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The evaluation of microbial contamination in platelet concentrates prepared by two different methods

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Abstract

The microbial contamination of platelet concentrates (PCs) prepared by two different methods both with a high risk of bacterial contamination during preparation and storage were evaluated. For apheresis platelets, the concentrates were obtained using the Haemonetics MCS 3P device. For the random method, platelets were obtained by two phase centrifugation, in the Heraeus Cryofuge 8500 I device using the Kansuk 3-way bags which permit storage for five days. 1620 plateletpheresis units prepared by apheresis, and 9838 units prepared by the random method, were included in the study. Of the 11,458 PCs studied, 32 (0.27%) were false positives and 24 (0.2%) were real positives. All of the positive results occurred in platelets prepared by the random method. *C. xerosis* and *S. epidermidis*, *S. hominis*, *Alpha-hemolytic streptococci*, all flora of the skin, were isolated in the contaminated concentrates. The risk of microbial contamination of PCs, prepared both by apheresis and from whole blood, continues at a low rate although the products were collected into specific bags following rules including appropriate disinfection of the skin, correct centrifugation collection time and optimal storage conditions including temperature and agitation. These results again emphasize the importance of: obeying phlebotomy rules and hand disinfection of the person who collects the blood as well as the need for careful skin decontamination of the donor, during donation.

1. Introduction

Bacterial contamination of blood components is an important cause of transfusion-associated morbidity and mortality [1]. While the incidence of bacterial contamination in all the blood transfusions is reported to range between 0.2% and 0.5%, bacterial contamination of blood components was responsible for 15.9% of 182 transfusion-associated fatalities reported to the food and drug administration (FDA) from 1986 to 1991 [2,3]. Skin contamination, chronic infection or asymptomatic donor bacteremia, and poor storage processes are implicated as potential origins of bacterial contamination of blood and blood products [4].

In this study, the microbial contamination of platelet concentrates (PCs) prepared by two

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different methods which have high risks of bacterial contamination during both preparation and storage were evaluated.

2. Materials and methods

A total of 11,458 units (U) of PCs including 1620 apheresis units (1 PU = 6 U PCs) and 9838 units from whole blood were investigated for microbial contamination between January 1999 and December 2000, in the Blood Bank of the Florence Nightingale Hospital. The PCs which were obtained by apheresis were prepared in the multicomponent system three pump device (MCS 3P, Haemonetics, USA) which works on the principle of intermittent flow centrifugation (IFC) in the platelet + plasma program (PLP) using REF 795 size set, in CLX bags (Cutter, USA) composed of polyvinyl chloride + tri-2-ethyl-hexyl-trimellitate (PVC + TOTM). The donors skin was disinfected before the procedure with 10% polyvinylpyrrolidon iode complex (PVPIC) and then 6 U of platelets and 2 U of plasma were obtained [5]. To obtain PCs from whole blood the skin was also disinfected with 10% PVPIC and then 450 ± 45 ml of whole blood was collected into bags (Kansuk, Turkey) of PVC + TOTM with sodium chloride, adenine, glucose monohydrate and mannitol (SAGEM) in which three components (platelet, plasma and an erythrocyte suspension) can be stored with the platelets kept for five days. The process of component preparation was completed in a maximum of 8 h; the whole blood was separated into its components of platelet-rich-plasma (PRP) and erythrocytes by centrifugation in the Cryofuge 8500 I (Heraeus, USA) for 7 min at 2700 revolutions per min (rpm). In the second phase, the PCs and also plasma and erythrocyte suspensions (ES) were obtained by centrifugation of the PRP at 3200 rpm for 15 min. On the first day, 3–5 ml taken from the platelet concentrates (PPC) obtained from each of the two groups were transferred into sterile test tubes under laminar air flow (LAF) and aseptic conditions. From these tubes, 3 ml was taken with disposable pipettes and inoculated into pediatric blood culture bottles with the number 2194, of the Bactec 9050 device (Becton–Dickinson, USA). The bottles were left to incubate at 35 ± 1.5 °C in the Bactec 9050 device and observed for five days.

Additionally Gram stains were prepared with the following criteria of evaluation: negative if there were no organisms in 20 fields, 1+ for one organism per 10–20 fields, 2+ for one organism per field, 3+ for 2–10 organisms per field and 4+ for 10 organisms per field [6]. On the fifth day after PCs were prepared for investigation, experimental platelet concentrate (EPC) samples of 50 ml were taken from the apheresis PCs and 15 ml from the PCs prepared from whole blood and were put into bags similar to the primary platelet bags, using a sterile connection device (SCD) 312 (Terumo, Japanese). At the end of this process, the bags containing EPCs of 50 and 15 ml were stored at 22 °C for five days on a circular agitator at 2–6 rpm (Helmer, PAS Model 40, Noblesvilla, USA).

On the fifth day, as on the first day, PC samples of 3 ml from the EPCs were taken and inoculated into the Bactec culture bottles with the bottles incubated for five days. Gram stains were prepared. If proliferation was seen on the first and the fifth days, the concentration of the bacteria in ml was determined by a quantitative culture with dilutions of $1/10 \times 10^4$ and $1/10 \times 10^6$. While the PCs which were prepared by the two methods and which were in the primary bags were transfused to patients; the EPCs which were intended only for research were not used for patients.

3 ml of samples from the plasma, the other product obtained from both apheresis and whole blood were used to check the true positivity of the PC cultures. For this purpose, 3 ml of the PC samples from each of the plasma bags were inoculated into the Bactec bottles, under aseptic conditions under laminar flow. They were stored in the Bactec device, from the preparation day until the 10th day, which was the last day of observation. If not used yet during the observation period, the samples were also used to check.

To evaluate the true microbial positivity of the PCs; the contamination of plasma and, if not used, of the PPC and ES, were taken into consideration.

If the samples were positive only in the EPC and not in the plasma or other samples, the result was accepted as a false positive.

Table 1
The relationship of bacteria contamination in the PCs prepared by single and random methods during the storage period

Method	*n*	Proliferation on the first day	Proliferation on the fifth day			
			True positive		False positive	
		n	*n*	%	*n*	%
Single	1620	0	1	0.06	0	0
Random	9838	0	7	0.07	24	0.24
Total	11,458	0	8	0.07	24	0.21

All of the transfused patients were observed for any complications until they were discharged from hospital.

The identification of microorganisms was carried out with a kit for Gram positive rods by using the staphylcoccus panel, number 414, of Sceptor (Becton–Dickinson, USA) and the API (Bio-Meriux, France) systems.

For statistical evaluation of the results, the confidence interval (CI) was calculated according to the poisson dissociation of the small stata 5.0 program.

Table 2
Gram and culture results of platelet concentrates which were prepared by single vs. random methods

Gram stain		Culture	
		Positive	Negative
Single			
Positive	1	1	0
Negative	1619	0	1619
Random			
Positive	7	7	0
Negative	9831	0	9831

3. Results

Bacterial contamination was determined in 32 (0.27%) of the 11,458 EPCs which were included in the study. Of the 32 samples, 24 (0.2%) were false and 8 (0.07%) were evaluated as true positives (Table 1).

Both Gram and culture positivity were determined in one of the 1620 U which were prepared by the apheresis method, CI: 95% (0.94–1.05) and seven of the 9838 U which were prepared by the random method, with a CI of 95% (6.97–7.02) (Table 2). Of the eight samples in which true proliferation was determined, positivity was determined in the sample taken from the EPC on the fifth day, by the 19th h and was maximum, by the 33rd h. The microorganisms which were true contaminants were mostly *Staphylococcus epidermidis*. On the other hand, *Bacillus spp*. were isolated from the false positives (Table 3).

Two of the platelets prepared by the two methods and determined as true positives, were transfused to patients; the others were not transfused because they were not the main product needed (ES and/or plasma were needed in these patients). There were no transfusion complications observed in the two patients to whom the contaminated PCs were transfused.

4. Discussion

Bacterial contamination of cellular blood components is defined as a transfusion complication [7]. The bacterial contamination rates and related septic reactions are reported as high and are generally related to platelet transfusion [7–9]. Marrow et al. [10] reported the incidence of sepsis related to transfusion of platelets and erythrocytes as 1/1700 and 1/500,000, respectively. Storing the platelets – which are suspended in plasma in gas-permeable bags for five days, on the agitator and at room temperature – is reported to supply optimal conditions for bacterial proliferation [4,11]. The bacterial contamination rates are reported to reflect variations in respect to the preparation process of the platelets. This is reported as 0.03%, 0% and 0.14%, 0.19% for single and random methods,

Table 3
Types of microorganisms which proliferated in true or false positive platelet concentrates

Microorganisms	*n*	Gram stain	The culture concentration (mean)
True positives			
C. xerosis	2	+3, +3	25×10^6
S. epidermidis	3	+3, +4, +3	1×10^7
S. hominis	1	+3	2×10^6
Alpha-hemolytic streptococci	2	+2, +1	5×10^5
Total	8		
False positives			
Bacillus spp.	13	+3, +3, +4, +2, +1, +4, +2, +3, +2, +3, +1, +4, +2	5×10^5
Micrococci	8	+2, +4, +1, +2, +3, +4, +1, +3	2×10^4
Candida spp.	2	+2, +3	7×10^2
Saccharomyces cerevisiae	1	+2	3×10^5
Total	24		

respectively, in different studies by Barrett et al. [4] and Yomtovian et al. [6] in 1993. Björk et al. [12], in their investigation which used the two methods, found bacteria contamination in 19 of 2084 PCs; of these, one was found by the single method and the rest (18) were found by the random method. When similar data are examined, this rate is seen to vary between 0–10% and 0–4.9% for random and single EPCs, respectively, [13–15]. In the investigation of Leiby et al. [16] in 1997, the contamination rate was reported as 0.08% in a total of 4995 units of RDPs.

In our study, as reported in the literature, this was determined to be 0.06% and 0.07% in SDP and RDP, respectively. Our data revealed that, although the microbial contamination rate of the platelets (which are prepared both with single and random methods and are kept in similar (chemical structure) storage bags [PVC + TOTM]) is very low, it is necessary to consider that the process of deterioration of the platelets is standardized and that asepsi rules are carefully obeyed, in order to be able to determine the seriousness of the clinical situation where contaminated platelets are transfused. It became possible to store platelets for up to five days after the addition of TOTM to the PVC bags. In PVC alone, until 1982, storage was only acceptable for up to three days depending on the O_2 and CO_2 permeability, as approved by the FDA [17,18]. In some studies, the storage period of platelets was increased to seven days due to the increased need for PCs in diseases such as leukemia; however, the FDA decreased the storage period to five days again due to the relationship between bacterial contamination and the storage period of the platelets. This was because of the reports between 1980 and 1983 [19] concerning increased mortality related to transfusion.

In some experimental studies, the proliferation of the bacteria, after inoculation into platelets, entered a stagnant phase at 24 and 48 h and then rapidly increased to 10^8 ml. It was reported that this proliferation process occurred in both Gram positive and Gram negative bacteria during the five-day storage period [20–22]. In our study, we also found no proliferation when the PCs, prepared by two different methods, were examined on day one; however, when examined on the fifth day, the cultures and microscopic observation were positive in 1 U of the PCs prepared with apheresis and in 7 U which were prepared from whole blood. In our study, normal microorganisms of the skin flora such as *S. epidermidis*, *Corynebacterium xerosis*, *Alpha-hemolytic streptococci* and *Staphylococcus hominis* were isolated in the PCs which were culture positive. In the literature, the most common agents causing bacterial contamination in platelets are reported to be Gram positive cocci like *S. epidermidis*, *Alpha-hemolytic streptococci*, *Staphylococcus aureus* and diphteroid rods [13–15]. Between 1985 and 1996, 21 deaths were reported due to transfusion-related *Yersinia enterocolytica*

sepsis [23]. Yomtovian et al. [6] isolated *S. epidermidis* in 0.19% of the cases of bacterial contamination which they found in 3141 random EPCs. Between 1994 and 1995, coagulase negative staphylococci were found in 12 of 19 samples in 2084 PCs [12]. *Corynebacteria spp*. were most often isolated during the investigation of Leiby et al. [16] in 1997. It was reported that bacteria like *staphylococci*, *micrococci*, etc. from normal skin flora are spread when appropriate antiseptic techniques were not applied. Bacteria such as *Pseudomonas spp*. and *Achromobacter spp*., etc. are contaminants during laboratory studies, and *Salmonella spp*. and *Yersinia spp*. are contaminants found in asymptomatic bacteremia [10].

In our study, the microorganisms which were isolated from the PCs were generally from the skin flora. We considered that these bacteria contaminated the PCs from the donors skin during the collection process. Also, the type of the microorganisms which proliferated in the false positive cultures led us to believe that an in vitro contamination occurred during the process of transferring the PC samples from the PPC bags into the EPC bags.

As a result, microbial contamination risk of PCs, which were prepared both by apheresis and from whole blood, continues to exist at a low rate although collected into specific bags under appropriate conditions with careful and correct disinfection of the skin, centrifugation at appropriate revolutions, a proper collection time and storage at optimal conditions of temperature and agitation.

Our results once again point out the importance of certain procedures such as: observance of phlebotomy rules and careful disinfection of the phlebotomists hands and the skin of the donor, during donation.

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