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ORIGINAL ARTICLE

Microbial contamination of hematopoietic progenitor cell products: clinical outcome

PA Patah[1], S Parmar[1], J McMannis[1], T Sadeghi[1], S Karandish[1], G Rondon[1], J Tarrand[2], R Champlin[1], M de Lima[1] and EJ Shpall[1]

[1]*Department of Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, TX, USA and* [2]*Department of Laboratorial Medicine, University of Texas MD Anderson Cancer Center, Houston, TX, USA*

We reviewed the results of routine microbiological assays of 3078 infused hematopoietic progenitor cell (HPC) products for autologous and allogeneic transplantation between January 2001 and December 2005. Thirty-seven (1.2%) contaminated products were found. All patients receiving contaminated infusions received empirical antibiotic prophylaxis according to the assay result. None of these patients developed a positive blood culture with the same agent, developed infections that could be attributable to the contaminated product or experienced any clinical sequelae. Coagulase-negative *Staphylococcus* was found in 32 (86.5%) products. Admission lengths and time to engraftment were within the expected time frame for autologous and allogeneic transplants. Microbial contamination of HPC products occurs at a low frequency; prophylactic use of antibiotics based on the microbiological assay appears to be effective in preventing clinical complications.

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Introduction

Microbial contamination of the infused hematopoietic progenitor cell (HPC) product is a potential source of infection following transplants. Microbial contamination of HPC products has been described in frequencies varying from close to 1% up to high contamination rates.[1–4] Harvesting, *ex vivo* processing, cryopreservation and the pre-infusion thawing process[5–7] can be responsible for contamination, owing to inadequate decontamination of skin at the needle puncture site, indwelling catheter site for peripheral blood progenitor cell (PBPC) collection or owing to contamination by laboratory personnel or equipment. Commonly documented species are part of the normal flora of the skin.[7,8]

Several small studies have reported favorable outcomes after infusion of contaminated products with minimal clinical consequences.[9–11] The US Food and Drug Administration (FDA) has recently established new rules for 'current good tissue practice for manufacturers of human cellular and tissue-based products', in which they describe a risk of 2.4% for contamination and raise concerns that there was a high rate of morbidity and mortality after receiving a contaminated product.[12] In this report, we retrospectively studied the outcomes of transplantation involving contaminated HPC product infusions performed during a 6-year period in a single institution.

Materials and methods

We reviewed 3078 infusions of HPC products given between 2001 and 2005 for autologous and allogeneic transplants at the University of Texas, MD Anderson Cancer Center, following Institutional Review Board approval of the study. Data were extracted from medical records and the Department of Stem Cell Transplantation and Cellular Therapy database. HPC products included marrow and PBPC.

Sterility testing was performed at the time of (1) marrow harvest or apheresis, (2) infusion, for freshly infused products, and (3) thawing and infusion, for cryopreserved products. Samples were obtained from HPC products by the MD Anderson Cell Therapy Laboratory. Aliquots from each product were inoculated directly into Bactec Standard/10 Aerobic/F or NR7 bottles (Becton Dickinson, Sharpsburg, MD, USA). The inoculum consisted of 2–3 ml of HPC product from each bag of component sent for culture: typically 2–5 components per product. On average, 1.6% of the transfused sample was submitted for culture (range, 0.15–4.4%), corresponding to 399 CFU/product, on average, required for positivity. Blood culture bottles were transported to the MD Anderson Microbiology Laboratory. These bottles were read on a continuous monitoring system for 14 days before being considered negative. Bottles that gave a positive signal were subcultured

Correspondence: Dr EJ Shpall, Department of Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, unit 423, Houston, TX 77035, USA.
E-mail: eshpall@mdanderson.org
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aerobically and anaerobically and all organisms were identified using standard instrumentation, methods and practices. In addition, antimicrobial susceptibility was generated on each isolate.

Patients routinely received systemic antibiotic prophylaxis post transplant, generally including a fluoroquinolone, acyclovir and fluconazole. For patients who received products known to have a positive culture, appropriate antibiotics were started before infusion, or as soon as a positive culture was reported for the recipients of fresh HPC products.

Clinical events were analyzed for all patients receiving HPC products with documented microbial contamination. We considered the patient's disease, cell source (BM or PBPC), positive cultures before infusion, infusion-related symptoms, occurrence of fever and positive cultures following the infusion, other complications including requirements for transfer to an intensive care unit, time to engraftment, length of admission and clinical status after discharge.

Results

From January 1, 2000 to December 31, 2005, 3078 patients received HPC products at MD Anderson Cancer Center. Thirty-seven products (1.2%) had microbial contamination detected at some point in the microbial testing process. Clinical data for the 37 patients who received contaminated infusions are summarized in Table 1.

The products were autologous for 21 patients and allogeneic for 16 (6 from related and 10 from unrelated donors); 15 patients received BM and 22 received PBPC. Eleven products were manipulated (mononuclear cell enriched, CD34-selected or plasma reduced) and eight were washed before infusion. Fifteen (40.5%) were fresh and 22 (59.5%) were cryopreserved. Contamination according to different steps of microbial sampling (harvesting or apheresis collection, before or after cryopreservation), number of positive samples and occurrence of fever or positive blood culture simultaneously with positive product culture are summarized in Table 2.

The most frequently isolated contaminating organisms were bacterial flora of normal skin, as shown in Table 3. Coagulase-negative Staphylococci (CNS) were detected in 31 of the 37 products at harvest or apheresis (both freshly infused or subsequently cryopreserved) and in one product (with previous sterile screening) only after thaw. Gram-positive cocci (non-specified; GPC) were positive in two products at harvesting or apheresis; *Enterococcus faecalis* was isolated in one frozen product at apheresis and after thaw; *Bacillus* species and *Micrococcus* species were isolated in one product each after thaw – both had previous sterile screenings. One product was found to have a CNS before cryopreservation and a GPC after thaw, and five products that had positive cultures for CNS before cryopreservation remained positive after thawing. Positive cultures for the fresh products were identified at a median of 4 days (range, 1–8). There were no instances of fungal contamination.

One patient was found to have a positive blood culture before infusion, identified as a Gram-negative rod; the infused HPC product was contaminated by a GPC. No further positive blood cultures were documented in that patient. None of the other patients who received microbially contaminated products had documented infections before HPC infusion.

Table 1 Characteristics of the 37 patients who received contaminated products

Age (years)	49 (20–68)
Male	23 (62%)
Diagnosis	
Hematological malignancies	32 (87%)
Aplastic anemia	2 (5%)
Non-hematological malignancies	3 (8%)
Type of transplant	
Autologous	21 (57%)
Allogeneic, unrelated donor	10 (27%)
Allogeneic, related donor	6 (16%)
HPC source	
Bone marrow	15 (41%)
Mobilized peripheral blood	22 (59%)
Processed[a]	
No	26 (70%)
Yes	11 (30%)
Fresh products	15 (41%)
Cryopreserved products	22 (59%)

Abbreviation: HPC = hematopoietic progenitor cell.
[a]Mononuclear cell enrichment, plasma reduced, volume reduced, CD34+ enrichment.

Table 2 Contamination at different microbial sampling steps

Positive in two or more different steps (fresh or frozen)	13 (35%)
Two or more samples sent to culture before cryopreservation, only one positive; negative after thaw (frozen)	8 (22%)
Two or more samples sent to culture, only one positive (fresh)	4 (11%)
One sample sent to culture before cryopreservation; negative after thaw (frozen)	2 (5%)
One sample sent to culture (fresh)	5 (13%)
Patient had fever and a positive blood or catheter culture with same agent (fresh or frozen)	3 (8%)
Positive only after thaw; negative at apheresis/harvest (frozen)	2 (5%)

Table 3 Results of positive cultures

Coagulase-negative Staphylococci	32[a]
Gram-positive cocci, nonspecified	3[a]
Bacillus species	1
Micrococcus species	1
Enterococcus faecalis	1

Abbreviations: CNS = coagulase-negative Staphylococci; GPC = Gram-positive cocci.
[a]One product had a positive culture for a CNS at apheresis and a GPC after thaw.

Two patients developed reactions during the infusion procedure. The first one had symptoms of chest pain with normal vital signs, physical examination and electrocardiogram. The other patient, who was known to have a cardiomyopathy, had acute pulmonary edema. All the other 35 patients were asymptomatic during infusions.

During the post transplant course, 6 of the 37 patients who had received contaminated HPC products had positive blood cultures, but all showed different bacteria than the ones isolated in their products. No patient had a documented bacteremia that could be ascribed to the contaminated infusion.

All autologous transplant patients had neutrophil recovery at a median of 10 days (range, 8–14). Among the allogeneic transplant patients, all but one engrafted in a median of 13 days (range, 5–21). The patient who did not engraft received an HLA-mismatched allograft and subsequently received her 'back-up' autologous HPC for treatment of presumed graft rejection.

The median length of in-patient admission was 21 days (range, 8–34) for autologous recipients and 28 days (20–65) for allogeneic transplant patients. One patient had prolonged hospitalization because of fever and hypotension on day +34, requiring transfer to the intensive care unit, but recovered and was discharged on day +44. No positive blood culture was identified; the patient was treated empirically with broad-spectrum antibiotics. No other recipients of contaminated infusions required intensive care unit admissions during their post transplant course.

At a median follow-up of 568 days (range, 49–1971), 8 or the 37 patients died, at a median of 166 days (range, 49–645). Causes of death were recurrence/persistence of disease in two patients, secondary AML after lymphoma treatment in one patient, acute GVHD in one patient, treatment-related toxicity after allogeneic transplant in two patients (pulmonary failure and multiple organ failure), brain abscess due to *Nocardia* in one patient and neutropenic fever with negative cultures leading to sepsis 75 days after transplant in one patient. None of the 37 patients experienced complications or had clinical sequelae attributable to the contaminated HPC infusions.

Discussion

This report is consistent with those of others published over the last decade, demonstrating that the rate of HPC product microbial contamination is low. The infusion of contaminated products is not associated with adverse clinical outcomes. In our group of 3078 patients receiving HPC products between 2001 and 2005, we found that 37 (1.2%) were contaminated with bacteria. Previous studies have reported microbial contamination rates within the same range, varying from 1.6 to 4.5%.[9,13,14]

Most of the bacteria isolated in this series are part of normal skin flora, predominately CNS – detected in 32 products (87%). The data are consistent with other published studies that also show skin bacteria as the most common microbial contaminant. Survival of CNS following cryopreservation has been variably reported. Some authors reported that they did not recover CNS from contaminated products after they were cryopreserved and subsequently thawed, while other authors stated that CNS can survive the cryopreservation and thawing procedures.[8,11,14] In our series, we found 5 products, among 13, which had positive cultures for CNS before cryopreservation and remained positive after thawing – making it important to take into account a positive culture before cryopreservation, when discussing antibiotic therapy.

All 37 patients who received contaminated products were treated with antibiotics appropriate for the specific organisms identified, in addition to the standard antibiotic prophylaxis, generally fluoroquinolone, acyclovir and fluconazole. Antibiotics were started before transplant, for patients who had a positive culture obtained before cryopreservation or as soon as a positive culture was reported after infusion. We do not know what would have happened had our patients not received antibiotics. Thus, our experience with the antibiotic administration policy as described is one of several strategies that could be useful in this setting.

The median length of in-patient admission for the 37 patients who received respectively the autologous- and allogeneic-contaminated products was 21 and 28 days, which is within the expected length for these procedures. Engraftment was not impaired by microbial contamination. All patients receiving an autologous transplant engrafted in a median of 10 days, while all but one patient receiving an allogeneic transplant engrafted at a median of 13 days. The one patient who did not engraft received a related allograft mismatched at two human leukocyte antigens, and this was probably the reason for rejection.

Among patients receiving contaminated infusions, only one died during that admission; the cause of death was acute GVHD. With a median follow-up of 568 days, the mortality rate was 21.6%; no deaths could be ascribed to contaminated infusions.

We conclude that microbial contamination of HPC products is primarily caused by normal skin flora. Similar results have been reported by others,[9,11,14] with favorable outcomes, and no major complications, following infusion of microbial-contaminated HPC products.

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