Figure 1) were in the range 1.1–49%, but the rate of major failures was 1.3% or lower in four of the lots. Higher rates of major failure, in the range 6.6–47%, were observed in the four

other lots (H00I19027, H00K29072, H01G18097, H01E07045).

Contribution of cryopreservation and storage variables to bag failures

The contribution of other variables to the bag failures was evaluated. Product type was not a factor, as both PBPC and lymphocyte-containing bags were similarly affected in number and severity of failures, with overall failure rates of 5.6% and 6.6% for PBPC and lymphocytes, respectively (data not shown). Programmed controlled-rate freeze curves were all within specifications, and there was no evidence of errors or irregularities in reagents or procedures used. Table 4 shows that failed bags had been stored in all three freezer storage tanks, 26 in Freezer I, 23 in Freezer II, and 19 in Freezer III, at all four rack levels (a, b, c, and d) within each tank. Roughly equal numbers had been stored in vapor or liquid phase: 34 had been stored in vapor (levels a, b) and 30 in liquid (levels c, d). Another consideration was the new freeze procedure, using 5% DMSO plus 6% pentastarch instead of 10% DMSO, introduced on October 1, 2000. However, review of failures by lot numbers (Table 3) showed that four Cryocyte

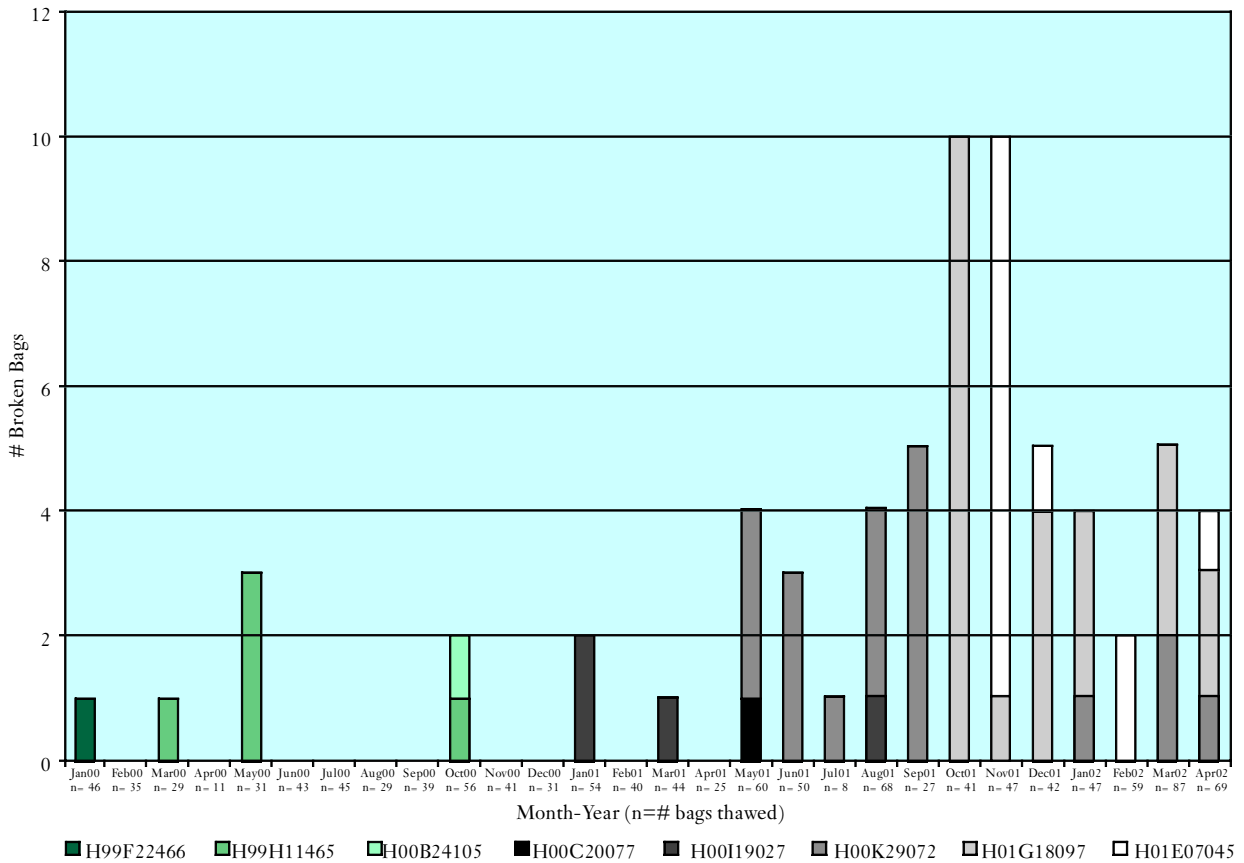


Figure 1. Broken bags by lot number over the 28-month study period. All bag failures occurred in one of eight manufacturing lots, each of which is represented by a different color. The number of bag failures per month peaked in October 2001 and November 2001, with 10 broken bags per month.

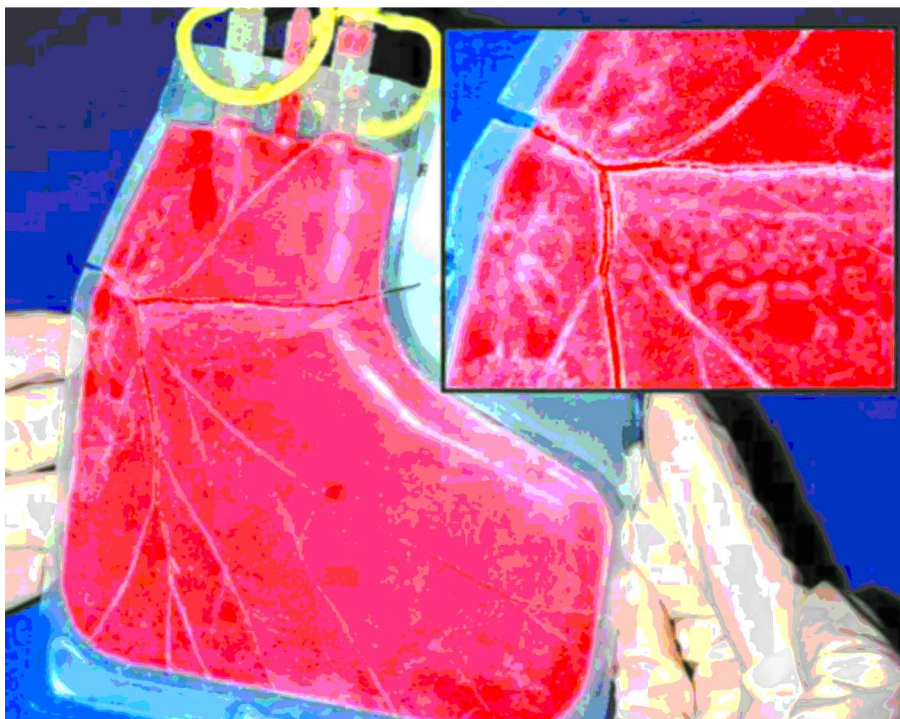


Figure 2. Photograph (with close-up of portion, inset) of a typical major bag failure. The starburst pattern in this bag was one of the most common fracture patterns among the major bag failures. This pattern demonstrates exposure of the product to the surrounding environment even prior to thaw. The plastic material shows a full thickness defect (inset).

Table 3. Major and minor bag failures by lot number

Bag lot	Dates of use	No. thawed	No. of failures		
			Minor	Major	Total
Other lots	Before Dec 1998	9	0	0	0
H98I10466	Dec 98–Aug 99	19	0	0	0
H99A25478	Apr 99–May 99	7	0	0	0
H99B02467	Jun 99–Aug 99	9	0	0	0
H99E12477	Sep 99–Oct 99; May 00	38	0	0	0
H99F22466	Nov 99–Feb 00	62	1 (1.7%)	0	1 (1.7%)
H99H11465	Dec 99–May 00	157	4 (2.5%)	1 (< 1%)	5 (3.2%)
H00A18026	May 00–Sep 00	87	0	0	0
H00B24105	Jun 00–Oct 00	96	0	1 (1.1%)	1 (1.1%)
H00C20077	Jul 00–Dec 00	76	0	1 (1.3%)	1 (1.3%)
H00F24023	Oct 00–Feb 01	94	0	0	0
H00I19027	Jan 01	35	0	3 (8.6%)	4 (11.4%) ¹
H00K29072	Nov 00–Sep 01	242	1 (< 1%)	16 (6.6%)	20 (8.3%) ²
H01G18097	Sep 01–Oct 01	45	1 (2.4%)	21 (47%)	22 (49%)
H01E07045	Oct 01–Nov 01	50	0	13 (26%)	13 (26%)
H99A04465	Nov 01–Jan 02	75	0	0	0
FEP	Jan 02–present	102	0	0	0

¹Type of failure not recorded for one bag.

²Type of failure not recorded for three bags.

Table 4. Bag failures by freezer location¹

Level	Freezer			Total
	I	II	III	
a	7	5	2	14
b	6	12	5	23
c	4	4	5	13
d	9	2	7	18
Total	26	23	19	68

¹Products were stored in one of three freezers (designated I, II, III) each of which had four levels (a–d, with a at the top and d at the bottom).

lot numbers, H00B24105, H00C20077, H00F24023 and H99A04465, were used after October 1, 2000 but were not associated with high failure rates, and lot H00F24023 was used exclusively after October 1, 2000, with the pentastarch method, without a single failure among 96 bags used.

Resolution of bag failures after introduction of alternate bags

The change in the severity and number of failures appeared to be associated with bag lots with serial numbers

indicating manufacture in 2000 and 2001. We therefore postulated that the use of bags manufactured before 2000 or bags from another manufacturer might resolve the problem. In November 2001, we introduced a Cryocyte bag lot manufactured in 1999 (H99A04465), followed by use of KryoSaf e FEP bags, from January 23, 2002 – present. No bag failures have occurred in the 75 Cryocyte 1999 bags and 102 KryoSaf e bags thawed through April 30, 2002.

Product salvage, microbial cultures, and adverse effects

Of 605 bags thawed between May 2001 and April 2002, none had positive microbial cultures on samples taken from the product immediately before cryopreservation. Of 58 broken bags thawed in that time-frame, 24 were salvaged for infusion. Of the 14 patients receiving salvaged products, six were already receiving antibiotics for neutropenic fever, and were switched to broader prophylactic antibiotic coverage; the other eight were placed on a new course of prophylactic antibiotics. As shown in Table 5, ten of the 24 salvaged/infused bags (42%) had positive bacterial cultures. Positive cultures were all common skin contaminants and sensitive to broad-spectrum antibiotics, without unusual resistance

Table 5. Bag fracture type and culture results of salvaged PBPC and lymphocyte products

Patient	Product type	Broken bags salvaged	Type of fracture	Culture result
1	PBPC	2	Port or tubing break	No growth
2	PBPC	1	Crack across top of bag	No growth
3	PBPC	2	Starburst across one face of bag	<i>Corynebacterium</i> species (2 of 2 bags)
4	PBPC	1	Starburst across one face of bag	No growth
5	PBPC	1	Starburst across one face of bag	No growth
6	PBPC	2	Starburst across one face of bag	No growth
7	PBPC	2	Starburst across both faces of bag	<i>Staphylococcus warneri</i> (1 of 2 bags)
8	PBPC	1	Crack down side of bag	No growth
9	PBPC	3	Starburst across both faces of bag	<i>S. epidermidis</i> and <i>S. warneri</i> (3 of 3 bags)
10	PBPC	3	Starburst across both faces of bag	<i>Micrococcus</i> species and <i>Bacillus</i> species (3 of 3 bags)
11	Lymph	2	Crack across front and side	No growth
12	Lymph	1	Crack across top and side of bag	No growth
13	Lymph	2	Starburst across one face of bag	No growth
14	Lymph	1	Crack down side of bag	<i>S. species</i> and <i>Bacillus</i> species (1 of 1 bag)
Total		24		10 bags with positive cultures

patterns. All 10 patients receiving PBPC products were either absolutely neutropenic or becoming neutropenic. All four patients who received contaminated PBPC products developed fever within 24 h (maximum temperature 39.4°C), but so did three of the other six patients who received salvaged PBPC products with negative cultures. The one patient who received a contaminated lymphocyte product was not neutropenic, but developed fever, chills, and malaise 2 days after the infusion, and was hospitalized for 5 days at another site. However, none of the five patients who received contaminated products and none of those with fever after receiving salvaged products had positive blood cultures, and none had permanent sequelae. In three patients, bag failure led to discard of the lymphocyte product, resulting in treatment delay until a replacement product could be collected from the donor.

Effect of bag failure on infused CD34⁺ cell dose and clinical engraftment

Over the 28-month period, 154 different patients (ages 7–68 years) were infused with PBPC products that had been cryopreserved in bags for autologous or allogeneic hematopoietic transplantation according to one of 18 different clinical protocols. As shown in Table 6, most of the patients were on treatment protocols for allogeneic

PBPCs after nonmyeloablative conditioning (n = 125). Among these, the 13 whose broken PBPC bags had been discarded received lower doses of CD34⁺ cells than the 106 patients whose PBPC products were unaffected by bag failures (mean $5.7 \times 10^6/\text{kg}$ versus $8.8 \times 10^6/\text{kg}$, $p < 0.01$). In all cases, however, infused CD34⁺ cell doses were adequate to meet the minimum infusion dose of $3 \times 10^6/\text{kg}$ specified in the protocols. The six patients who received PBPC salvaged from broken bags had comparable CD34⁺ cell doses to patients who had no broken bags (mean $7.5 \times 10^6/\text{kg}$ versus $8.8 \times 10^6/\text{kg}$, $p = 0.5$).

Among autologous transplant patients, the five who received salvaged PBPC products had lower doses of CD34⁺ progenitor cells than the two patients who had broken bags discarded, but the difference was not statistically significant (mean $2.9 \times 10^6/\text{kg}$ versus $8.8 \times 10^6/\text{kg}$, $p = 0.4$). The five patients receiving salvaged products had significantly lower doses than the 23 patients who had no broken bags (mean $2.9 \times 10^6/\text{kg}$ versus $10.4 \times 10^6/\text{kg}$, $p < 0.01$). This was clearly a reflection of the fact that transplant physicians were not willing to discard autologous PBPC products if the available CD34⁺ cell dose in all cryopreserved bags was relatively low and close to the required minimum cell dose specified in the treatment protocols. Of interest, the starting total CD34⁺ cell doses

Table 6. CD34⁺ cell doses for transplant patients by protocol type and bag use

			n	Mean CD34 ⁺ ($\times 10^6/\text{kg}$) \pm SD		Total
				Infused	Discarded	
allo n = 125	NM	Broken bags infused	6	7.5 \pm 3.9	1.9 ¹	9.5 ¹ \pm 3.8
		Broken bag discarded	13	5.7 \pm 1.9	2.1 \pm 2.1	7.8 \pm 2.6
		No broken bags	106	8.8 \pm 4.8	na	8.8 \pm 4.8
allo n = 4	myelo	Broken bags infused	0	na	na	na
		Broken bag discarded	1	8.3	1.5	9.8
		No broken bags	3	10.8 \pm 5.7	na	10.8 \pm 5.7
auto n = 30		Broken bags infused	5	2.9 \pm 1.0	0.5 ²	3.0 \pm 1.0
		Broken bag discarded	2	8.8 \pm 6.8	2.5 \pm 2.8	11.3 \pm 9.6
		No broken bags	23	10.4 \pm 8.6	na	10.4 \pm 8.6

¹Two patients with broken bags, some were infused; others discarded.

²Partial salvage.

auto = autologous; allo = allogeneic; myelo = myeloablative; NM = nonmyeloablative; na = not applicable.

were comparable for patients whose broken bags were discarded and for patients with no broken bags (mean $11.3 \times 10^6/\text{kg}$ versus $10.4 \times 10^6/\text{kg}$, $p = 0.7$), and high enough such that discard of broken bags did not have a negative impact on the cell dose administered.

To evaluate clinical consequences of bag failures, neutrophil engraftment was evaluated for all patients on nonmyeloablative allogeneic transplant protocols; the other two transplant groups were not evaluated because their numbers were too small for meaningful analysis. In the overall group of 125 patients, who had a median CD34⁺ cell dose of $7.52 \times 10^6/\text{kg}$ (range 2.1–30.0 $\times 10^6/\text{kg}$), the overall median time to ANC $> 500/\mu\text{L}$ was 10 days, with a range of 7–20 days (Figure 3). There were no engraftment failures and, given the range of CD34⁺ cell doses infused, the infusion of a minimum CD34⁺ cell dose of $2 \times 10^6/\text{kg}$, and the relatively low number of patients whose doses were $< 4 \times 10^6/\text{kg}$, a definite dose–response effect cannot be demonstrated. The 13 patients who had broken bags discarded, and who therefore received lower CD34⁺ doses, had a median neutrophil engraftment time of 11 days, which was comparable to the median of 10 days for the 103 patients in the control group with no broken bags. The longest time to engraftment among the broken bag recipients was 14 days. Several control patients also had more prolonged neutrophil engraftment times. Among the patients receiving products from broken bags for nonmyeloablative transplant protocols, three received products with positive microbiology cultures, and had engraftment times of

9, 13, and 15 days, while three received culture-negative products, and had engraftment times of 8, 9, and 9 days. The small numbers in these subgroups make definitive statistical comparisons impossible, but it can be stated that all engraftment times for these six patients fell within the expected range for the given cell-dose administered.

Discussion

Container integrity is critical for ensuring safety and efficacy of drug and biologic products. In this report, we documented a low background rate of failures in containers used for storage of cryopreserved PBPC and lymphocyte products. Before May 2001, no vials and less than 2% of bags failed, with the majority considered minor failures. From May 2001–April 2002, however, we observed an overall bag failure rate of 10%, the majority of which were major, with severe cracking along bag seams or across the face of the bag, and requiring complex salvage procedures.

The most obvious and disturbing consequence of major bag failures was the high rate of bacterial contamination (42%), despite use of aseptic recovery methods. Bacterial contamination of cellular therapy products may originate from the donor, from reagents used in their manufacture, or from contact with solid surfaces, humans, or airborne particulates in the manufacturing environment. Published reports suggest that contamination of apheresis-derived PBPC products before manipulation is relatively unusual, in the range of 0.2–1.67%, but more common for bone marrow, range 1.1–17% [3–8], and

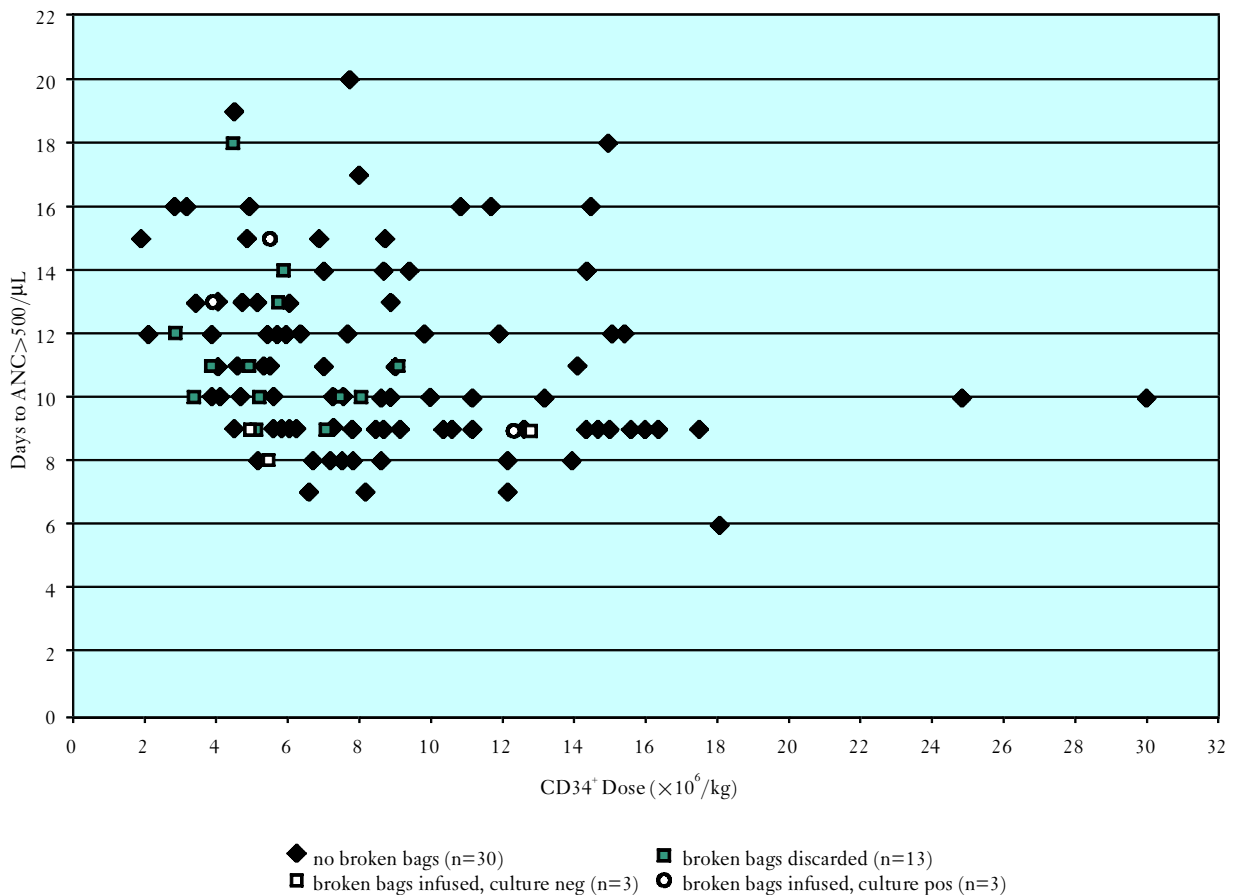


Figure 3. Neutrophil engraftment data for nonmyeloablative allogeneic transplant patients, demonstrated by days to ANC > 500/μL after graft infusion (y-axis), plotted against the dose of CD34⁺ cells in the graft (x-axis). Data are given for 103 control patients who had no broken PBPC bags, ◆; 13 patients with broken bags discarded, ■, and six patients with broken bags infused (three with positive cultures, □; three with negative cultures, ○). There was no evidence of delayed engraftment in the patients whose broken bags were discarded, or patients who were infused with products from broken bags—whether bacterially contaminated or not.

typically consists of organisms from donor skin. Introduction of bacteria during processing is unusual in a controlled environment, but has been associated with open-system processing and use of contaminated reagents [3,4,9,10]. In one report, loss of container integrity during thaw in a contaminated waterbath resulted in product contamination with a variety of organisms, including *Staphylococcus* and gram negative bacteria, including *Pseudomonas* species [11]. We did not observe a single positive bacterial culture in 416 products sampled before cryopreservation, and the use of overwrap bags protected the broken bags from exposure to water during thawing. The most likely source of contamination in our series was therefore contact of the thawed product with the non-sterile external surface of the bag.

The decision to infuse a product from a broken bag was made only when the risks of infusing the product were

considered lower than the risks of not infusing the product (i.e. prolonged myelosuppression) and after consent of the patient. In 15 instances, the physician decided to withhold products from broken containers. For PBPC, this resulted in CD34⁺ cell doses that still met pre-defined infusion criteria, but were lower than those infused to patients whose products were not affected by broken containers. For lymphocytes, this resulted in an additional leukapheresis procedure for each of three patients' donors, and subsequent product preparation, associated with use of additional resources and inconvenience to the donors.

The cause of the bag failures is uncertain at present. They were not related to product type, storage in a particular freezer, specific horizontal or vertical position within a freezer, or storage in liquid versus vapor phase. Our data rule out the use of the pentastarch-containing freeze mix as the sole cause of the phenomenon, because there were

no breaks among 96 bags from one lot (H00F24023) used only with this freeze mix. High failure rates were associated with four of 19 bag lots. We observed that the four implicated bag lots were manufactured in 2000 and 2001, and that the phenomenon disappeared when we substituted a Cryocyte bag lot manufactured in 1999 or another manufacturer's bag (KryoSafe). This led us to speculate that a subtle change in the Cryocyte manufacturing process occurring in late 1999 or early 2000 could have affected the bags. This possibility is currently being pursued by the manufacturer.

Container integrity is critical, not only for maintaining sterility of the individual product, but also for minimizing risks to laboratory staff and to patients whose products are handled and stored in the same facility. These concerns were highlighted by a report of hepatitis B transmission from one cryopreserved cellular product to others stored within the same LN₂ freezer, after loss of container integrity [12]. That report prompted standards requiring use of methods to minimize the risk of microbial cross-contamination of products stored in LN₂ [13,14]. Our facility has adopted the use of overwrap bags which, although not free from breakage themselves, may provide a higher level of product protection in the LN₂ environment.

Despite the high rate of bacterial contamination and the immunocompromised status of our patient population, we did not observe serious infusional toxicities (other than fevers, which were not definitely attributable to contaminated products) or permanent clinical sequelae. These data are in concert with previously published data [3,4,6,8,9,11]. All of our contaminants were skin organisms, and the lack of patient symptoms suggests that the number of organisms infused was relatively low and that contaminations were not associated with significant endotoxin or exotoxin production. However, because of uncertainty about the presence and extent of bacterial contamination prior to infusion of a salvaged product, all patients required prophylactic antibiotic coverage.

This report has implications for design of quality programs and tracking of manufacturing data for laboratory incidents. We believe that the bag failure phenomenon was noticed relatively quickly in our facility because of the convergence of several factors. First, our facility has a high volume of product cryopreservation procedures. Second, bag failure is a relatively unusual event in our laboratory, and is always cause for investigation. Third, the time interval from a product entering the freezer to removal for thaw

is relatively short—a median of 27 days for PBPC and 135 days for lymphocytes, so that the consequences of faulty cryopreservation would be observed close to the time of initial processing. Finally, the use of bag lots in a roughly sequential fashion made it possible to observe bag failures in clusters, which were subsequently related to specific bag lots. It is likely that, in facilities with lower cryopreservation activity or longer intervals between cryopreservation and infusion, individual bag failures might be considered isolated incidents. Our observations suggest a role for computerized systems for capturing, tracking, and analyzing product manufacturing data.

Despite the role of the US Food and Drug Administration (FDA) in regulating plastic bags for cellular products as Class II medical devices that are subject to GMP requirements [15–18], specific standards for bag performance have not been established in the USA—other than those proposed and outlined by the US Pharmacopeia for blood and blood components [19]. Those standards are similar to those published in 1993 by the International Standards Organization (ISO) for 'Plastic collapsible containers for human blood and blood components' [20], and used throughout Europe. The ISO standards specify biological, chemical, and physical requirements, including sterilization procedures, proof of thermal stability (placement in -80°C , followed by immersion in 50°C water, then return to room temperature), and resistance to leakage during centrifugation. Given increasing emphasis on ensuring safety and efficacy of cell and tissue products by the FDA [21], we suggest that further studies to define minimum and optimal performance characteristics of these cryopreservation containers should be pursued.

Disclaimer

The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

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References

- 1 Rowley S. Processing and storage. In: Haley R, Snyder E, editors. *Hematopoietic progenitor cells: a primer for medical professionals*. Bethesda, MD: AABB Press, 2000:55–67.
- 2 Read EJ. Quality assurance for cell processing: no more blind faith. *Transfusion* 1996;36:1–4.
- 3 Rowley SD, Davis J, Dick J *et al*. Bacterial contamination of bone marrow grafts intended for autologous and allogeneic bone marrow transplantation. *Transfusion* 1988;28:109–12.
- 4 Webb IJ, Coral FS, Andersen JW *et al*. Sources and sequelae of bacterial contamination of hematopoietic stem cell components: implications for the safety of hematotherapy and graft engineering. *Transfusion* 1996;36:782–8.
- 5 Padley D, Koontz F, Trigg ME *et al*. Bacterial contamination rates following processing of bone marrow and peripheral blood progenitor cell preparations. *Transfusion* 1996;36:53–6.
- 6 Prince HM, Page SR, Keating A *et al*. Microbial contamination of harvested bone marrow and peripheral blood. *Bone Marrow Transplant* 1995;15:87–91.
- 7 Espinosa MT, Fox R, Creger RJ *et al*. Microbiologic contamination of peripheral blood progenitor cells collected for hematopoietic cell transplantation. *Transfusion* 1996;36:789–93.
- 8 Schwella N, Zimmermann R, Heuft HG *et al*. Microbiologic contamination of peripheral blood stem cell autografts. *Vox Sang* 1994;67:32–5.
- 9 Stroncek DF, Fautsch DK, Lasky LC *et al*. Adverse reactions in patients transfused with cryopreserved marrow. *Transfusion* 1991;31:521–6.
- 10 Reported contamination of heparin sodium with *Pseudomonas putida*. *MMWR Morb Mortal Wkly Rep* 1986;35:123–4.
- 11 Lazarus HM, Magalhaes-Silverman M, Fox RM *et al*. Contamination during *in vitro* processing of bone marrow for transplantation: clinical significance. *Bone Marrow Transplant* 1991;7:241–6.
- 12 Tedder RS, Zuckerman MA, Goldstone AH *et al*. Hepatitis B transmission from contaminated cryopreservation tank. *Lancet* 1995;346:137–40.
- 13 *Standards for hematopoietic progenitor cells*. 1st edn. Bethesda, MD: American Association of Blood Banks, 1996.
- 14 *Standards for hematopoietic progenitor cell collection, processing, and transplantation*. 1st edn. Vancouver, BC, Canada: Foundation for Accreditation of Hematopoietic Cell Therapy (FAHCT), 1996.
- 15 The Medical Device Amendments of 1976 (1976 Amendments). *Publ Law* 94–285. (Available at <http://thomas.loc.gov/home/thomas.html>, visited 16th September 2002.)
- 16 *Premarket notification 510(k): Regulatory requirements for medical devices*. FDA 95–4158. (Available at <http://www.fda.gov/cdrh/manual/510kprt1.html>, visited 16th September 2002.)
- 17 *Medical Device Quality Systems Manual*. FDA 97–4179, section 8. (Available at http://www.fda.gov/cdrh/dsma/gmp_man.html, visited 16th September 2002.)
- 18 *Code of Federal Regulations. Title 21 CFR Part 820.160*. (Available at <http://www.access.gpo.gov/nara/cfr/cfr-table-search.html>, visited 16th September 2002.)
- 19 Sterile, single-use plastic large-volume containers for human blood and blood components. Drugs and pharmaceutical ingredients monograph (USP 23). *Pharmacoepial Forum* 1997;23:4651–5.
- 20 *Plastics collapsible containers for human blood and blood components. International Standard ISO 3826: 1993*. International Organization for Standards. 1st edn, 1993. (Available at www.iso.ch/en.)
- 21 Current good tissue practice for manufacturers of human cellular and tissue-based products; inspection and enforcement; proposed rule. *Fed Reg* January 8, 2001. (Available at <http://www.fda.gov/cber/rules/gtp010801pr.pdf>, visited 16th September 2002.)