Bacterial Contamination of Cellular Blood Components*

A Retrospective Review at a Large Cancer Center

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ABSTRACT

Concern over increased bacterial contamination prompted us to conduct a retrospective review of all bacterial cultures performed on cellular blood components at our institution between December 1989 and June 1993. Sterility checks were accomplished by using the Bactec® blood culture system. The breakdown of the units cultured versus units produced was as follows: Packed Red Blood Cells (PRBCs): 626 (0.6 percent)/102,593; Random Donor Platelets (RDPs): 523 (0.6 percent)/95,005; and Single Donor Platelets (SDPs): 97 (0.7 percent)/13,641. The units were divided into groups with the following results (cultured/positive): (I) PRBCs implicated in transfusion reactions (159/5); (II) PRBCs issued and returned to lab after 30 minutes (155/0); (III) PRBCs expired on shelf (276/3); (IV) PRBCs used for quality control (QC) (36/0); (V) RDPs implicated in transfusion reactions (309/12); (VI) RDPs used for QC (214/3); (VII) SDPs involved in transfusion reactions 43/2); and (VIII) SDPs used for QC (54/0). Identification of isolates yielded: Group I = 4 coagulase negative Staphylococcus (CNS) and 1 Enterobacter agglomerans; Group III = 2 gram negative bacilli and 1 CNS; Group V = 12 CNS; Group V = 12 CNS; Group VI = 2 CNS and 1 Pseudomonas paucimobilis: Group VII = 1 gram variable bacilli and 1 Enterococcus species. Overall, 1.3 percent of all PRBCs, 2.9 percent of all RDPs, and 2.1 percent of all SDPs cultured were positive for bacterial contamination. Although these percentages are low, given the increased susceptibility of immunosuppressed cancer patients, more intensive monitoring of bacterial contamination must be implemented to identify the source of infection.

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Introduction

Bacterial contamination of blood components and transfusion-transmitted sepsis have always been two of the most important and critical untoward events in the collection, processing, storage, and infusion of blood.\(^1\) Recently, numerous published articles and studies have reported a rise in the number of bacterially contaminated units in blood bank inventories.\(^2,3,4\) If true, the impact would be particularly critical on our transfusion-dependent patient population, because of the unique immunosuppression and susceptibility to infection of cancer patients, and its potentially fatal outcome.\(^5\) Thus, all cellular blood components subjected to bacterial cultures at our institution between December 1989 and June 1993 were reviewed retrospectively to determine the incidence of bacterially contaminated units of packed red blood cells (PRBCs), random-donor platelet concentrates (RDPs), and single-donor platelet concentrates (SDPs).

Materials and Methods

Our review covered all bacterial cultures performed on cellular blood components during the 43 months from December 1989 to June 1993. The cultured units were segregated into three main categories (table I): PRBCs, RDPs, and SDPs. These categories were then subdivided into the following groups: (I) PRBCs implicated in transfusion reactions; (II) PRBCs checked out and returned to the transfusion service after our self-imposed 30-minute limit; (III) PRBCs that expired on the shelf; (IV) PRBCs randomly selected for quality control (QC); (V) RDPs implicated in transfusion reactions; (VI) RDPs randomly selected for QC; (VII) SDPs implicated in transfusion reactions; and (VIII) SDPs randomly selected for QC.

The units were sampled using sterile techniques under a laminar flow biological safety cabinet hood in the microbiology section of our laboratory. Sterility was determined by bacterial culture of all specimens using the Bactec Plus 26 blood culture system* Each specimen was cultured both aerobically and anaerobically by inoculating two Bactec bottles with 2.0 ml of sample each and then incubating for a minimum of 14 days at 35°C and 22°C, respectively. The cultures were checked daily for increases in the CO\(_2\) production which, in this system, is equated with bacterial growth. If no evidence of growth was detected after 14 days, the bottles were discarded and the cultures considered negative for bacterial contamination. On the other hand, if growth was detected, the contents of contaminated bottles were then subplated onto 5 percent Trypsin Soy Broth blood, McConkey's, and CNA agar culture media, and organisms identified by using the Vitek and API systems.\(^\dagger\)

Results

To interpret better the results of our study, they were analyzed individually

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by component type so that appropriate consideration would be given to variables such as storage temperature and age of the component. A summary review of the records of all cellular blood components cultured during the study can be found in table II.

**Packed Red Blood Cells**

Of 102,593 PRBCs produced from December 1989 to June 1993, 626 (0.6 percent) were cultured. These 626 units belonged to either of Group I, II, III (approximately half of Group III units were either autologous or directed), or IV. Among these four groups, only two groups, groups I and III, showed any kind of bacterial growth upon culture. Of 159 PRBC units in Group I, five (3.1 percent) were positive for bacterial culture with four instances of coagulase-negative *Staphylococcus* species (routinely, no further identification is indicated by our microbiology protocol) and one occurrence of *Enterobacter agglomerans*.

A review of our records showed that visual inspection of these units revealed no turbidity or any other overt sign of contamination at the time of issue for transfusion. Also, all units with positive cultures in this category were less than 25 days old. None of the patients who experienced the transfusion reactions and who were transfused with culture-positive units showed evidence of sepsis. All blood cultures obtained post-transfusion were negative. The patient transfused with the unit from which *E. agglomerans* was isolated was already septic and receiving antibiotic therapy before being transfused. Blood cultures obtained with the aid of antibiotic removal devices (ARDs) after infusion of the unit in question, failed to isolate *E. agglomerans*.

Group III (units expired on the shelf) was the only other cluster of PRBCs that

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PRBC = packed red blood cells.
RDP = random donor platelets.
SDP = single donor platelets.
QC = quality control.
a Most of the units cultured were autologous or directed donors.
b Three pools of four RDPs each; there is low probability that all platelets in the pool were initially contaminated.
c Left past five days to allow bacterial growth, if present.
had positive cultures. Of 276 units cultured, three (1.1 percent) were positive: two occurrences of gram-negative bacilli and one instance of coagulase-negative *Staphylococcus* species. It should be noted that these units were the oldest in the study (>42 days) and while this cannot be construed as a causative factor, it may have contributed to our findings. The rest of the PRBCs cultured were negative for bacterial contamination, bringing the total of cultured units showing the presence of bacterial growth to eight of 626 (1.3 percent).

**PLATELETS**

The platelets reviewed in the study were divided into RDPs and SDPs. From a total of 95,005 RDPs and 13,641 SDPs produced, 523 (0.6 percent) and 97 (0.7 percent) units, respectively, were checked for bacterial contamination.

From the cluster of 309 RDP units in Group V (RDPs involved in transfusion reactions), three pools of four RDPs each (12 units), or 3.9 percent were found to be positive for bacterial growth, with three instances of coagulase-negative *Staphylococcus* species. One should note that given the rest of the platelet results in this study, the statistical probability of all 12 units involved being contaminated is almost null. Most likely, only one of the RDPs in each pool of four units was responsible for the positive culture results, which would make the percentage of contamination much smaller for that group. Nevertheless, since proving this idea would have been impossible, for the purpose of this study the higher percentage was recorded as if all 12 units were contaminated.

Of a total of 214 units in Group VI, composed of RDPs randomly selected for QC, only three (1.4 percent) showed evidence of bacterial contamination, with two occurrences of coagulase-negative *Staphylococcus* species plus an unclassified gram-negative bacilli and one instance of coagulase-negative *Staphylococcus* species plus *Pseudomonas paucimobilis*. In total, of 523 RDPs cultured (309 from Group V and 214 from Group VI), 2.9 percent showed evidence of bacterial contamination.

Of a total of 43 units in Group VII (SDPs implicated in transfusion reactions), only two (4.7 percent) showing evidence of bacterial contamination. One unit grew gram-variable bacilli, and the other grew an *Enterococcus* species. As with the PRBCs involved in transfusion reactions, follow-up blood cultures showed negative results and no evidence of transfusion-transmitted sepsis.

Of 54 units in Group VIII (SDPs randomly selected for QC), none was positive for bacterial growth or signs of contamination. As a whole, of 97 SDPs cultured from 13,641 units produced, two units (2.1 percent) showed evidence of contamination.

**Discussion**

Our study yielded some interesting results which, although comparable with recently reported levels of contamination (1 to 10 percent on average), differ particularly in the type of organisms isolated. In table III the number of positive bacterial cultures in our study are summarized by group. Notable for its absence is *Yersinia enterocolitica*, the leading cause of concern in most recent studies. Goldman and Blachman cited a steady increase in the number of deaths associated with bacterial sepsis between 1976 and 1988 and the presence of *Y. enterocolitica* as an insidious contaminant with devastating consequences. The concern over *Yersinia* is legitimized by its ability to proliferate at refrigerated temperatures (1 to 6°C) and its predilection for an iron-rich environment, which makes stored blood a preferred growth medium. Also, studies by Arduino et al.
and Kim et al\textsuperscript{8} agreed in concluding that even low-level \textit{Yersinia} contamination can result in a blood product containing high concentrations of endotoxin and bacteria after 2 to 3 weeks of storage, thus making it a very serious threat to the blood bank inventory. At this institution, 1 percent of the stool cultures from adults with diarrhea yield \textit{Y. enterocolitica}, and it could be inferred that the donor base would follow along the same lines. But with the geographic distribution of patient population (more than 70 percent of our patients are either international or from outside the Houston Metro Area) our findings can not be extrapolated to those from the Microbiology Department. Also owing to the nature of cancer treatment and the high demand for transfusion, as much as 20 to 25 percent of the RDP’s and 5 percent of PRBC’s transfused at this institution are purchased from blood centers across the country. This fact prevents us from equating the culture results from our patient population with those of the donors. That no \textit{Y. enterocolitica} was isolated in any of our cultures is significant and could be directly related to our donor selection protocol, which, based on commentary by the U. S. Food and Drug Administration Blood Product Advisory Committee,\textsuperscript{10,11} includes questions dealing with recent gastrointestinal ailments, diarrhea, fever, etc. Admittedly, and as Grossman et al\textsuperscript{12} state, the efficacy of this approach is limited and only expected to identify 50 percent of the cases of \textit{Yersinia} infection eliminating 11 percent of donors\textsuperscript{10,11} This also implies that on occasion blood donors may be in a state of incubation or recovery from bac-
teremia (asymptomatic) and thus able to slip through the donor selection process, placing a potentially contaminated unit into the blood bank's available inventory. In most of these cases, direct visual inspection of the unit does not reveal turbidity, icterus, or any other signs of contamination, even though, according to Glick et al, this is not a reliable method for determining if contamination is present. Many of the units in our study were released prior to the description by Kim et al of a visual inspection method involving the comparison of segment color to the bags of red blood cells preserved in additive solution (AS-3), as a means of identifying gross contamination. This method is also limited since it cannot detect low levels of bacteria.

Other factors, such as possible contamination during pooling of components, transport of units to the transfusion site, and procedures directly related to the bedside infusion of blood components, must also be addressed. While the last two are usually "off limits" to our blood bank personnel as far as ensuring proper handling, the pooling of components is directly under our control, and new efforts have been directed toward procuring a more sterile blood component.

Manual pooling of RDPs can be a source of contamination when strict adherence to the use of sterile technique is not followed. A busy blood-distribution area may also aggravate the problem since time may be a precious commodity. The advent of sterile connecting devices has had, in our case, considerable impact of our ability to maintain a smooth flow of blood components through our blood distribution area by allowing us to pool RDPs a short time ahead of their release. This has in turn created a less stressful environment and also ensured the sterile handling of the product, thus reducing the possibility of contamination or transfusion-transmitted sepsis.

It should be noted that the highest percentage of positive cultures in our study involved SDPs (4.7 percent), that were 1 to 2 days old and were implicated in transfusion reactions. These reactions were mostly febrile in nature with few instances of hypotension or urticaria. In contrast, none of the SDPs in the quality control group that were held for up to 7 days before culture showed any evidence of bacterial contamination. This suggests that the positive results were a consequence of contamination during collection or manipulation of the product by nursing personnel.

Next, the unit testing procedure itself must be analyzed and put into perspective. Given the manipulation involved in sampling the units, and given the fact that the possibility of contamination during inoculation and culturing cannot be completely discounted, a positive culture does not necessarily reflect direct contamination of the product from the original donor. It is notable that six of 18 positive products in our study grew gram-negative bacilli. The cold storage conditions at which some blood products are kept may favor the growth of environmental gram-negative bacilli such as P. paucimobilis and facilitate contamination from outside of the collection bags. Contamination with unusual organisms, such as P. paucimobilis, is occasionally seen and has previously been traced to ice baths, cooling blocks, etc. It is possible, but not confirmed, that transport coolers may serve as a source for this organism. The organism could be introduced at the time of tubing splicing. Thus, a potential control measure may be to disinfect the storage cabinets more frequently.

The patient population and the type of medication being administered can also have an impact on the morbidity and
mortality of the potential bacterial contaminants. In the case of cancer patients requiring constant transfusions of platelets and RBCs, antibiotic therapy is also common, and this may influence the clinical outcome associated with the transfusion of bacterially contaminated blood products.

Another aspect of our research seems to agree with previous findings by Hamill et al.\textsuperscript{15} with regard to the amount of time a unit of PRBCs may stay outside the blood bank after release for infusion before being returned to the blood distribution area and safely placed back into the available inventory. Hamill et al.\textsuperscript{15} have suggested that a 2-hour cutoff limit be adopted since no significant increase in bacterial proliferation seems to occur and since extension of the currently used 30-minute "grace period" would allow the recapture of most units being discarded while providing a reasonably safe margin. In our study, none of the units in group II (PRBC units returned to the blood bank after 30 minutes of release) showed any evidence of bacterial contamination; this supports the assertion of Hamill et al.\textsuperscript{15}

More research and the implementation of comprehensive preventive measures such as the extension of donor screenings; the avoidance of scarred phlebotomy sites, which may be difficult to disinfect; and the development of a rapid, reliable test for the detection of bacterial contaminants are needed as described by Goldman et al.\textsuperscript{3} Furthermore, as mentioned by Hoppe,\textsuperscript{16} the addition of antibiotics to blood containers in order to suppress bacterial growth remains unsettled, given the possibility of untoward reaction induced by the added antibiotics. Also the use of prestorage filtration devices to remove WBCs\textsuperscript{8,17,18,19,20} continues to be evaluated to achieve optimal effectiveness. However, in the age of cutbacks and increased demand for cost-cutting measures, expensive new technology must be weighed against its overall effectiveness and the impact it would have on the operational capabilities of the transfusion service.

In closing, transfusion-transmitted sepsis is of primary concern; it should be noted that of the patients receiving culture-positive units in this study, none showed evidence of transfusion-related infection or any consequence thereof. Although the percentage of positive cultures was low in this study, given the increased susceptibility of immunosuppressed cancer patients, more extensive monitoring of bacterial contamination must be implemented to identify the source of the contaminants.

References


