Original article

Autologous transplant: microbial contamination of hematopoietic stem cell products

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ABSTRACT

Hematopoietic progenitor cells from peripheral blood (HPCPB) are commonly used for autologous and allogenic transplants in patients with most various onco-hematological diseases, and despite the utilization of sterile techniques during collection and processing of these products, bacterial contamination can occur. This study aimed to investigate the microbial contamination of HPCPB products. Microbial cultures of 837 HPCPB products between the year 2000 and 2009 were retrospectively analyzed to determine the incidence of culture positivity and identify the main organisms that cause contamination. The microbiological studies were performed with an automated system (BacT/Alert® bioMérieux Corporate).

Thirty-six (4.3%) of 837 microbial cultures were contaminated. Coagulase-negative Staphylococcus was the most frequent bacteria isolated from HPCPB products (20 [56%] of the 36 positive microbial cultures). Considering the 36 contaminated samples, 22 HPCPB products were infused and 14 discarded. Pre- and post-infusion antibiotic therapy of the patients transfused with contaminated products was established based on the isolated microorganism and its antibiogram. Microbial contamination rate of HPCPB products was low. Clinically significant outcomes after infusion of contaminated HPCPB products were not observed.

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Introduction

Hematopoietic progenitor cells from peripheral blood (HPCPB) are commonly used for autologous and allogenic transplants in patients with various onco-hematological diseases. The progenitor hematopoietic cells are capable of self-renewal and differentiation in all blood cells lineages. Bone marrow is the traditional source for obtaining HPCPB, collected by multiple punctures and aspirations of the posterior iliac crests. The aspirated material contains red blood cells, leukocytes, platelets, mast cells, plasma, and pluripotent hematopoietic progenitor cells. In recent years, the collection of HPCPB via apheresis has been increasingly used. The combination of

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Rapidly growing organism
The donor may be
Fig. 2 presents the annual contamination rate of the
tamination at the time of water-bath defrosting. Cultures were
of the infusion. Such action serves to verify a possible con-
samples were collected for microbial cultures at the moment
Corporation–Durham, USA). In addition, after blood bag thawing,
tles with 20 mL of activated charcoal (BacT/Alert®)
of the product were inoculated in pediatric blood culture bot-
were reviewed.
charts of the donors with positive HPCPB microbial cultures
the main organisms causing contamination. In addition, the
incidence of microbial culture positivity and identification of
containing HPCPB, which were infused or discarded according to
2000 to 2009 were retrospectively analyzed to determine the
addition, the blood culture results after thawing the bag con-
therapy for the contaminated cells were also described. In
bacterial contamination of these cells.
56% of the 837 collected samples (4.3%) yielded positive cultures for
the 837 collected samples (4.3%) yielded positive cultures for
from 2000 to 2009. As shown in Fig. 2, the

high doses of chemotherapy with subsequent transplantation
of these cells constitutes the standard treatment for many
onco-hematological diseases.
Obtaining, processing, storing and transplantation of
HPCPB involve many steps, which are normally performed
in different environments and may result in microbial con-
tamination. In fact, HPCPB manipulation during processing
and pre- and post-cryopreservation are important sources of
bacterial contamination of these cells.1 The donor may be
the source of microbial contamination of HPCPB. Donors with
asymptomatic bacteremia or who are recovering from a bac-
terial infection may develop episodes of transient bacteremia,
which can lead to product contamination. In addition, HPCPB
collected by apheresis often requires the insertion of central
venous catheters (CVC). Infections associated with CVCs are
an important source of transient bacteremia and a possible
cause of HPCPB contamination.2
Thus, in order to ensure a final product appropriate for
transplant, it is essential to adhere to a quality control policy.
Such controls should include CD34+ cell count, cell viability
assessment, and microbiological monitoring.3
The main objective of this study was to investigate the
incidence of positive microbial cultures for HPCPB products
from donors attending a tertiary care hospital in the period
from 2000 to 2009. In parallel, the major bacteria contaminat-
ing HPCPB products and the pre- and post-infusion antibiotic
therapy for the contaminated cells were also described. In
addition, the blood culture results after thawing the bag con-
taining HPCPB, which were infused or discarded according to
medical decision, were also analyzed (Table 1).

<table>
<thead>
<tr>
<th>Table 1 – Management considerations of HPCPB products with positive microbial cultures.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administer the product</td>
</tr>
<tr>
<td>Slow growing organism</td>
</tr>
<tr>
<td>Skin or environmental contaminant</td>
</tr>
<tr>
<td>Donor or patient is not available for recollection</td>
</tr>
<tr>
<td>New product requires remobilization or central line placement</td>
</tr>
<tr>
<td>Product contains majority of total cell dose</td>
</tr>
</tbody>
</table>

HPCPB, hematopoietic progenitor cells from peripheral blood.
* Culture positivity beyond 30 hours of incubation.

Material and methods

Microbial cultures of 837 HPCPB products of donors attend-
ing a tertiary care hospital located in southern Brazil from
2000 to 2009 were retrospectively analyzed to determine the
incidence of microbial culture positivity and identification of
the main organisms causing contamination. In addition, the
charts of the donors with positive HPCPB microbial cultures
were reviewed.
For the sterility control of the HPCPB products, after the
cryopreservation process and before freezing, 3 mL samples
of the product were inoculated in pediatric blood culture bot-
tles with 20 mL of activated charcoal (BacT/Alert®) bioMérieux
Corporation–Durham, USA). In addition, after blood bag thawing,
samples were collected for microbial cultures at the moment
of the infusion. Such action serves to verify a possible con-
tamination at the time of water-bath defrosting. Cultures were
sent to the microbiology department, where they were incu-
bated for five days. When positive, microscopy and bacterial
isolation were performed and identified through standard bio-
chemical tests.
Data were organized and analyzed using the Microsoft
Excel 2007® software, according to the distribution of fre-
quency.
Microbiological surveys were performed with automated
BacT/Alert® at 36 °C. The products were added into a class I
laminar-flow cabinet with HEPA filters.
The study was approved by the local ethics committee,
which is accredited by the National Committee of Ethics in
Research of the National Health Department and the Office
for Human Research Protection (OHRP) of the United States.

Results

A total of 837 HPCPB collections and microbial cultures
were performed at the hemotherapy section from 2000 to
2009. The average volume drawn and time for collection
and processing were 255 mL and 206 minutes respectively.
The underlying diseases and the main characteristics of
the patients that received HPCPB products are presented in
Table 2. The main underlying diseases included multiple
myeloma (n = 314), followed by Hodgkin lymphoma (n = 143),
non-Hodgkin lymphoma (n = 132), acute myeloid leukemia
(n = 63), neuroblastoma (n = 52), Wilms tumor (n = 28), medu-
loblastoma (n = 23), and Ewing sarcoma (n = 16). Thirty-six of
the 837 collected samples (4.3%) yielded positive cultures for
bacteria. Fig. 1 presents the annual contamination rate of the
HPCPB products from 2000 to 2009. As shown in Fig. 2, the

![Fig. 1 – Annual rate of contamination in samples collected in the period from 2000 to 2009. Distribution of the 36 contaminated samples of total 837 made in this period.](image-url)
most frequently isolated organism was coagulase-negative Staphylococcus (56%), followed by Staphylococcus aureus (17%), Bacillus sp. (8%), coryneform Gram-positive bacilli (8%), non-coryneform Gram-positive bacilli (6%), Enterobacter sp. (3%), and Citrobacter freundii (3%). Coagulase-negative Staphylococcus isolates were 100% resistant to beta-lactams antibiotics including oxacillin.

Twenty-two of the 36 HPCPB products with positive microbial cultures were infused, and 14 were discarded based on the medical staff's decision. Considering that HPCPB products contamination has an impact in reducing the number of CD34+ cells, and therefore reducing hematopoietic engraftment after peripheral blood stem cell transplantation, Table 3 presents the CD34+ counts of the discarded contaminated HPCPB collections and the remaining stock collections for each patient.

Table 4 presents pre-infusion and post-infusion antibiotic therapy of the patients transfused with contaminated products. Although nine of the 22 infusions had not received antimicrobial therapy prior to the infusion of HPCPB products, all of the patients received such therapy during or after the HPCPB infusion.

Twelve (55%) of the 22 contaminated HPCPB products presented positive microbial cultures after the freeze-thaw

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**Table 2 – Profile of underlying diseases and HPCPB collections performed at the Hemotherapy Section from 2000-2009.**

<table>
<thead>
<tr>
<th>Underlying disease</th>
<th>n (collections)</th>
<th>Age (average)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>Amiloidosis</td>
<td>3</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>Erythroid series aplasia</td>
<td>1</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>Pleuropulmonary blastoma</td>
<td>3</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>CNS germimoma</td>
<td>1</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Immunocytoyma</td>
<td>1</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>2</td>
<td>17</td>
<td>–</td>
</tr>
<tr>
<td>Acute lymphoide leukemia</td>
<td>5</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>63</td>
<td>31</td>
<td>47</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>2</td>
<td>42</td>
<td>–</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td>1</td>
<td>32</td>
<td>–</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>143</td>
<td>26</td>
<td>59</td>
</tr>
<tr>
<td>Mantle-cell lymphoma</td>
<td>2</td>
<td>57</td>
<td>2</td>
</tr>
<tr>
<td>B-cell granulocytic lymphoma</td>
<td>3</td>
<td>41</td>
<td>3</td>
</tr>
<tr>
<td>T-cell lymphoblastic lymphoma</td>
<td>1</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>132</td>
<td>43</td>
<td>82</td>
</tr>
<tr>
<td>Peripheral T lymphoma</td>
<td>2</td>
<td>33</td>
<td>–</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>23</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Myelohyperbrosis</td>
<td>1</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>314</td>
<td>54</td>
<td>170</td>
</tr>
<tr>
<td>Tumor of suprarenal gland</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Thoracic malignant neoplasm</td>
<td>1</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Malignant neoplasm of kidney and renal pelvis</td>
<td>2</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>52</td>
<td>4</td>
<td>41</td>
</tr>
<tr>
<td>Pancreatoblastoma</td>
<td>1</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td>Pineaoblastoma</td>
<td>2</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Renal cell sarcoma</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>16</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Wilms sarcoma</td>
<td>1</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Poems syndrome + Castleman disease</td>
<td>1</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Askin tumor</td>
<td>5</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Renal cell tumor</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Ewing tumor</td>
<td>1</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>Ovarian tumor</td>
<td>2</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>Endodermal sinus tumor</td>
<td>4</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Testicle tumor</td>
<td>3</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Wilms tumor</td>
<td>28</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Germinative tumor</td>
<td>4</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>837</strong></td>
<td><strong>470</strong></td>
<td><strong>367</strong></td>
</tr>
</tbody>
</table>

**Fig. 2 – Bacteria isolated in the 36 contaminated hematopoietic progenitor cells from peripheral blood products.**
### Table 3 – CD34+ count of contaminated and remaining stock HPCPB collections.

<table>
<thead>
<tr>
<th>Donor-bacteria</th>
<th>nX10^6 CD34/Kg (discarded collection)</th>
<th>nX10^6 CD34/Kg (remaining stock collections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Citrobacter freundii</td>
<td>0.35</td>
<td>1.43</td>
</tr>
<tr>
<td>2- Staphylococcus coagulase neg.</td>
<td>0.13</td>
<td>0.29</td>
</tr>
<tr>
<td>3- Staphylococcus coagulase neg.</td>
<td>3.21</td>
<td>3.33</td>
</tr>
<tr>
<td>3- Staphylococcus aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3- Staphylococcus aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3- Staphylococcus aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4- Staphylococcus aureus</td>
<td>1.67</td>
<td>2.56</td>
</tr>
<tr>
<td>5- Staphylococcus aureus</td>
<td>0.32</td>
<td>1.39</td>
</tr>
<tr>
<td>6- Staphylococcus coagulase neg.</td>
<td>3.2</td>
<td>7.61</td>
</tr>
<tr>
<td>7- Staphylococcus aureus</td>
<td>1.01</td>
<td>4.11</td>
</tr>
<tr>
<td>8- Coryneform Gram+ bacilli</td>
<td>1.33</td>
<td>-</td>
</tr>
<tr>
<td>8- Coryneform Gram+ bacilli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9- Staphylococcus coagulase Neg.</td>
<td>1.27</td>
<td>3.14</td>
</tr>
</tbody>
</table>

HPCPB, hematopoietic progenitor cells from peripheral blood.

* Total of 14 discarded collections; each number represents a patient; patients 3 and 8 had more than one collection.

### Table 4 – Antibiotic therapy of pre- and post-infusion of contaminated HPCPB.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Non-coryneform Gram + bacilli</td>
<td>Non-administered</td>
<td>Norfloxacin</td>
</tr>
<tr>
<td>2- Staphylococcus coagulase neg.</td>
<td>Non-administered</td>
<td>Ampicillin + cefepime</td>
</tr>
<tr>
<td>3- Bacillus sp.</td>
<td>Ciprofloxacin</td>
<td>Vancomycin + ciprofloxacin</td>
</tr>
<tr>
<td>4- Staphylococcus coagulase neg.</td>
<td>Sulfa + trimethoprim</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>5- Enterobacter sp.</td>
<td>Norfloxacin + sulfa + trimethoprim</td>
<td>Cefepime</td>
</tr>
<tr>
<td>6- Staphylococcus coagulase neg.</td>
<td>Non-administered</td>
<td>Gentamicin + cefepime</td>
</tr>
<tr>
<td>7- Staphylococcus coagulase neg.</td>
<td>Non-administered</td>
<td>Vancomycin + cefepime</td>
</tr>
<tr>
<td>8- Bacillus Gram+ coryneform</td>
<td>Ciprofloxacin</td>
<td>Vancomycin + cefepime</td>
</tr>
<tr>
<td>9- Staphylococcus coagulase neg.</td>
<td>Clindamycin</td>
<td>Cefepime + amikacina</td>
</tr>
<tr>
<td>10- Staphylococcus coagulase neg.</td>
<td>Ciprofloxacin</td>
<td>Oxacillin + cefepime</td>
</tr>
<tr>
<td>11- Staphylococcus coagulase neg.</td>
<td>Oxacillin</td>
<td>Vancomycin + cefepime</td>
</tr>
<tr>
<td>12- Bacillus sp.</td>
<td>Cefepime</td>
<td>Amikacin</td>
</tr>
<tr>
<td>13- Bacillus sp.</td>
<td>Non-administered</td>
<td>Vancomycin + cefepime</td>
</tr>
<tr>
<td>14- Staphylococcus coagulase neg.</td>
<td>Clindamycin</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>15- Staphylococcus coagulase neg.</td>
<td>Non-administered</td>
<td>Oxacillin + cefepime</td>
</tr>
<tr>
<td>16- Staphylococcus coagulase neg.</td>
<td>Clindamycin</td>
<td>Cefepime + oxacillin</td>
</tr>
<tr>
<td>17- Staphylococcus coagulase neg.</td>
<td>Sulfa + trimethoprim</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>17- Staphylococcus coagulase neg.</td>
<td>Sulfa + trimethoprim</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>18- Staphylococcus coagulase neg.</td>
<td>Non-administered</td>
<td>Cefepime + clindamycin</td>
</tr>
<tr>
<td>19- Staphylococcus coagulase neg.</td>
<td>Oxacillin</td>
<td>Cefepime</td>
</tr>
<tr>
<td>20- Staphylococcus coagulase neg.</td>
<td>Non-administered</td>
<td>Cefepime + vancomycin</td>
</tr>
<tr>
<td>21- Non-coryneform Gram+ bacilli</td>
<td>Non-administered</td>
<td>Cefepime</td>
</tr>
</tbody>
</table>

HPCPB, hematopoietic progenitor cells from peripheral blood.

* The above contaminated infusions were performed in different patients, except nr. 17.

### Discussion

Similar to the present results, previous studies have reported microbial contamination rates varying from 1.6% to 4.5%. The incidence of microbial contamination of HPCPB products in those studies varied according to the source of the cells. Kamble et al. have shown contamination in four of the 26 collections (15%) from core blood, eight of 177 (4.5%) from bone marrow, and 21 of 532 (3.9%) from peripheral blood. Coagulase-negative Staphylococcus was the organism predominantly isolated in this study, with 20 (56%) of the 36 positive microbial cultures. Most of the previous studies also identified the coagulase-negative Staphylococcus and other bacteria that often colonize the skin and are water contaminants. The potential contamination sources of HPCPB products include reagents, venous access-catheters, aseptic failure, cell processing, bag disruption, equipment used for water-bath, incubators, and centrifuges.

Even though contaminated HPCPB products are often discarded, 22 of the 36 contaminated collections were infused. Authors have reported success achieved after the infusion of the contaminated HPCPB products, with few
Contamination of HPCPB products with clinically significant adverse outcome occurs especially with potentially pathogenic bacteria, but is rare, with an incidence of 0.3% of notified cases. Klei et al. have reported a patient that died due to multi-organ-system failure, after having received HPCPB product contaminated with <i>Burkholderia cepacia</i>. Even though the infusion was initiated with proper antimicrobial therapy. Moreover, contaminated HPCPB products with methicillin-resistant <i>Staphylococcus aureus</i> have been shown to cause severely disseminated infection in patients. Interestingly, bacterial contamination of those products does not affect the patients’ transplant kinetics. It was previously demonstrated by Schwella et al. that there was no significant differences in hematopoietic recovery time, duration of fever, and number of days of antimicrobial administration in patients who had received contaminated HPCPB products when compared with those who had received products free of contamination. In fact, previous studies have shown that the CFUs of different bacteria diminished after the cryopreservation process. For instance, the CFU of <i>Staphylococcus epidermidis</i>, decreased approximately 13.7% after addition of DMSO. Moreover, the presence of active phagocytic cells in the frozen products could additionally eliminate existing bacteria. On the other hand, Gram-positive bacteria such as <i>Staphylococcus</i> sp. are known to survive after the cryopreservation process. Different studies have shown conflicting results regarding the survival of coagulase-negative <i>Staphylococcus</i> after the cryopreservation process.

Data have shown that aseptic conditions impact bacterial contamination in areas where HPCPB products are handled and processed. There is a 5.2% decrease in HPCPB products contamination at a clean bench compared with 0.8% decrease at a bench in laboratory implementing good manufacturing practices with certified conditions. Thus, quality control and good practices of handling and conservation of reagents and equipment used in cell cryopreservation are essential to provide safer products for patients, with reduction of the probable contamination sources.

In summary, our study has shown that the contamination rate of HPCPB products is overall low and it is usually caused by the normal skin microbiota, which could survive the cryopreservation process. No clinically significant outcomes were observed in patients transfused with contaminated HPCPB products. Continuous monitoring of HPCPB products is essential to assure the success of the transplantation.

Conflict of interest

All authors declare to have no conflict of interest.

REFERENCES