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ORIGINAL ARTICLE

Influence of harvest bacterial contamination on autologous peripheral blood progenitor cells post-transplant

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Microbiological contamination of manipulated blood products, including hematopoietic progenitors obtained from peripheral blood, is an infrequent but persistent problem in transplant units. The relevance of such contamination in causing patient infection has been reported as insignificant, but the effect on the post-transplant course has not been well documented. We studied the incidence of bacterial contamination in autologous peripheral blood progenitor cell transplants in two of the bench processing steps, as well as the repercussions in the post-transplant course affecting incidence of infections, transfusion requirements and time to engraftment. A total of 365 aphereses performed on 152 patients were cryopreserved in 617 bags. In 31 of these bags (5.0%), bacterial cultures were positive for *Coagulase-negative Staphylococcus* (31.1%), *S. epidermidis* (21.9%), *Corynebacterium* sp. (6.3%), *S. warneri* (6.3%), *Stenotrophomonas maltophilia* (6.3%), *Streptococcus* sp. (9.4%), *Viridans group Streptococcus* (3.1%) and more than one bacteria (*Coagulase-negative Staphylococcus* plus *Corynebacterium)* (15.6%). Half of the bags were contaminated at the time of freezing and the others at the time of thawing. The 31 contaminated bags were infused into 17 patients. In five of these the same contaminating bacteria was found. No difference between the two groups of patients (contaminated and non-contaminated) was found on the day the fever started, length of fever, blood transfusion requirements and engraftment, but length of hospitalization was significantly greater in patients receiving contaminated transplants.

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Introduction

Bacterial contamination of blood products for transfusion during processing is a potential risk and has been the cause of serious sepsis and some deaths.[1–4] Microbial contamination has also been reported in blood processed for immunotherapy[5] and in hematopoietic progenitor cell transplants. In these cases, the contamination may be, according to the source of stem cells, from 0 to 4.5% in peripheral blood to as high as 26% in bone marrow.[5–10] Peripheral blood progenitor cells (PBPCs) have been increasingly used for both autologous and allogeneic transplants. In autologous PBPC transplants (APBPCT), cells are obtained frequently by apheresis through a central venous line, but this is also known to be a possible route for infection.[11,12] Not infrequently, several aphereses per patient are necessary to obtain sufficient PBPCs to ensure engraftment. PBPCs for APBPCTs are manipulated *ex vivo* in several steps: apheresis, freezing, thawing and infusion, and sometimes also by positive or negative cell selection. In each one of these procedures, bacterial contamination is possible, even if high standards of asepsis are maintained.[6,11] When microbial contamination occurs in red blood cells, platelets or plasma, it is possible to take the decision to discard them, because it is easy to obtain more from donors. However, this is a very difficult decision where contaminated PBPCs are concerned, because it is likely to be virtually impossible to obtain more PBPCs from the patient in most cases. The administration of contaminated progenitor cell grafts has been claimed to be harmless by several authors, whether or not prophylactic antibiotics are used.[6,13–18] There are few studies on the effects of contaminated APBPCTs post-transplant apart from documented infection episodes.[9] It is important to know how the infusion of infected products affects the patient's post-transplant course. The aim of this study was to analyze the incidence of bacterial contamination in patients receiving APBPCTs and to assess how it affected post-transplantation infection rate, duration of fever, engraftment, transfusion requirements and length of hospitalization.

Materials and methods

From January 1996 to December 2005, we performed 367 apheresis procedures on 152 patients, for APBPCTs.

The cells obtained were frozen and stored in 617 freezing bags.

Mobilization was with cyclophosphamide 1.5 g/m^2 (day 1) plus 10 μg/kg/day of human recombinant granulocyte-colony stimulating factor (G-CSF) (Neupogen; Amgen, Thousand Oaks, CA, USA) from day 6 until collection was completed in 78% of the cases, or with G-CSF (10 or 20 μg/kg/day) alone in the rest.

Central venous catheters (Hickman or Shaldon's type) were inserted in all patients for apheresis. PBPCs were obtained using a continuous blood cell separator (Fenwal CS3000; Baxter, Deerfield, IL, USA); four times the calculated blood volume was processed in each apheresis. At the time of harvest, no patient included in the study had clinical signs of infection.

Positive selection of CD34$^+$ cells was performed in some cases, with the aim of malignant cell purging, using monoclonal antibodies and immuno-magnetic beads (Isolex 300i; Nexell Therapeutics, Irvine, CA, USA).

For cryopreservation, PBPCs were placed in dedicated bags (Cryocyte Freezing Container; Nexell Therapeutics, Irvine, CA, USA), mixed with dimethyl sulfoxide (DMSO) plus autologous plasma at a final concentration of DMSO of 9–10%. Cryopreservation was performed with controlled rate freezing equipment (CM-25; Carburos Metálicos, Barcelona, Spain), lowering the temperature at a rate of 1–2°C/min. Freezing was performed immediately, or within 20 h post apheresis,[7,19] and the cells were stored in liquid nitrogen until infusion. A distilled water bath at 40°C was used for thawing. All steps were carried out using an air flow cabinet (Telstar CV-100; Telstar Industrial, Terrasa, Spain) except for positive selection and thawing.

PBPCs were infused through a central venous catheter, using blood transfusion sets (EMC0349 Baxter; Deerfield, IL, USA) and 60 ml sterile syringes for filtering, with no more than 15 min elapsing from thawing to the end of infusion, in line with Gorin's recommendation.[20]

Bacterial cultures were done immediately after mixing PBPCs with DMSO (pre-freezing sample) and at the end of PBPC infusion (post-thawing sample) through a sampling site coupler. We considered the bacteria growing in the post-thawing sample to be the contaminant in the infusion product when it was different from the pre-freezing sample. Patient blood cultures were started when fever developed, and repeated every 24 h, if the fever persisted.

For bacteriological cultures, a 1 ml sample was injected in Bact/alert aerobic and anaerobic culture bottles (Bio-Merieux, Marcy-l'Etoile, Rhône, France), which was then incubated and checked for bacterial growth daily for 1 week. Gram stain was carried out on positive samples and they were seeded according to this result (chocolate agar, blood agar, SCS, McConkey and Sabouraud dextrose agar) for identification and antibiotic sensitivity.

All APBPCTs were carried out in hospital. The high-dose chemotherapy protocol administered was the same for patients with the same diagnosis. Radiotherapy was not used. All patients received Acyclovir, Fluconazole and Ciprofloxacine from the first day of high-dose chemotherapy, as well as granulocyte-colony stimulating factor 5 μg/kg/day from day +5 until granulocyte recovery.

Granulocyte engraftment was described as the day when peripheral blood granulocyte count was greater than 0.5×10^9/l. Platelet engraftment was considered to have occurred when the platelet count was higher than 20×10^9/l for two consecutive days, without platelet transfusion.

Cumulative incidence and non-parametric Mann–Whitney *U*-tests were used in statistical analysis (SPSS 12 for Windows, Statsoft, Tulsa, CA, USA).

Results

Diagnoses of the 152 patients receiving APBPCTs are shown in Table 1. Positive CD34$^+$ cell selection was performed in 29 patients: 20 breast cancer (BC), four multiple myeloma (MM), two non-Hodgkin's lymphoma, two multiple sclerosis and one Hodgkin's Disease (HD).

Of the 617 bags infused, 31 (5%) had bacterial contamination, 16 of them in pre-freezing samples and 15 in post-thawing samples. In 10 contaminated pre-freezing bags, the same organism grew in the culture performed post-thawing and different bacteria grew in the other six. No bag rupture or leakage occurred.

Five hundred and five non-contaminated bags were administered to 135 patients (median 3; range: 1–20). In the group of 17 patients receiving contaminated PBPCs, a total of 71 bags were administered (the 31 contaminated ones referred to above, plus 40 uncontaminated bags) (median 5; range: 1–9); a median of 1 contaminated bag per patient (range 1–5). Bacteria found are shown in Table 2; no fungal

Table 1 Patient's diagnosis

Diagnosis	*Contaminated* n *(%)*	*Noncontaminated* n *(%)*	*Total* n *(%)*
Multiple myeloma	11 (64.7)	34 (25.2)	45 (29.6)
Breast cancer		41 (30.4)	41 (27.0)
Non-Hodgkin's lymphoma	3 (17.6)	31 (23.0)	34 (22.4)
Hodgkin disease	1 (5.9)	8 (5.9)	9 (5.9)
Acute myeloid leukemia		7 (5.2)	7 (4.6)
Acute lymphoid leukemia	1 (5.9)	5 (3.7)	6 (3.9)
Chronic lymphoid leukemia	1 (5.9)	3 (2.2)	4 (2.6)
Multiple sclerosis		2 (1.5)	2 (1.3)
Edwing sarcoma		2 (1.5)	2 (1.3)
Testicular germinal cancer		1 (0.7)	1 (0.7)
Chronic myeloid leukemia		1 (0.7)	1 (0.7)
Total	17 (100)	135 (100)	152 (100)

contamination was found. The distribution of diagnoses in these patients is shown in Table 1.

None of the 29 $CD34^+$ positively selected cell products had evidence of microbiological contamination. Hundred and forty one out of 152 transplanted patients developed fever. Fever was present in all patients receiving contaminated infusions; seven patients had fever before the infusion (from days −5 to −1), and one of them was in the group receiving contaminated material (Table 2).

Table 2 Bacterial growth found in 32 bags used for the 17 contaminated transplants, and patients with the same bacteria in blood culture

Bacteria	*Bags* n *(%)*	*Transplants* n *(%)*	*Patient's blood-cult (%)*
Coagulase-negative Staphylococcus	10 (31.1)	4 (23.5)	
Staphylococcus epidermidis	7 (21.9)	4 (23.5)	3 (60.0)
Corynebacterium sp.	2 (6.3)	2 (11.8)	1 (20.0)
S. warneri	2 (6.3)	2 (11.8)	
Stenotrophomonas maltophilia	2 (6.3)	1 (5.9)	1 (20.0)
Streptococcus sp.	3 (9.4)	2 (11.8)	
Viridans group Streptococcus	1 (3.1)	1 (5.9)	
Coagulase-negative Staphylococcus plus *Corynebacterium*	5 (15.6)	1 (5.9)	
Total	32 (100)	17 (100)	5 (100)

Table 3 Bacterial growth in patient's blood cultures

Bacteria	*Cases (%)*
None	59 (45.4)
S. epidermidis	38 (29.2)
Coagulase-negative Staphylococcus	9 (6.9)
Corynebacterium	3 (2.3)
S. aureus	3 (2.3)
Viridans group Streptococcus	4 (3.1)
S. haemolyticus	1 (0.8)
Enterococcus faecalis	1 (0.8)
E. coli	2 (1.5)
Enterobacter cloacae	1 (0.8)
Candida parapsilosis	1 (0.8)
S. epidermidis, Bacillus sp. plus *Pseudomonas maltophilia* plus *Candida sake*	1 (0.8)
S. epidermidis plus *S. maltophilia*	1 (0.8)
S. epidermidis plus *S. hominis*	1 (0.8)
S. epidermidis plus S. *warneri*	1 (0.8)
S. haemolyticus plus *Pseudomonas aeruginosa*	1 (0.8)
S. aureus plus *Pseudomonas spp*	1 (0.8)
Streptococcus mitis plus *Candida parapsilosis*	1 (0.8)
Streptococcus pneumoniae plus *S. epidermidis*	1 (0.8)
Total	130 (100)

In 59 febrile patients, blood cultures gave no bacterial growth, and in 71 patients they were positive. Bacterial growth in these cases is depicted in Table 3. The other 11 patients had positive bacterial cultures from other samples (urine, feces, etc.). Interestingly, in all patients transplanted from contaminated bags, positive bacteriological blood cultures were found, although in only five of these 17 patients were the isolated bacteria identical to those found in the bag culture (Table 2).

Four patients died within the first 90 days post transplant (transplant-related deaths): one with BC died on day +12 of *Streptococcus mitis* sepsis, one with BC of *Enterobacter cloacae* sepsis on day +13, one with HD died on day +18 of *S. epidermidis* sepsis and one MM patient died of veno-occlusive-disease 60 days post APBPCTs. None of these patients had received contaminated PBPCs.

The type and degree of toxicity (mucosal, hepatic, gastrointestinal, etc.), related to conditioning treatment, did not differ between the group of patients receiving contaminated and non-contaminated cells.

No statistical significance was found between patients transplanted with, and without contaminated PBPCs in a comparison of the first day of fever, duration of fever, requirement for red blood cell and platelet transfusions, granulocyte and platelet engraftment (Table 4 and Figure 1). However, duration of hospitalization was significantly higher in the group transplanted with contaminated products (Table 4). This significance remained when the subgroups of patients with, and without, positive blood cultures were compared with those receiving bacterially contaminated transplants. Comparison of these parameters between the groups of patients with and without fever showed no difference, neither did comparison between patients with, and without, bacterial isolates (data not shown). Because in the group of patients transplanted with contaminated PBPCs, there were no BC cases, comparison was repeated excluding these patients, but the result remained unchanged.

As in the contaminated group there was a higher proportion of MM than in the non-contaminated one, we repeated the comparison in only MM patients, and the duration of hospitalization remained higher in the MM contaminated group ($P = 0.04$).

No statistical difference in toxicity and in post-transplant course was found between the pre- and post-freezing bacterial contamination subgroups.

Table 4 Post-transplant course of patients receiving contaminated and non-contaminated transplants

	[a]*1st day of fever*	*Days of fever*	*Days in hospital*	[a]*Granulocyte engraftment*	[a]*Platelet engraftment*	*Packed RBC transfusion*	*Plat. Units transfusion*
Cont. ($n = 17$)	7 (−3–+17)	4 (1–11)	27 (16–51)	10 (+10–+18)	13 (+8–+25)	2 (0–6)	18 (6–64)
Non-cont. ($n = 124$)	5 (−5–+16)	4 (1–23)	22 (14–56)	11 (+8–+21)	12 (+1–+36)	2 (0–44)	21 (0–80)
P	NS	NS	**0.01**	NS	NS	NS	NS

Abbreviations: RBC = red blood cell; NS = non-significant.
[a]Day of infusion is considered day 0. RBC: red blood cell. Data are expressed as median (range).

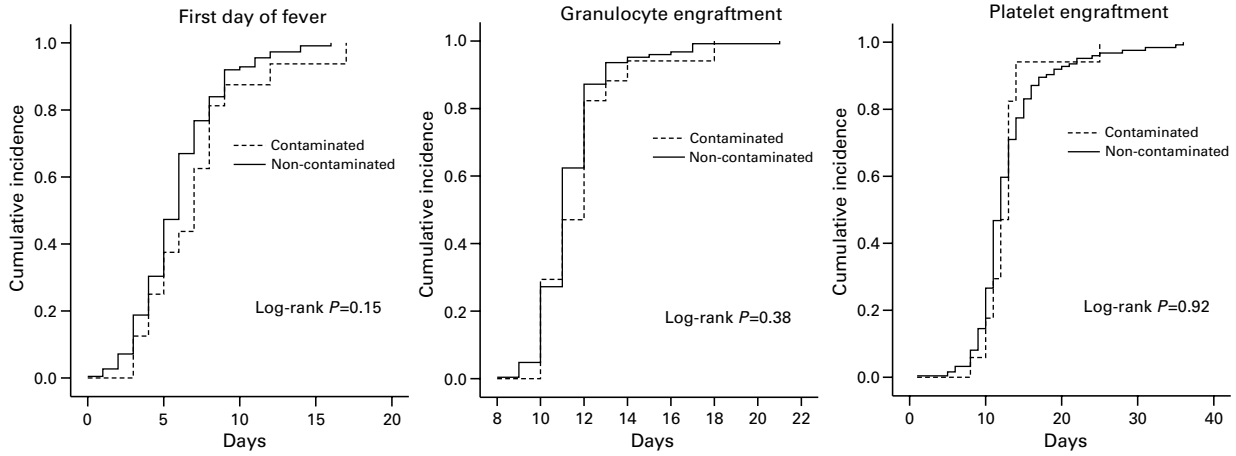

Figure 1 Cumulative incidence of first day of fever and granulocyte and platelet engraftment. No differences were found between the contaminated and non-contaminated group.

Discussion

PBPCs are used with increasing frequency in autologous as well as in allogeneic transplantation. Bacterial contamination is a well-known risk in PBPC transplants, especially autologous,[6,12,13] because these require more *ex-vivo* manipulation, and this complication seems very difficult to avoid even though the strictest standards of hygiene were adopted.[21] In our cases, bacterial PBPC contamination was more frequently caused by normal skin flora, as is the case in most of the previous reports.[1,6,7,14] As patients receiving APBPCTs have been treated with high-dose therapy, the temptation to discard contaminated PBPCs is reasonable;[12] but difficulty in obtaining sufficient PBPCs in subsequent aphereses, together with the fact that almost half of bacterial contaminations occur at the time of thawing, and results of these cultures are not available at the time of infusion, make this decision very difficult. Nonetheless, it is interesting that patients transplanted with positive bacterial products experienced no difference in infectious episodes compared with those transplanted with non-contaminated ones. Additionally, there was no difference with respect to the day the fever developed in either patient group. One possible explanation for lack of problems associated with contaminated PBPCs might be that granulocyte counts remain at adequate levels for about three days following infusion, possibly destroying contaminating bacteria.

In six bags, bacterial growth from frozen samples differed to the growth from thawed ones. This phenomenon has been reported by other authors[6,13] although no explanation is given in these reports. It is possible that storing the product in liquid nitrogen and DMSO decreases the bacterial burden[4,22] and this makes it impossible to detect bacteria present pre-freezing, whereas a new contamination occurs during the thawing process.

Although in five of the 17 patients transplanted with contaminated PBPCs the same bacteria were isolated from blood cultures, it was impossible to ascertain that it was the same infection because such infections are most common in this patient group. It is thus possible that these were new infections.

In our patients, no bacterial contamination was found among $CD34^+$ positively selected cells, in contrast to other publications,[5,6] probably because this technique is performed within closed systems with little manual manipulation.

Many authors have concluded that the use of bacterially contaminated products has no clinical importance among post-transplant infectious events.[6,7,9,12,14,16,23] In our experience, none of the patients receiving contaminated grafts developed clinical symptoms associated with them, and there was no effect on the post-transplant course. This accords with the study by Schwella *et al*.[9] of bone marrow transplants, except for the issue of longer hospitalization, not studied in that paper. As diagnoses were heterogeneous, and as MM was the most frequent diagnosis and the only one with sufficient cases in the contaminated PBPC group for a separate study, a comparison between MM patients in both groups was undertaken. The results remained the same; duration of hospitalization was again significantly longer after a contaminated transplant in MM, although the statistical significance was less than in the whole study ($P = 0.04$ vs 0.01). In our opinion, this difference should be interpreted with caution, and a study of more cases with a more homogeneous patient group could clarify this issue.

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