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Quality control of bacterial contamination in autologous peripheral blood stem cells for transplantation

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A B S T R A C T

Background and Objectives. Microbiological follow-up is part of quality control of peripheral blood stem cell (PBSC) manipulation.

Design and Methods. We prospectively studied microbiological cultures performed in 865 consecutive untreated autologous PBSC harvests from 348 patients. Our aim was to know the rate of microbiological contamination, the optimum moment to evaluate the sample and the clinical significance of the positive findings.

Results. Fifty-nine of the 852 samples (6.9%) yielded a positive culture after PBSC collection (sample 1) and 62 samples also yielded positive results before cryopreservation (7.2%) (sample 2). At the time of the analysis, a total of 520 aphereses had been infused and the number of positive cultures after thawing (sample 3) and after washing (sample 4; 82 aphereses) was 5.4% and 2.3%, respectively. Most of the positive cultures were due to coagulase-negative staphylococci (48 isolates). After thawing 15 coagulase-negative staphylococci and 2 enterococci isolates were recovered. Comparison between samples using a marginal homogeneity test showed no differences in the rate of contamination observed at the different sampling points.

Interpretation and Conclusions. Positive microbiological findings in collected PBSC are not due to contamination within the laboratory. Cryopreservation using DMSO does not eradicate bacteria and manipulation does not seem to affect results. To simplify the procedure it would be possible to eliminate the microbiological controls performed immediately before cryopreservation.

Key words: microbiological contamination, quality control, peripheral blood stem cells, apheresis

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Peripheral blood stem cells (PBSC) are extensively used as a source of hematopoietic progenitors for autologous transplantation in patients with a wide range of malignant diseases.[1] To ensure the availability of an adequate product for transplantation, a policy of strict quality control of the collected product is essential. The controls should include $CD34^+$ cell count, cell viability assessment, clonogenic assays and follow-up of microbiological sterility.

With regards to microbiological contamination of PBSC products, several studies have demonstrated that some species of bacteria and dermatophyte fungi can survive cryopreservation and that microbiological contamination of apheresis products, although very rare, may occur. Furthermore, contamination has been occasionally associated with a generalized infection in the recipient.[2,3] So, any possible source of contamination should be avoided during the processing of PBSC products. Contamination of the PBSC graft can occur at several stages during harvesting,[4] processing and thawing,[5] and differentiating between clinically significant pathogens and laboratory contaminants may be difficult. This fact highlights the importance of proper microbiological analysis during cryopreservation and thawing as an important part of the quality control procedure. The ideal moment when cultures should be done does, however, remain to be determined. We studied the results of microbiological cultures prospectively performed in 865 consecutive untreated autologous PBSC harvests from 348 patients with several malignant diseases. Samples for bacterial cultures were taken at different time points during processing.

Design and Methods

From March 2000 to August 2003, microbiological analyses were prospectively done in 865 consecutive autologous PBSC collections undergoing cryopreservation in our laboratory. Samples were obtained after collection, before controlled-rate freezing and after thawing, immediately prior to infusion. All components were collected and processed using sterile techniques without the addition of antibiotics. The objective of the study was to know the rate of microbiological contamination, the optimum moment to evaluate the sample and the clinical significance of the positive findings.

Patients

A total of 348 patients from five different centers undergoing 865 rounds of PBSC collection form the basis of this analysis. There were 180 males and 168 females. Diagnoses included multiple myeloma (n=103), non-Hodgkin's lymphoma (n=68), acute leukemia (n=64), solid tumor (n=58), Hodgkin's disease (n=21), chronic lymphocytic leukemia (n=6) and chronic myeloid leukemia (n=4). Mobilization of PBSC consisted of chemotherapy followed by granulocyte colony-stimulating factor (G-CSF) or G-CSF alone.

PBSC harvest and processing

PBSC harvest

PBSC collections were performed with continuous-flow blood cell separators (COBE Spectra, Gambro or CS-3000 Plus, Fenwal), according to standard techniques.[6,7] The volume processed was up to three times the patient's blood volume and large volume leukapheresis were not performed in any case. No patient had fever or clinical signs of infection at the time of PBSC harvest. Leukapheresis was performed when blood $CD34^+$ cell or leukocyte counts were considered adequate according to each institution's protocol. Records of venous access were reported for 538 of the 865 leukaphereses; in 340 collections (63%) patients carried a central venous catheter and a peripheral venous access was used in the remaining 198 cases (37%). The median and mean numbers of PBSC collections were 3 and 2.5±1.4, respectively (range 1-10). *In vitro* purging of the autografts was not done in any case.

Cryopreservation

Aphereses were cryopreserved within the first 24 hours after collection in every case. Cryopreservation was performed after buffy-coat preparation. Cells were suspended in a medium containing dimethylsulfoxide (DMSO; B Braun Medical, Bolougne, Cedex), at a final concentration of 10% and 5% albumin (Albúmina humana 20% Grifols, Barcelona, Spain). Cells were frozen at a controlled rate of 1°C per minute and stored at less than -120°C in the vapor phase of nitrogen until use.

PBSC thawing and transfusion

Five hundred and twenty aphereses had been infused. PBSC from adult patients were thawed in a 37°C waterbath in hospital wards and rapidly transfused through a central venous line. For children and to minimize toxicity during infusion, PBSC were washed after thawing to reduce volume and to remove DMSO; this policy was extended to the adult population from May 2002. At the time of the study, 82 aphereses had been thawed and washed. Briefly, cells were diluted V/V with a 2.5% albumin, 5% ACD (ACD-A, Grifols, Barcelona, Spain) and 82.5% dextrane solution (Rheomacrodex, Grifols, Barcelona, Spain). After 5-10 minutes, cells were washed with albumin-saline solution as above using a cellular separator device (IBM-COBE 2991) and centrifuged at 3000 rpm for 7 minutes. The supernatant was then removed and cells were resuspended in an albumin saline solution (2.5% albumin). The washing procedure was performed with a 1 2991™ blood cell processing set.

Bacterial cultures

Bacterial cultures were performed on all PBSC products at the following times: after collection, i.e., when the apheresis arrived at our facility from the hospital (sample 1), immediately before controlled-rate freezing (sample 2) and at the time of thawing (sample 3). Furthermore, in PBSC aphereses from children a sample was taken after washing (sample 4). For microbiological cultures, a 0.5 mL sample was obtained and inoculated into 20 mL activated charcoal media (BacT/ALERT®PF, Organon Teknica Corporation, Durham, North Carolina, USA). Samples were incubated for a minimum of 7 days or until considered positive. Positive cultures were then subcultured in aerobic and anaerobic conditions and the bacterial species identified through standard biochemical testing.

Microbiological studies were performed with a BacT/Alert culture system (Organon Teknica) at 35°C. Products were processed in a laminar air flow clean bench with HEPA filters (Telstar BH-100 and AV-100). Samples for bacterial cultures were taken under the same conditions.

Follow-up of patients

The clinical records of the patients who received contaminated PBSC were reviewed to assess the patients' clinical outcome. Fever was defined as an axillary temperature of 38°C on two occasions at least

1 h apart or 38.5°C on one occasion. Febrile episodes were classified according to the guidelines issued by the HIS consensus conference and the ESCMID as follows: microbiologically documented infection (MDI) with or without bacteremia, clinically documented infection (CDI), fever of unknown origin (FUO) and non-infectious fever.[8,9] Single blood culture isolates were considered sufficient to classify an episode as bacteremic, except for coagulase-negative staphylococci and *Corynebacterium spp.*, which required at least two positive blood culture specimens. Finally, the type and duration of antibiotic therapy administered and bacterial isolations were also checked.

Statistical analysis

Any correlation of contamination among samples at different stages was determined by the marginal homogeneity test, a non-parametric test for two related ordinal variables. This test is an extension of the McNemar test from binary response to multinomial response. It tests for changes in responses using the chi-square distribution. It is useful for detecting changes in responses due to experimental intervention in before-and-after designs. The χ^2 analysis was used to test the relationship between venous access and bacterial findings. Calculations were performed using the Statiscal Package for Social Science (SPSS; v 8.0). Data were analyzed as of December 31, 2003.

Results

Fifty-nine of the 851 samples (6.9%) yielded a positive culture immediately after PBSC collection (sample 1) and 62 samples also yielded positive results before cryopreservation (7.2%) (sample 2). In both cases, a coagulase-negative staphylococcus was the micro-organism most frequently isolated (Table 1). At the time of the analysis, 520 aphereses had been infused and the number of positive cultures after thawing (sample 3) and after washing (sample 4) was 5.4% and 2.3%, respectively (Table 2).

Microbiological analyses were done, not only as part of quality control, but also to assess the possible influence of *in vitro* processing in an open system and whether the product contained bacteria at collection or became contamined subsequently. The correlations of bacterial isolates among samples taken at different times are shown in Table 3; differences were not statistically significant in any case. After thawing, bacterial isolates were the same in 17 and 18 cases as those cultured in samples 1 and 2, respectively.

Overall, 28 patients received contaminated PBSC products. A review of the medical records of these cases showed that all of them had previously received prophylactic oral antibiotics (quinolones). Likewise, four patients had received vancomycin immediately prior to infusion because of the isolation of coagulase-negative staphylococcus. Nineteen of the 25 patients (68%) developed fever in the first month after transplant (early post-transplant period): MDI (9 cases), FUO (7 cases) and CDI (3 cases). Ten of the 19 patients developed positive blood cultures, but in two of them the bacterial isolation was considered contamination (a single isolate for coagulase-negative staphylococci). In the remaining eight cases only three of the bacterial isolates corresponded to the initial one founded in the apheresis product (coagulase-negative staphylococci in every case). Every patient received standard treatment for febrile neutropenic episodes (usually a combination of a β-lactam plus an aminoglycoside) and none of them died as a consequence of

Table 1. Bacterial contamination of PBSC after collection and before cryopreservation.

	After collection		*Before cryopreservation*	
	No.	*%*	*No.*	*%*
Negative	792	93.1	803	92.8
Positive	58	6.9	62	7.2
Coagulase-negative Staphylococci	45	5.3	48	5.5
Micrococcus	4	0.5	3	0.3
Enterococcus faecalis	2	0.2	3	0.3
Corynebacterium spp.	2	0.2	2	0.2
Enterobacter spp.	2	0.2	2	0.2
P. acnes	1	0.1	1	0.1
Proteus mirabilis	1	0.1	1	0.1
Bacillus spp.	–	–	2	0.2
Streptomyces spp.	1	0.1	–	–
Gram-negative bacillus	1	0.1	–	–
Missing	40	4.5	26	2.9

Table 2. Bacterial contamination of PBSC after thawing and washing.

	No. thaw/wash	% thaw/wash
Negative	492/84	94.6/97.7
Positive	28/2	5.4/2.3
Coagulase-negative Staphylococci	19/1	3.7/1.2
Enterococcus faecalis	3/0	0.6/0
P. acnes	2/0	0.4/0
Corynebacterium spp.	1/0	0.2/0
Proteus mirabilis	1/0	1/0
Brevundimonas vesicularis	1/0	0.2/0
Salmonella D no tiphy	1/0	0.2/0
Anaerobic bacillus	0/1	0/5.6
Total	520/86	

Table 3. Comparison of isolates at sequential points of the process.

		Sample 2	
	Negative	*Positive*	*Total*
Sample 1			
Negative	774	10	784
Positive	7	51	58
Total	781	61	842
p=0.885			
		Sample 3	
	Negative	*Positive*	*Total*
Sample 1			
Negative	452	9	461
Positive	8	17	25
Total	460	26	486
p=0.240			
		Sample 4	
	Negative	*Positive*	*Total*
Sample 1			
Negative	78	2	80
Positive	2	0	2
Total	80	2	82
p=0.414			
		Sample 3	
	Negative	*Positive*	*Total*
Sample 2			
Negative	465	10	475
Positive	8	18	26
Total	473	28	501
p=0.463			
		Sample 4	
	Negative	*Positive*	*Total*
Sample 2			
Negative	80	2	82
Positive	1	0	1
Total	81	2	83
p=0.329			
		Sample 4	
	Negative	*Positive*	*Total*
Sample 3			
Negative	79	2	81
Positive	1	0	1
Total	80	2	82
p=0.866			

the infectious episodes. Hematopoietic recovery was similar to that observed among patients receiving uncontaminated grafts, and no irreversible clinical sequelae were observed in recipients of contaminated PBSC grafts. Finally, we did not find any correlation between the rate of positive cultures in the product and different patients' characteristics such as age, diagnosis, number of cycles of prior chemotherapy, and the administration of steroids. Likewise, no correlation was found between the positive findings observed at the different collection times (sample 1, 2, 3 or 4) and the type of venous access used for PBSC collection ($p > 0.05$).

Discussion

We found that bacterial contamination affects 7.2% of the products during PBSC collection and processing. Most of the positive cultures were due to common skin flora or environmental micro-organisms including coagulase-negative staphylococci (48 isolates). Although Gram-positive bacteria are said to survive cold incubation poorly[12] we observed a high rate of bacterial contamination due to Gram positive bacteria at thawing, indicating that in most cases these Gram positive bacteria had withstood very low temperatures. Furthermore, the contamination rate for any given bacteria fell slightly from 7.2% (sample 1 or 2) to 5.4% after thawing, probably reflecting the same previous bacteria plus previous false positive or late false negative isolates. When intetrpreting the dat the limitations of the bacterial test should be taken into account; for example, a sample volume less than 4 mL reduces the probability of recovery when there is a small population of an organism, but smaller amounts (e.g. 0.5 mL) can be used, thus mini-minimizing cellular waste, an aspect that can have a very important impact on the graft recipient's outcome. Several series have reported a bacterial contamination rate of 0% to 18% during *ex vivo* manipulation of PBSC.[3,13,15-20]

At our laboratory, the rate of contamination of autologous PBSC was higher than previously mentioned.[3,12,15,17,20] Possible reasons for this may be a more sensitive microbiological detection technique, more patients with indwelling catheters, a higher number of previously infected patients and poorer sterile working conditions. However, none of the patients were septic or had signs of catheter infection at the time of PBSC harvesting and no differences of bacterial contamination were observed depending on the type of venous access used for PBSC collection. Furthermore, the fact that most isolates were already present at collection rules out a bad manipulation technique. Thus, we believe that this higher contamination rate is due to better microbiological detection and perhaps to particular features of our population of patients. Microbial contamination of the PBSC products can occur at four different points: infection of the apheresis venous line before harvest, during

manipulation of the venous access during the separation procedure, during *ex vivo* processing of the product before cryopreservation, and during thawing and infusion. Initially we conducted this study to evaluate the number of contamined samples arriving at our laboratory. When we reported the results of the microbiological tests performed prior to cryopreservation back to hospitals, the clinicians tended to consider them as not clinically relevant, especially in the case of samples containing coagulase-negative staphylococci, not adopting any specific therapeutic measure. So, determining the real clinical impact of positive microbiological findings became our main concern. By testing samples taken at different times we tried to determine whther positivity remained equal through all steps of the process. As has been shown, the correlation of results observed after collection (sample 1) and just prior to cryopreservation (sample 2) was good. Likewise, a very good correlation was observed between sample 2 (before freezing) and 3 (post-thawing). Overall, these data strongly suggest that microbiological isolates are real pathogens, and that DMSO and temperatures below 0°C do not eradicate bacteria completely. Our results also show that eliminating tests on either the samples obtained after collection or those before cryopreservation will reduce costs without affecting efficacy.

In vitro addition of antibiotics to PBSC grafts has been shown to be ineffective.[2] We therefore consider that in case of contaminated PBSC grafts, two prophylactic antibiotics active against the pathogen should be simultaneously administered up to 72 hours after infusion. In the case of appreciable growths of coagulase-negative staphylococci, vancomycin alone should be the drug of choice.[4] In this regard, Schwella reported on five patients who received from 1 to 5 culture-positive stem cell concentrates without serious sequelae. In this report no micro-organism present in the stem cell autograft was recovered *in vivo* in the post-transplantation period, although fever as a sign of infection occurred in all the patients but one.[16] In our series, no life-threatening infections were observed among recipients of contaminated grafts, suggesting that clinical consequences of positive cultures are less when information about the isolate is available. However, it should be taken into account that the current management of fever in neutropenic patients consists of rapid and early measures including the use of broad-spectrum antibiotics which probably diminish the importance of inoculating bacteria with the graft.

In recent years, the burden of work in a cryopreservation laboratory has increased in parallel to the number of products stored and to the use of more complex laboratory manipulation techniques. This increase in activity is associated with many potential dangers, such as microbiological contamination, making the need for dedicated staff and properly managed clean room facilities especially important.[3,14-18,20] Laboratories undertaking this work should be able to show that their routine procedures and results comply with guidelines and standards. Ritter *et al.* stressed the impact of clean room conditions on the bacterial contamination rate of PBSC products, showing an extraordinary decrease in this rate after implementation of good manufacturing practice conditions, from 5.2% using a clean bench in a normal laboratory room to 0.8% under certified clean room conditions.[20]

In conclusion, our results strongly suggest that bacteria found in collected PBSC are not due to contamination within the laboratory. Likewise, our study shows that cryopreservation using DMSO does not eradicate bacteria completely. Finally, manipulation does not seem to affect results, and in order to simplify the procedure without affecting efficacy, it would be possible to eliminate the microbiological controls performed just before cryopreservation.

LL was the principal investigator and wrote the paper. RR, MAS and JdlR critically reviewed and approved the final version of the paper. JdlR, PR, IP, RG and JMF were responsible for clinical data referred from the hospitals. CGB was responsible for the microbiological data. PS helped with the study design. The authors reported no potential conflicts of interest.

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