From the donor's arm to blood product: a study on bacterial contamination of apheresis platelet concentrates

Anna Maria Leo[1], Maria Monica Salvadego[1], Maria Grazia Piva[1], Graziano Ruffato[1], Sara Valverde[2], Ernesto Trabuio[2], Francesco Antico[2], Gianluca Gessoni[1-2]

[1] *Centro Trasfusionale A-ULS 14*
[2] *Dipartimento di Patologia Clinica A-ULS 14 Chioggia, Italy*

Background. Transfusion-associated bacterial infections are a quite frequent collateral effect of administration of platelet concentrates (PC). We carried out a microbiological surveillance of bacterial contamination of apheresis platelet concentrates by studying microbial flora on donors' arms before and after skin disinfection, through blood cultures with the diversion volume and with the PC.

Materials and methods. Platelet aphereses were carried out using two Haemonetics MCS+ instruments. Cutaneous swabs were examined by the direct plate technique and blood cultures were performed using Bact/ALERT aerobic bottles. In the 5 years from January 2001 to December 2005 we tested 481 PC.

Results. Cutaneous swabs showed significant bacterial growth in 89% of cases before skin disinfection and in 44% after. None of the blood cultures performed on diversion blood was positive, one (0.2%) PC was positive on the fifth day after collection and the presence of a *Staphylococcus epidermidis* strain was demonstrated.

Conclusions. Our results suggest that the skin disinfection protocol adopted in our structure is not fully satisfactory. The cultures performed on the PC showed a low prevalence of contamination, and the only positive sample was contaminated by a common skin contaminant (*S. epidermidis*). The culture became positive on the fifth day after collection, but on the second day the PC had been transfused to a patient, without any adverse reaction.

In our experience a culture method using Bact/ALERT aerobic bottles was not able to prevent transfusion of the only contaminated PC identified in this study.

Key Words: apheresis, platelet concentrates, microbiological contamination, arm disinfection

Introduction

Transfusion-associated bacterial infections are the most frequent reported cause of post- transfusional infections in Western Countries. The risk of bacterial contamination of labile blood products is estimated to be around 1/100,000 but is very variable. Bacterial contamination is an exceptional event for fresh-frozen plasma (FFP), but the risk is estimated to be 1/3,000 for platelet concentrates (PC) and for red blood cell concentrates (RCC). The risk of a post-transfusional sepsis is estimated to be about 1/25,000 for PC and 1/250,00 for RCC[1] . To reduce the risk of sepsis due to bacterial contamination of blood units, in March 2004 the American Association of Blood Banks (AABB) adopted a new standard, that requires implementation of measures to detect and limit bacterial contamination in all platelet components[2].

Gram-positive skin commensals such as coagulase-negative *Staphylococci* and *Bacillus cereus* are the organisms most often recovered from donated blood (and implicated in bacterial contamination of platelets). Such contamination is thought to occur principally during phlebotomy, as a result of incomplete disinfection and/or skin core removal (including skin appendages, where skin

Blood Transfus 2007; 5: 130-133 DOI 10.2450/2007.0003-07

disinfectants may not penetrate) by the collection needle. These organisms typically do not grow at 1 to 6 °C but survive and multiply readily at the platelet storage temperature of 20 to 24 °C. In the case of Gram-negative bacterial contamination, asymptomatic donors with transient bacteraemia are presumed to be responsible for most cases of contamination[3].

A number of methods have been suggested for detecting bacterial contamination of PC. The main methods are culture systems, using bottles designed for blood cultures with an aerobic medium and automated equipment to detect positive samples in a completely automated manner[4]. Some institutions have implemented non-culture methods based on reagent strips (glucose and pH) to test platelets[5] or the use pH screening of whole-blood platelets with a pHmeter[6] (a pH>7.0 is required for the PC to be released), as surrogate tests for bacterial contamination.

There are also methods that detect the presence of bacteria in leucodepleted PC by measuring the reduction of oxygen in the sample, caused by aerobic bacterial growth[7] or by using solid-phase laser cytometry[8]. Moreover, real-time polymerase chain reaction (PCR) methods based on the detection of 16S ribosomal DNA have recently been described[9].

The aim of this study is to report the 5-year data from a surveillance project on bacterial contamination of PC obtained by apheresis in a small hospital-based Italian Transfusion Service. Moreover, we considered some other aspects such as the efficiency of the skin disinfection protocol used in our service, the prevalence of asymptomatic bacteraemia in blood donors in our area, the prevalence of bacterial contamination in apheresis PC concentrates, and the efficiency of an automated culture method for detecting contaminated PC with the aim of preventing the release of positive units.

Materials and methods

We performed a 5-year surveillance study, from January 2001 to December 2005, on bacterial contamination in all the apheresis platelets units collected in our Transfusion Service.

We adopted two Haemonectis MCS+ (Haemonetics Italia, Milan, Italy), using kits with leucodepletion filters, for the apheresis procedures. In the considered period, 532 platelet aphereses were performed. For each procedure, we obtained four samples: skin swabs before and after disinfection, a blood sample taken after venipucture (diversion volume), collected into a satellite bag integrated within the apheresis kit, and a 4 mL sample from the PC. Blood and platelet samples were cultured individually at 37 °C for 7 days using the Bact/ALERT system (Biomerieux Italia, Milan, Italy) of aerobic bottles. Positive samples were subcultured in agar plates (chocolate blood, Columbia CNA, mannitol salt, McConkey's medium) and standard biochemical identification of bacterial strains, performed using automated equipment: Dade Microscan (Dade Italia, Milan, Italy).

A direct swabbing and plating technique was used to enumerate bacteria present at the venipuncture site before and after disinfection.

A dry cotton swab was scrubbed on the skin in the antecubital fossa, swabs were shipped in Stuart medium to our reference microbiology laboratory and plated onto agar plates (chocolate blood, Columbia CNA, mannitol salt, McConkey's medium) to enumerate the bacterial colonies after incubation at 37 °C for 24 hours. We considered as negative (non-significant bacterial growth) a sample with less than 10 colony forming unit (CFU) / plate, if no coagulase-positive *Staphylococci* (CPS) or *Enterobacteriaceae* strains were observed.

Results

In the considered period we performed 532 platelet aphereses, but the four culture tests required for this study were only performed in 481 (90%), which are, therefore, the procedures suitable for evaluation.

Before arm disinfection, only 97 (21%) cutaneous swabs were negative (non-significant bacterial growth); after arm disinfection, 319 (66%) of the cutaneous swabs were negative. None of the skin swabs was positive for an *Enterobacteriaceae* strain, whereas 67 (14%) of the swabs obtained before and 45 (9%) of the swabs obtained after skin disinfection were positive for CPS.

None of the blood cultures performed from blood collected in the diversion bag gave a positive result.

Only two bottles, inoculated with leucodepleted PC, gave an initially positive result, using Bact/ALERT aerobic bottles, within 7 days of incubation, one after 5 days, the other after 7 days. Subcultures were positive only for the first sample and a coagulase-negative *Staphylococcus epidermidis* was identified.

The prevalence of contaminated platelet units was 0.2%.

Discussion

In our study the prevalence of positive cutaneous swabs obtained before skin disinfection (79%), which is

compatible with, but slightly lower than, the rate reported in the literature (98%)[10] . In our opinion, this discrepancy had two main causes. One was the use of dry swabs shipped in Stuart medium to the centralised microbiology laboratory, located in a hospital 25 Km away from our Transfusion Service, so some of the swabs were plated the day after collection. The second was the evaluation criteria: when interpreting the results of the skin swabs in our study, we considered as negative samples those with non-significant bacterial growth, that is to say a low bacterial load (under 10 colony-forming units), and the absence of potentially relevant micro-organisms, such as CPS or *Enterobateriaceae*.

The procedure for disinfecting the donors' arm, that is used in our Transfusion Service skin, is conducted in two stages: first the antecubital fossa is cleaned with alcohol, then the skin is treated with povidone iodine. After skin disinfection, the prevalence of positive swabs (66%) agreed well with prevalences reported in the literature (54-63%)[10].

None on the culture performed with the whole blood obtained from the diversion bag was positive after 7 days of culture in Bact/ALERT aerobic bottles. These data suggest that, in our area where all the blood units and apheresis products were collected in the morning, the rate of transient asymptomatic bacteraemia in blood donors is very low. Of the two initially reactive PC, only one was confirmed, by agar plate subcultures, to be contaminated. The recurrence of falsely reactive results obtained using Bact/ALERT aerobic bottles inoculated with leucodepleted platelets has been reported in the literature and was due mainly to malfunctioning of Bact/ALERT incubation units; indeed, culture bottles for this specific purpose are now available[11] .

The prevalence of bacterial contamination of apheresis PC in our study was 0.2%; this result is in good agreement with data from the literature[1,3-5] . The bacterial strain isolated from the positive units was a *S. epidermidis*, a common skin contaminant. The microbiological study performed in this blood donor showed that the cutaneous swab obtained before skin disinfection was positive but the one obtained after disinfection was negative for *S. epidermidis*, so it is possible that bacteria were collected by the needle removing a skin core (including skin appendages, which the skin disinfectants may not penetrate). The bottles became positive during the fifth day of incubation, which is suggestive of a very low level of initial bacterial contamination. The PC was transfused, a day after collection, into a 63-year old leukaemic patient without any adverse reaction.

The most relevant aspect of our study was the fact that we performed four microbiological tests in each considered procedure. Two cutaneous swabs were obtained before and after skin disinfection, to assess the load of contaminant flora and to evaluate the efficiency of the donors' arm disinfection protocol used in our Transfusion Service. The results showed the our arm disinfection protocol needs to be improved. Two Bact/ALERT aerobic bottles were inoculated, the first with about 5 mL of whole blood obtained from the diversion bag, integrated in the apheresis circuit, the second inoculated with about 4 mL of the final product, obtained after leucodepletion. The cultures performed on the whole blood were considered with the aim of studying two aspects of contamination of blood components: the presence of transient asymptomatic episodes of bacteraemia in blood donors and the presence of a higher bacterial load (due to collection of contaminated skin with the needle) in the first millilitre of collected blood. The results suggested that, in our practice, these aspects were of only slight relevance. The cultures performed on the PC showed a prevalence of contamination of 0.2%, and the only positive sample was contaminated by a common skin contaminant (*S. epidermidis*). The culture became positive on the fifth day after collection; on the second day, the PC had been transfused into a patient without causing any adverse reaction. In our experience, a culture method performed using Bact/ALERT aerobic bottles was not able to prevent transfusion of the only contaminated PC identified in this study. Indeed, it was recently reported that false negative results are also possible with culture methods[12] . Other screening methods proposed to reduce the risk of sepsis due to contaminated platelets have shown lack of sensitivity or specificity[6-8] or are too expensive[9] . It has, therefore, been suggested that, in compliance with regulatory agencies, pathogen inactivation systems suitable for cellular components should be a more effective approach to reducing the risk of transfusion-associated sepsis than an approach based upon the screening tests currently available[1] .

References

1) Blajchman A, Beckers A, Dickmeiss E, et al. Bacterial detection of platelets: current problems and possible resolutions. Transfus Med Rev 2005; **19**: 259-72.
2) AABB. *Standards for Blood Banks and Transfusion Services*. Bethesda, MD: AABB 2004.
3) Brecher M, Hay S. Bacterial contamination of blood components. Clin Microbiol Rev 2005; **18**: 195-204.
4) Castro E, Bueno J, Barea L, Gonzalez R. Feasibility of implementing an automated culture system for bacteria

screening in platelets in the blood bank routine. Transfus Med 2005; **15**: 185-95.

5) Morbidity and Mortality Weekly Report. Fatal bacteria infections associated with platelet transfusion. United States 2004. CDC 2005; **54**: 168-70. Available at: http//www.cdc.gov/mmwr/preview/mmrhtlm/mm5407a2.htm
6) Yazer M, Triulzi D. Use of a pH meter for bacterial screening of whole blood platelets. Transfusion 2005; **45**: 1133-7.
7) McDonald C, Pearce S, Wilkins KC, et al. Pall eBDS: an enhanced bacterial detection system for screening platelet concentrates. Transfus Med 2005; **15**: 259-68.
8) Schmidt M, Weis C, Heck J, et al. Optimized Scansystem platelet kit for bacterial detection with enhanced sensitivity: detection within 24 h after spiking. Vox Sang 2005; **89**: 135-9.
9) Mohammadi T, Pietersz R, Vandenbroucke-Grauls C, et al. Detection of bacteria in platelet concentrates: comparison of broad-range real-time 16S rDNA polymerase chain reaction and automated culturing. Transfusion 2005; **45**: 731-6.
10) McDonald C, Lowe P, Roy A, et al. Evaluation of donor arm disinfection techniques. Vox Sang 2001; **80**: 135-41.
11) Hundhausen T, Muller T and the GERMS Group. False positive alarms for bacterial screening of platelet concentrates with BacT/ALERT new generation plastic bottles: a multicentric pilot study. Transfusion 2005; **45**: 1267-74.
12) Larsen C, Ezligini F, Hermansen N, Kjeldsen-Kragh J. Six years' experience of using the BacT/ALERT system to screen all platelet concentrates, and additional testing of outdated platelet concentrates to estimate the frequency of false-negative results. Vox Sang 2005; **88**: 93-7.

Received: 22 January 2007 –Revision accepted: 20 April 2007
Correspondence: Dr. Gianluca Gessoni MD
Centro Trasfusionale Ospedale Civile
Via Madonna Marina 500
30015 Chioggia - Venezia - Italy
e-mail: ggessoni@asl14chioggia.veneto.it