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Does microbial contamination influence the success of the hematopoietic cell transplantation outcomes?

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ABSTRACT

Introduction: Microbial contamination can be a marker for faulty process and is assumed to play an important role in the collection of hematopoietic progenitor cell (HPC) and infusion procedure. We aimed to determine the microbial contamination rates and evaluate the success of hematopoietic cell transplantation (HCT) in patients who received contaminated products.

Patients-methods: We analyzed microbial contamination records of HPC grafts between 2012 and 2015, retrospectively. Contamination rates of autologous donors were evaluated for at three steps: at the end of mobilization, following processing with dimethyl sulfoxide, and just before stem cell infusion. Grafts of allogeneic donors were assessed only before HCT.

Result: A total of 445 mobilization procedures were carried out on 333 (167 autologous and 166 allogeneic) donors. The microbiological contamination of peripheral blood (323/333 donations) and bone marrow (10/333 donations) products were analyzed. Bacterial contamination was detected in 18 of 1552 (1.15 %) culture bottles of 333 donors. During the study period 248 patients underwent HCT and among these patients microbial contamination rate on sample basis was 1.3 % (16/1212). Microbial contamination detected in nine patients (7 autologous; 2 allogeneic). In 8 of 9 patients, a febrile neutropenic attack was observed. The median day for the neutropenic fever was 4 days (0–9). None of the patients died within the post-transplant 30 days who received contaminated products.

Conclusion: The use of contaminated products with antibiotic prophylaxis may be safe in terms of the first day of fever, duration of fever, neutrophil, platelet engraftment and duration of hospitalization.

1. Introduction

Autologous and allogeneic hematopoietic cell transplantations (HCT) are commonly used for the treatment of hematological diseases. In stem cell products, microbial contamination incidence was 0.2–26.3% [1–14]. It can be caused by inadequate decontamination of skin at the needle puncture site, indwelling catheter site during the harvesting, ex vivo processing, cryopreservation, and the pre-infusion thawing process and contamination by laboratory staff or equipment [1–10]. Microbial contamination can be a marker for faulty process and is assumed to play an important role in the collection of hematopoietic progenitor cell (HPC) and infusion procedure. On the other hand, its requirement is

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unclear for the success of HCT and infusion procedure. In a variety of studies, it was reported that microbial contamination of stem cells did not cause any adverse events in patients who received contaminated HPC products [1–8]. Therefore, in this study, we aimed to determine our microbial contamination rates during collection, processing and infusion steps of HPC products and then evaluate the success of HCT in patients who received contaminated HPC products.

2. Material and method

2.1. Patients

Microbial cultures of 445 HPCs of 333 donors (167 autologous and 166 allogeneic) with various hematologic disorders between February 2012 and January 2015 were retrospectively analyzed. The age of the patients ranged between 16 and 72 years. The demographic characteristics of the patients were presented in Table 1. Clinical outcomes of the patients who received contaminated products were recorded and analyzed.

2.2. Stem cell collection

Stem cells were collected by bone marrow (BM) harvesting and peripheral blood progenitor cell (PBPC) apheresis. PBPCs were mobilized with 10 μg/kg daily doses of recombinant human granulocyte colony stimulating factor (GCSF; filgrastim or lenograstim). The insertion of a central venous catheter under sterile conditions was succeeded by the collection of cells. In collecting the HPCs, a continuous-flow blood cell separator spectra optia apheresis system (TerumoBCT, USA), was used. The cell separator was included in processing three total blood volumes at each of the collections, which was made in order to collect at least 3×10^6/kg and 5×10^6/kg CD34 + cells for multiple myelomas and other indications, respectively. Collections were continued until the above threshold dose of PBPCs was obtained unless any donor complication related to mobilization was observed necessitating termination of the apheresis procedure. When mobilization with G-CSF failed, further mobilization with G-CSF plus chemotherapy, G-CSF and plerixafor or BM harvesting was performed in autologous donors. Also, HPC collections were performed via BM harvesting under general anesthesia for the donors of patients diagnosed with aplastic anemia or donors refusing PBPC mobilization.

2.3. Microbial sampling

Microbial cultures of HPCs for autologous HCT candidates were performed in three phases: (1) subsequent to the marrow harvesting or PBPC apheresis, (2) following the process with cryoprotective agent DMSO (Dimethyl sulfoxide), and (3) just before the infusion of thawed HPCs. On the other hand, HPCs of allogeneic donors were analyzed only following marrow harvesting or PBPC apheresis. Two culture bottles each consisting 1 ml of HPC product were evaluated at all phases. For each patient who could receive autologous or allogeneic HCT, a total of 6 or 2 culture bottles (samples) were analyzed for microbial contamination. HPC products were inoculated into BacT/ALERT 3D automated system blood culture bottles. These bottles were incubated in the same automated system for at least seven days. The bottles with positive signals were subcultured in 5% sheep blood agar, chocolate agar, eosin methylene blue agar and Sabouraud dextrose agar. An automated identification system (VITEK 2 Compact, bioMerieux, Marcy-10 Etoile, France) was used to identify the microorganisms. Antimicrobial susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guideline. Patients routinely received systemic antibiotic prophylaxis, generally including a fluoroquinolone, trimethoprim- sulfamethoxazole (TMP-SMX) and acyclovir, fluconazole. For patients who received products with documented microbial contamination, appropriate antibiotics such as vancomycin were started as soon as an occurrence of febrile neutropenia or any sign of blood stream infection. Otherwise, no preemptive therapy was commenced following HCT with contaminated HPC product.

2.4. Statistical analysis

Descriptive statistics were presented as median and range. Mann–Whitney U test was used for the comparisons of the first day of fever, duration of fever, engraftment days and duration of hospitalization between groups. $P < 0.05$ was considered to be of statistical significance.

3. Results

Between February 2012 and January 2015, a total of 333 donors (167 autologous and 166 allogeneic) were mobilized and 445 HPC products were obtained. We analyzed 1552 culture bottles and bacterial contamination was

Table 1
Characteristics of patients.

Gender		Age	Diagnosis				Donor type		HPC* source	
Male	Female		Leukemia	Lymphoma	Multiple myeloma	Others†	Allogeneic	Autolog	Bone marrow	PBPC**
132	116	32	135	49	47	17	166	82	10	238
53%	47%	(16–72)	(54%)	(20%)	(19%)	(7%)	(67%)	(33%)	(4%)	(96%)

* HPC: Hematopoietic progenitor cell.
** PBPC: Peripheral blood progenitor cell.
† Others: Paroxysmal nocturnal hemoglobinuria, myelodysplastic syndromes, aplastic anemia, testicular tumor.

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Table 2
Distribution of bacteria isolated from hematopoietic progenitor cell products in different periods.

Bacteria	Apheresis	Cryopreservation	Infusion
S. aureus	1		
S. epidermidis	5	3	2
S. hemolyticus	1	1	1
Proteus mirabilis	1		
Sphingomonas paucimobilis	1		
Shigella spp.		1	
Diphteroid bacilli	1		

detected in 18 of 1552 (1.15 %) samples from 333 donors. During the study period, 248 patients underwent HCT. Microbial contamination rate on sample basis among patients who treated with HCT was found to be 1.3% (16/1212). Demographic and clinical data of patients who received HSC products were summarized in Table 1. Microbial contamination was detected in 9 patients (3.6%) (7 autologous; 2 allogeneic) who received HCT. Out of the 16 samples among the recipients of HCT, 8 (1.1%) were culture positive after mobilization, and 5 (1.3%) after cryopreservation; 3 (1.3%) were found to be culture positive before infusion. However, one of them was sterile during the periods of apheresis and processing, while there was detected bacterial contamination before infusion. Whereas eight out of 16 products (50%) were sterile in the post-thawing period, contamination was detected in them after apheresis and cryopreservation. *Staphylococcus epidermis* was the most common isolated pathogen in apheresis (5/9; 55%), cryopreservation (3/6; 50%) and infusion (2/3; 66%) phases. Table 2 indicated the distribution of microbial isolates from the samples.

After the infusion of contaminated stem cell products, 8 of 9 patients had a febrile neutropenic attack, and the median day for the neutropenic fever was 4 days (range 0–9). There were positive blood cultures in 5 out of 8 patients at the first febrile attack. In two of these five patients, the same species of bacteria were isolated as those found in the contaminated HPC product. Median day for neutrophil engraftment was 12 days (8–16), median day for platelet engraftment was 16 days (11–24) and median day for the duration of hospitalization was 21 days (15–37) who received contaminated products. In 10 patients who received uncontaminated products, neutrophil or platelet engraftment was not observed. These patients were not evaluated for neutrophil and platelet engraftment days. Comparing of HCT with contaminated and uncontaminated products were summarized in Table 3. Furthermore, none of the patients who received contaminated products died within the post-transplant 30 days.

4. Discussion

Microbial contamination incidence may change in various hospitals. The contamination rate in HPC products in our hospital was 13% between 2010 and 2012. In the 2010–2012 period, microbial contamination rate on sample basis among patients who were treated with HCT was found to be 8%. After 2012, we made efforts for decreasing the contamination rates in our hospital. We took precautions for the collection such as freezing, and storage of samples such as frequent replacement of filters of biological cabinets, disinfection of bain-marie, cleaning of the biological cabinet after each process, the wiping of sample bags with chlorhexidine, adherence to aseptic rules, and education of apheresis team. In current study conducted between 2012 and 2015 among 248 patients underwent HCT, microbial contamination rate on sample basis among patients who were treated with HCT was found to be 1.3 % and overall contamination rate was 1.15%. We suggested that applying of these precautions was necessary to reduce the microbial contamination rates in HCT products. Kozlowska-Skrzypczak M et al. evaluated the collection and preparation steps by a retrospective analysis of the microbial cultures of 330 donations. They reported 9 (2.73%) contaminated HSC products. Bacillus species were the most frequently isolated bacteria from PB and BM products. On the other hand, coagulase-negative staphylococci and Micrococcus species were the most frequent microorganisms detected in the air microbial control. They suggested that microbial control results were necessary for the safety of hematopoietic stem cell products transplantation [15]. Kamble et al. studied a retrospective analysis of 735 consecutive BM and PBPC harvests between 1998 and 2003. In their study, thirty-three of 735 (4.5%) harvests were contaminated. The incidence of microbial contamination varied with the source of the graft (4 of 26 [15%] was cord blood, 8 of 177 [4.5%] were marrow, and 21 of 532 [3.9%] were peripheral blood). Coagulase-negative Staphylococcus and Propionibacterium acnes were most frequently isolated bacteria [2]. In a Turkish study of Donmez et al. microbial contamination was detected in 28 (5.7%) products of the postprocessing period and in 18 (3.66%) products of the post-thawing period. Large volume leukapheresis and high numbers of stem cell culture sampling were found to be risk factors for postprocessing bacterial contamination [3]. In our study following mobilization, after cryopreservation and before infusion steps 1.1%, 1.3%, and 1.3% out of 16 samples among HCT recipients were found to be culture positive, respectively. One out of 16 samples was sterile during apheresis and processing periods but bacterial contamination was detected before infusion. In the post-thawing period, 50% of the products were

Table 3
Comparing of hematopoietic cell transplantation with contaminated and uncontaminated products.

	*HCT with contaminated products	HCT with uncontaminated products	*p* value
Median day for the neutropenic fever	4 days (0–9)	5 days (0–10)	$p > 0.05$
Median Neutrophil engraftment days	12 days (8–16)	13 days (9–19)	$p > 0.05$
Median Platelet engraftment days	16 days (11–24)	17 days (13–26)	$p > 0.05$
Median day for duration of hospitalization	21 days (15–37)	20 days (14–40)	$p > 0.05$

* HCT: Hematopoietic cell transplantation.

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sterile although they were detected as contaminated following apheresis and cryopreservation. Similar to other studies Staphylococcus epidermis was the most common isolated pathogen which is a normal skin flora member [2–4]. Patah PA et al. reviewed the results of routine microbiological assays of 3078 infused HPC products for autologous and allogeneic transplantation. Thirty-seven (1.2%) contaminated products were found. All patients receiving contaminated infusions received empirical antibiotic prophylaxis and 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. Admission lengths and time to engraftment were within the expected time frame for autologous and allogeneic transplants [6]. In our study, we observed that after the infusion of contaminated stem cell products, 8 of 9 patients had febrile neutropenic attack and median day for the neutropenic fever was 4 days. The limitation of our study may be the small number of the cases who received contaminated products. On the other hand, our patients routinely received systemic antibiotic prophylaxis during until neutrophil engraftment.

In conclusion, skin flora members were the most isolated bacteria from the HPC products. The contamination rates can be reduced with the precautions on collection, freezing, and storage of samples such as frequent replacement of upper and lower filters of biological cabinets, disinfection of bain-marie, cleaning of the biological cabinet after each process, the wiping of sample bags with chlorhexidine, adherence to aseptic rules. We also concluded that the use of contaminated products with antibiotic prophylaxis may be safe in terms of the first day of fever, duration of fever, neutrophil, platelet engraftment and duration of hospitalization. However, the large-scale studies are needed to determine the success of HCT in patients who received contaminated HPC products.

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