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HAEMATOLOGY

Microbial contamination of autologous peripheral blood stem cell products: incidence, clinical outcome, quality control and management strategies

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Summary
Background: Microbial screening is part of quality control for autologous peripheral blood stem cell (PBSC) manipulation. The incidence of culture positive products varies widely. The clinical significance of infusing culture positive products is uncertain, with no consensus on the interventions required.
Methods: Microbial cultures of 606 consecutive autologous PBSC products from 278 patients between 1995 and 2005 were retrospectively analysed to determine the incidence of culture positivity and classified according to likely clinical significance. Clinical outcomes of patients who received culture positive products were reviewed. Using the baseline probability of contamination, a quality control method to detect a systematic source of contamination during product manipulation was developed.
Results: Eleven (1.8%) of 606 products were culture positive in eight (2.9%) patients. Ten products yielded coagulase negative Staphylococcus species and one Corynebacterium accolens. One patient had catheter sepsis during collection. Five patients received culture positive products, with prophylactic antibiotics when there was known coagulase negative Staphylococcus species. None had clinical evidence of infection following product infusion.
Conclusions: The incidence of culture positive products was comparable to published series. Only skin commensals were isolated. Infusion of skin commensal positive products appears safe with antibiotic prophylaxis. Using local and published experience, clinical and laboratory policies were developed for culture positive products.

Key words: Bone marrow transplantation, haematopoietic progenitor cells, microbial contamination, quality control.

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INTRODUCTION

Haematopoietic progenitor cells (HPC) are a specialised transfusion product. Microbial contamination can occur during product collection, processing or storage. Microbial screening by culture is undertaken to identify individual contaminated products, which may assist in decision making about the product and the patient, and also as a means of quality assurance, verifying the integrity of stem cell collection and processing. There are no consensus guidelines on interpretation of positive microbial cultures. Differentiating between clinically significant and insignificant contamination is difficult. There is variable practice regarding management of these products, including administering (with or without antibiotics) or discarding. Although infusion of culture positive products rarely results in unfavourable outcomes, it continues to be a potential source of sepsis and even death in the severely immune-compromised recipient. Unlike other blood products, HPC products are usually not easily replaceable.

The lack of consensus on interventions required is highlighted by differing recommendations by authoritative bodies. In Australia, institutions collecting and infusing HPC must comply with the latest National Pathology Accreditation Advisory Committee (NPAAC) Requirements for Procedures Related to the Collection, Processing, Storage and Issue of Human Haemopoietic Progenitor Cells. These state that ‘issue of product with positive microbial culture requires exceptional release’. However, there is a need for guidance on when exceptional release or stem cell recollection are justified and the clinical precautions to be taken when infusing culture positive products.

Culture positive products are unavoidable in transfusion services, despite efforts to limit contamination. In addition to contamination from skin commensals, bacteraemia, particularly following chemotherapy, and indwelling venous catheter colonisation may increase the risk of culture positive products in autologous haemopoietic stem cell donors. In addition to providing information on each individual PBSC product, monitoring the rate of occurrence of positive cultures may provide information on process integrity. There is a need for a simple strategy to monitor and detect changing frequency of contamination.

We report on The Canberra Hospital experience with autologous PBSC products. Our aim is to describe the frequency of culture positive products, identify the organisms involved and review the clinical outcomes of infusion. We then propose a local management strategy to respond to positive cultures, focusing on the potential needs of the donor, the safety of the product and implications for the laboratory’s quality management.

METHODS

We performed a retrospective analysis of 873 microbial cultures for 606 consecutive PBSC products obtained from 278 patients undergoing autologous stem cell transplantation at Canberra Hospital, Australia, between 1995 and 2005. The frequency of positive cultures and the organisms were identified. Medical and laboratory records of patients who received culture positive PBSC products were reviewed for evidence of infection during infusion and in the post-transplantation period until discharge from hospital. Statistical analysis was primarily descriptive, with the Chi-squared test used for comparisons.
Culture positive products were classified in accordance with the schema for paired culture positive HPC products proposed by Palley et al., which can be understood in three groups:

1. Patient positive product: both culture samples turn positive rapidly and the donor has blood or line cultures with the same organism, indicating the presence of organisms from the bacteraemic donor.
2. True positive product: both culture samples turn positive or one sample turns positive rapidly but the donor has no signs of infection, indicating introduction of organisms into products during collection or laboratory manipulation.
3. False positive product: only one sample turns positive and slowly, indicating likely introduction of organisms by sampling procedures.

Using this approach, false positive products are thought to most likely represent contamination during micropipette sampling, as the organism is not found on repeat testing. However, they may be due to a low inoculum within the product, and this possibility needs to be considered when dealing with virulent organisms.

Apheresis collection
PBSCs were mobilised by administration of chemotherapy followed by a course of granulocyte colony-stimulating factor (G-CSF) to donors (Fig. 1). Antibiotics were not given prophylactically. Collection was via a central or peripheral venous catheter, connected to a cell-separator machine (COBE Spectra; CardianBCT, USA). Daily G-CSF injections and collection continued until the harvest endpoint for each intended transplant was achieved.

Initial processing
All PBSC products were processed in the on-campus Bone Marrow Transplant laboratory. Processing scientists were clean gowns and sterile gloves. All open processing procedures were performed inside a Class II biological safety cabinet. Prior to laboratory manipulation, each product had a 1 mL sample removed for cell counting.

Cryopreservation
Products were then transferred into a transfer pack for volume reduction via centrifugation (1500 rpm for 10 min). Volume reduction removed up to 75% of the total product volume. An equal volume of cryoprotectant (20% dimethyl sulfoxide (DMSO), 30% albumin and 50% saline) was added to the concentrated products. Upon completion of laboratory manipulation, a 1 mL sample was removed for cryopreservation. The final products were divided equally into cryocyte bags for cryopreservation in a controlled-rate freezer in liquid nitrogen tanks at ~190°C.

Microbial culture
A 2 mL sample of the product was injected into a single Bectec Peds Plus blood culture bottle (Becton Dickenson, USA) and incubated for 14 days using the BACTEC 9240 continuous monitoring system (Becton Dickinson). Anaerobic cultures were not undertaken. Positive bottles were subcultured onto Columbia horse blood, chocolate, MacConkey and Sabouraud dishes (Oxoid, Australia) for microbial identification and sensitivity testing. Before 2000, microbial culture occurred only after manipulation (in conjunction with cryopreservation). From 2000 onwards, paired microbial culture was performed, before manipulation (in conjunction with cell counting) and again after manipulation (with cryopreservation). Post-harvest microbial culture was not performed at any time.

RESULTS
Product culture positive frequency and isolated microorganisms
Over the 10 year period, 11 (1.3%) of 873 routine microbial cultures were positive. This corresponded to 11 (1.8%) of 606 products, from eight (2.9%) of 278 patients, having positive cultures (Table 1). Ten isolates were coagulase negative staphylococci (CNS) and one was Corynebacterium accolens (Table 2).

Points of microbial introduction
Of the eight patients (11 products) with positive microbial cultures, five patients (five products) had paired microbial cultures and three patients (six products) had post-manipulation cultures only. None of the 11 products were positive in both paired cultures (Table 2). Of the five products with paired cultures, one was positive only pre-manipulation and four were positive post-manipulation (p > 0.05 for comparison between pre- and post-cultures). Two products were collected from a patient with evidence of catheter-related bacteraemia at the time of collection (Patient 3, Table 2), with the same organism grown from the two PBSC products and the patient’s peripheral blood. No symptoms of infection were present in any of the other seven patients during collection.

Patient outcome
Five patients had been infused with seven culture positive products (Table 2). Two patients received two culture positive products each during a single infusion period. Four patients received products cultured positive with CNS and one patient received a product positive with Corynebacterium accolens. Prophylactic vancomycin was administered for products known to be positive with CNS. Vancomycin was commenced at the time of infusion, with patients receiving up to a total of four doses over a 48 h period.

No clinical signs of infection, defined as fever (temperature ≥38.3°C), rigors or hypotension within 24 h of PBSC infusion, followed infusion of any culture positive product.

During the post-transplantation course of the five patients who received culture positive products, four patients developed

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Incidence of culture positive PBSC product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive frequency (%)</td>
</tr>
<tr>
<td>Microbial cultures</td>
<td>11/873 (1.3)</td>
</tr>
<tr>
<td>PBSC products</td>
<td>11/806 (1.8)</td>
</tr>
<tr>
<td>Patients</td>
<td>8/278 (2.9)</td>
</tr>
</tbody>
</table>

PBSC, peripheral blood stem cell.
febrile neutropenia within 5–7 days after infusion. However, none developed bacteremia with the same organism in the blood cultures as in the infused product. Two patients had bacteremia with different organisms from the infused product (Streptococcus viridans and Escherichia coli). All patients had a successful engraftment.

Three patients did not proceed to transplant. One had a collection interrupted by catheter sepsis, leading to an inadequate number of stem cells harvested. Another developed cardiomyopathy. The last had lack of disease progression. No transplant was withheld due to a contaminated product.

**DISCUSSION**

**Product culture positive frequency and isolated microorganisms**

In our laboratory, the frequency of culture positive products was 1.8%, which was comparable to reported series, ranging from 1.0% to 21.0%. All microbial isolates were skin commensals, similar to other reported series. None of our isolates were pathogens, consistent with the low incidence reported in the literature, 0.28%.

**Points of microbial introduction**

Microbial introduction into product bags can occur at several points during collection, processing and cryopreservation. Bacteremic donors, infected skin plugs or catheters, improper execution of aseptic techniques or contaminated reagents or laboratory equipment are all potential sources. Contamination may also occur during thawing, but this has not been evaluated in the present study as post-thaw cultures were not routinely performed. Whilst post-thaw cultures may validate the thaw procedure, culture results would not be available for immediate patient management.

Of the five products with paired microbial cultures in which at least one was positive, four were positive only after processing. Larrea et al. found approximately equal rates of culture positivity before and after processing, and statistically our findings are consistent with that. Unlike previous studies there were no paired culture positive samples in this cohort. This could result from insensitivity of microbial culture or low levels of contamination. Patients 1 and 2, who were both noted to be well at the time of collection, had two consecutive culture positive products each, suggesting a microbial source from an asymptomatic catheter infection or asymptomatic bacteremia. No blood cultures were taken directly from the patients. Patient 3 had two consecutive culture positive products due to bacteremia with catheter-related infection, thus accounting for the positive cultures.

**Interpretation of culture positivity**

Interpretation of positive microbial cultures from HPC products is confounded by the difficulty in determining whether the organism originated from the HPC product itself or during the microbial testing procedure. Traditionally, the misleading word 'contamination' has been used to describe either situation, but the descriptive term 'culture positive product' is better. The latter term simply describes the status of the culture, which could either reflect the presence of organisms within product bags or the inadvertent introduction of organisms into blood culture bottles alone, during sampling. Paired microbial culture (pre-manipulation and post-manipulation samples) can help in
Table 3  Clinical outcomes: infusion of pathogen-positive products

<table>
<thead>
<tr>
<th>Authors</th>
<th>Cultured pathogen</th>
<th>No of positive cultures (stage of manipulation)</th>
<th>Prophylactic antibiotics</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelly et al.</td>
<td><em>Burkholderia</em> sp.</td>
<td>2/3 (post-processing and post-graft treatment)</td>
<td>Yes</td>
<td>No sepsis</td>
</tr>
<tr>
<td></td>
<td><em>Enterococcus</em> sp.</td>
<td>1/2 (pre-processing)</td>
<td>No</td>
<td>No sepsis</td>
</tr>
<tr>
<td>Klein et al.</td>
<td><em>Escherichia coli</em> and <em>Klebsiella pneumonia</em> (both within 1 product bag)</td>
<td>Uncertain</td>
<td>Yes</td>
<td>No sepsis</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas cepacia</em></td>
<td>1/2 (post-processing)</td>
<td>Yes (Piperacillin and Bactrim)</td>
<td>Death from septic shock (7 days post-transplant)</td>
</tr>
</tbody>
</table>

interpretation. In the model of blood culture contamination, important factors in predicting true bacteremia include time to growth, presence of multiple positive cultures and the identification of the organism, with the latter two factors as stronger predictors. Likewise, in paired microbial culture of HPC products, when duplicate samples are culture positive, it is likely that organisms are truly present within products. Also, when time to culture positivity is rapid (i.e., arbitrarily less than 36 hours), it is likely that a significant number of organisms are present. Several studies have shown that blood cultures turning positive more than 3–5 days after incubation have been more likely to represent a false positive (i.e., a contaminant) because the smaller inoculum associated with a contaminant results in slower growth than larger inoculums associated with a true bacteremia.

Using the criteria proposed by Padley et al., Patient 7 who had *Corynebacterium accolenos* in the pre-manipulation sample only, was a false positive culture, likely to have arisen from contamination during microbial sampling itself.

Patient outcome

There are limited published data on the clinical significance of infusion with culture positive products. Although the general experience is that adverse sequelae are rare, this remains uncertain, resulting in variability in practice. None of our recipients of culture positive products had adverse effects related to the cultured organism.

With regard to skin commensals, we assessed clinical outcomes of transplanting culture positive products from our own institution and reported series. From our study, four patients received six ‘true positive products’ (CNS) with prophylactic antibiotics, without subsequent signs of infection, and all successfully engrafted. Another patient received one ‘false positive product’ (*Corynebacterium accolenos*) without prophylactic antibiotics, also without subsequent signs of infection (Table 2). Nifong et al. reported a patient with line sepsis from CNS during collection, who received four culture positive products, all of which had been positive with CNS at multiple sampling stages. This case was an example of infusing a ‘patient positive product’. Vancomycin was administered at infusion. The patient developed bacteremia within 24 h of infusion, but remained asymptomatic. Engraftment was successful.

With regard to potential pathogens, we assessed clinical outcomes of transplanting culture positive products from published reports (Table 3), since our study did not isolate pathogens. Infusion of pathogen positive products was reported less commonly than skin commensal positive products. Reported cultured pathogens included *Staphylococcus aureus*, *Bacillus cereus*, *Burkholderia* species and *Enterobacter cloacae*. Prophylactic antibiotics were not universally given and most infusions were uncomplicated. However, Klein et al. reported one death from septic shock and multi-organ failure following infusion with a product cultured positive for *Pseudomonas cepacia* (now known as *Burkholderia cepacia*), despite appropriate prophylactic sulfamethoxazole/trimethoprim and piperacillin.

Padley et al. presented the largest series of HPC products to date. They found a culture positive frequency of 1.6% with the predominant isolate being CNS. Of 69 patients who received culture positive culture products (both skin commensals and pathogens), only 23 were given prophylactic antibiotics. There was only one case of post-transplant bacteremia with the same organism infused, but no differences in infusion-related toxicities or survival when culture positive HPC recipients were compared to all HPC recipients. In their summary of 25 previous published series, 434 patients were infused with culture positive product (both skin commensals and pathogens), with variable prophylactic antibiotic use. Only 14 patients had bacteremia with the same organism post-transplantation (with either skin commensal or pathogens), with one case of septic shock but with no irreversible sequelae or deaths reported. Another review of published series by Kamble et al. also found that most infusions, both for products positive with skin commensals or pathogens did not translate into sepsis and recommended transfusion without antibiotics.

Whilst transfusion of culture positive products rarely causes unfavourable outcomes, it has the potential to cause sepsis and death, particularly with Gram negative organisms.

Table 4  Recommended clinical management policy

| False positive/clinically insignificant | Administer, without prophylactic antibiotics | Administer, with prophylactic antibiotics | Administer, with prophylactic antibiotics at the minimum |
| True positive/clinically significant  | Administer, with prophylactic antibiotics | Administer, with prophylactic antibiotics | Consider an alternative product if possible |
| Patient positive/clinically significant | Administer, without prophylactic antibiotics | Administer, with prophylactic antibiotics | Consider an alternative product if possible |

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Clinical management of culture positive products

There are several important factors impacting on decisions whether a culture positive product could be administered, with or without prophylactic antibiotics, or should be discarded altogether. These factors include: (1) the patterns of paired microbial culture, (2) the nature of organisms cultured (skin commensals or pathogens), and (3) the feasibility of product replacement (whether the patient can tolerate a delay and recollection). Our recommendations for clinical management of culture positive products (Table 4) are based on our data and also that of published series. Ultimately, the decision to administer or discard a culture positive product is at the physician’s discretion, with informed consent from patients.

Laboratory quality control

While all positive cultures would result in a clinical review of the particular patient for possible sepsis, not all positive cultures need to be addressed by an extensive process review. In a well controlled system, the frequency of positive cultures should be relatively constant. Ideally, there should be no positive cultures, but practically contamination may occur during collection, processing and bacterial sampling and low culture positive frequencies are expected. A change in the frequency of positive cultures may indicate a change in the quality of the underlying processes, impacting on the quality of the product. Quality control rules are well described for continuous variables produced by automated laboratory analysers, prompting investigation to find or exclude changes in the testing process. Likewise, frequent positive cultures may reflect a systematic contamination source and should trigger extensive investigation to find such and eliminate the source. However, extensive investigation for a process error is potentially time consuming and unnecessary if there has been no change in the low frequency of positive cultures.

Therefore, we developed a method to identify an increased frequency of positive cultures, suggesting a potential change in the process. As cultures are either positive or negative, we used the binomial theorem to calculate the probability of $X$ number of culture positive products, occurring in $n$ number of consecutive PBSC harvests by random contamination,

$$P(X) = \frac{\pi^X (1 - \pi)^{n-X} n!}{X! (n-X)!}$$

where $\pi =$ probability of a positive culture (0.018).

We then defined an arbitrary threshold of probability <1% to investigate for systematic contamination. Using tabulated values for binomial probability we determined the probability of at least $X$ number of culture positive products occurring in $n$ number of consecutive PBSC harvests by random contamination. By plotting these we were able to determine when our threshold for investigation had been reached for at least two, three and four positive cultures (Fig. 2). The following quality control (QC) rules were derived:

1. Rule A: Investigate if at least two culture positive products occur in eight consecutive harvests.
2. Rule B: Investigate if at least three culture positive products occur in 24 consecutive harvests.
3. Rule C: Investigate if at least four culture positive products occur in 46 consecutive harvests.

We then developed a computer model to evaluate the performance of our QC rules, using random number generation in a spreadsheet (Excel 2007; Microsoft, USA). We simulated positive cultures occurring at frequencies of 1.8% (baseline), 5.0% and 10.0%. As expected, the proportion of culture positive products triggering an investigation for systematic contamination increased as the culture positive frequency increased (Table 5). Applying different combinations of QC rules (Rule A, Rule A or B, Rule A or B or C), the proportion of culture positive products triggering an investigation for systematic contamination increased when multi-rule QC (Rule A or B or C) was used instead of single-rule QC (Rule A), implying that the use of a multi-rule QC method increased the likelihood of a systematic source of contamination being investigated and found if it existed.

At baseline culture positive frequencies of 1.8%, the multi-rule QC will trigger investigation into 19% of culture positive products unnecessarily, a significant improvement over
investigating all culture positive cases; however, if there is a change in the process leading to increased frequencies of culture positivity of 5.0% or 10.0%, the multi-rule QC will cause a much higher proportion of culture positive products (94% at a 10.0% culture positive frequency) to trigger an investigation compared to a single or double rule QC (52 and 73%, respectively, at a 10.0% culture positive frequency). Thus, the advantage of this multi-rule QC method is that it is sensitive to systematic changes in culture positive frequencies whilst reducing significant numbers of unnecessary investigations at baseline culture positive frequencies due to random contamination.

Investigation for systematic contamination involves initial review of apheresis unit medical records. If the donor had line cultures or blood cultures with the same organism, then the source of culture positivity is the patient. Otherwise, a further review of processes, including aseptic technique, storage conditions for consumables and laboratory processing, should be undertaken. All efforts to minimise microbial introduction must be maintained to produce a safe product for HPC transplantation in the severely immune-compromised recipient. This involves ongoing personnel training and regular revalidation in aseptic techniques of collection and manipulation, maximising use of closed systems, minimising the duration of indwelling catheters and considerations for disposal of the initial few millilitres of collection product to eliminate skin plugs.

CONCLUSION

Our study builds on previous experience with HPC products, demonstrating that the incidence of culture positive autologous peripheral blood stem cell products is low, with skin contaminants being the predominant microbial isolate. However, clinicians and laboratories need to have plans in place to deal with positive cultures when they arise. These should be three-fold, considering the implications for the patient (and donor), the product, and processing procedures. For the patient, the major implication is whether an infection has been identified that requires treatment. As with other sterile site cultures, treating clinicians should be notified of positive results for immediate action if required. For the product, the major decision is whether to infuse the stem cells. Infusion with skin commensal positive products is safe with antibiotic prophylaxis, although others have rein infused without antibiotics, also with apparent safety. In contrast, caution is needed when Gram negative pathogens are isolated. Co-administration of antibiotics or product replacement should be considered. Variables that may impact on the decision to transfuse stem cell products include the number of cultures positive, the organism identification and time for cultures to flag as positive.

In terms of the process, positive bacterial cultures may signal a breakdown in normal sterility procedures. While low frequencies of contamination may be expected, from skin plugs for example, stem cell laboratories should develop policies to monitor the frequency of culture positivity as part of quality control, in order to identify systematic breaches or variations in the process. We propose one such method using a multi-rule QC that allows the capability for triggering laboratory investigation for systematic contamination when culture positive frequencies increase from baseline due to such a source arising.

REFERENCES


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