

Bacterial Reduction by Cell Salvage Washing and Leukocyte Depletion Filtration

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Background: Blood conservation techniques are being increasingly used because of the increased cost and lack of availability of allogeneic blood. Cell salvage offers great blood savings opportunities but is thought to be contraindicated in a number of areas (e.g., blood contaminated with bacteria). Several outcome studies have suggested the safety of this technique in trauma and colorectal surgery, but many practitioners are still hesitant to apply cell salvage in the face of frank bacterial contamination. This study was undertaken to assess the efficacy of bacterial removal when cell salvage was combined with leukocyte depletion filtration.

Methods: Expired packed erythrocytes were obtained and inoculated with a fixed amount of a stock bacteria (*Escherichia coli* American Type Culture Collections [ATCC] 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, or *Bacteroides fragilis* ATCC 25285) in amounts ranging from 2,000 to 4,000 colony forming units/ml. The blood was processed via a cell salvage machine. The washed blood was then filtered using a leukocyte reduction filter. The results for blood taken during each step of processing were compared using a repeated-measures design.

Results: Fifteen units of blood were contaminated with each of the stock bacteria. From the prewash sample to the postfiltration sample, 99.0%, 99.6%, 100%, and 97.6% of *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. fragilis* were removed, respectively.

Discussion: Significant but not complete removal of contaminating bacteria was seen. An increased level of patient safety may be added to cell salvage by including a leukocyte depletion filter when salvaging blood that might be grossly contaminated with bacteria.

THE high cost and decreasing availability of blood and blood products have made blood conservation strategies of growing importance. During many surgical procedures, cell salvage technology is applied to decrease transfusion of allogeneic blood. Cell salvage involves the collection of shed blood in a sterile collection reservoir followed by washing of the blood with normal saline or lactated Ringer's solution. A limitation of cell salvage is the numerous contraindications to its use. One of the common manufacturer-recommended contraindications to cell salvage use is in surgery where bowel contents may be mixed with salvaged blood.^{1,2}

The impact of cell salvage processing in blood that has been bacterially contaminated was investigated by Bou-

dreux *et al.*,³ who inoculated expired units of blood with bacteria and found that the blood after cell salvage washing had from 5% to 23% of the starting contamination. Despite this reduction, cell-salvaged blood with known bacterial contamination is still viewed as potentially harmful to patients. For this reason, we proposed to combine cell salvage washing with a leukocyte depletion filter in the production of a cell salvage blood product. These filters have been found to reduce bacterial contamination in allogeneic blood.⁴⁻⁶ Thus, the hypothesis of this study is that the combination of leukocyte depletion filtering along with cell salvage will further reduce bacterial contamination of the blood product.

Materials and Methods

Expired packed erythrocytes (autologous donation) were obtained from the blood bank. Each unit was inoculated with a standard bacterial cell line obtained from American Type Culture Collections (ATCC), which is a repository for a diverse collection of microorganisms. The four bacterial strains used were *Escherichia coli* ATCC 25922 (aerobic, Gram-negative bacilli), *Pseudomonas aeruginosa* ATCC 27853 (aerobic, Gram-negative bacilli), *Staphylococcus aureus* ATCC 29213 (aerobic, Gram-positive cocci), and *Bacteroides fragilis* ATCC 25285 (anaerobic, Gram-negative bacilli). Fifteen units of blood were obtained for each of the four bacterial types. Each bacterial cell line was processed in a group as discarded blood became available, rather than randomly choosing a bacteria.

Inoculation of each unit began by suspending the bacteria in 2 ml saline, 0.85%, at a turbidity of a 0.5 McFarland unit, which is $\approx 1.5 \times 10^8$ colony forming units (cfu)/ml. Using a micropipette, 0.01 ml was diluted into 2 ml saline, 0.85%, and was mixed well. One milliliter was removed and inoculated into the blood bank unit, and the unit was mixed for 1 h on a rocker table at room temperature. Just before cell salvage processing, a sample of this inoculated blood was taken for quantitative bacterial culture (prewash). For a control unit, 1 ml saline, 0.85%, was injected into the blood unit.

After inoculation and mixing, the blood was processed via a Sequestra 1000 cell salvage machine (Medtronic, Inc., Minneapolis, MN). The blood was drawn into the Latham bowl directly from the blood bag rather than dumping the unit into a cell salvage reservoir. Thus, no macroaggregate filtration took place. Cell processing occurred using a 125-ml Latham bowl with fill rates of

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Table 1. Characteristics of Blood Used

| Organism | Mean Days after Expiration \pm SD | Mean Volume (g) \pm SD | Blood Type | Rh |
|-------------------------------|-------------------------------------|--------------------------|---------------------|-----------|
| <i>E. coli</i> (n = 15) | 6.1 \pm 4.7 | 358 \pm 3 | 5 O, 7 A, 2 B, 1 AB | 13 +, 2 - |
| <i>S. aureus</i> (n = 15) | 4.9 \pm 4.7 | 343 \pm 36 | 6 O, 5 A, 3 B, 1 AB | 13 +, 2 - |
| <i>P. aeruginosa</i> (n = 15) | 5.5 \pm 2.4 | 344 \pm 15 | 3 O, 9 A, 3 B | 11 +, 4 - |
| <i>B. fragilis</i> (n = 15) | 8.9 \pm 12.8 | 347 \pm 17 | 4 O, 8 A, 3 B | 12 +, 3 - |
| Control (n = 5) | 13 \pm 12 | 325 \pm 57 | 1 O, 3 A, 1 B | 5+ |

300 ml/min, a wash rate of 300 ml/min, and a total wash volume of 1,000 ml. No erythrocyte pack flexing or manipulation of the manufacturer-specified centrifuge speed was performed. After cell washing, a sample of the washed blood (postwash) was collected for culture. The washed blood was then filtered utilizing a leukocyte reduction filter (LeukoGuard RS; Pall Biomedical Products Company, East Hills, NY) with 300 mmHg pressure applied to the collection bag *via* a 500-ml pressure bag. A sample of the filtered blood (postfiltration) was collected for culture.

After the processing, the blood samples from each time point, prewash, postwash, and postfiltration, were taken to the microbiology laboratory for immediate bacterial quantitation. One milliliter and 0.1 ml (1:10 dilution) of each blood sample were plated directly onto sheep blood agar or Mueller-Hinton agar in duplicate. In addition, 0.1 ml each sample was added to 0.9 ml saline (1:10 dilution) and serially diluted in saline to 1:100. Of the 1:10 and 1:100 dilutions, 0.1 ml was inoculated onto the sheep blood agar in duplicate, resulting in final dilutions of 1:100 and 1:1,000 of each sample. The inoculum was evenly distributed on the plates using disposable spreaders. The plates were inoculated at 35°C in ambient air for *E. coli*, *S. aureus*, and *P. aeruginosa* and anaerobically for *B. fragilis*. Colonies were counted at 24 h for *E. coli* and *P. aeruginosa* and at 48 h for *S. aureus* and *B. fragilis*.

Sample Size Calculation

In a preliminary trial, 99% of *S. aureus* was filtered from a blood sample. For a 95% confidence interval (under the assumption that a normal distribution holds), it was estimated that 15 cases would be needed to determine the filter efficacy of *S. aureus* (99 \pm 5%).

Table 2. Bacterial Reduction

| Bacteria | Inoculum Concentration (cfu/ml) | Prewash Concentration* (cfu/ml) | Postwash Concentration† (cfu/ml) | Postfiltration Concentration‡ (cfu/ml) | Reduction, % (Prewash to Postfiltration) |
|----------------------|---------------------------------|---------------------------------|----------------------------------|--|--|
| <i>E. coli</i> | 596,000 \pm 57,200 | 1,920 \pm 452 | 440 \pm 113 | 19 \pm 16 | 99.0 |
| <i>S. aureus</i> | 741,333 \pm 889,163 | 3,691 \pm 5,152 | 436 \pm 256 | 4 \pm 7 | 99.9 |
| <i>P. aeruginosa</i> | 611,000 \pm 273,000 | 1,970 \pm 1,020 | 227 \pm 113 | 0.6 \pm 2 | 100 |
| <i>B. fragilis</i> | 754,000 \pm 84,751 | 4,603 \pm 1,480 | 1,039 \pm 236 | 111 \pm 74 | 97.6 |
| Control (n = 5) | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | - |

* $P < 0.03$ for comparison of prewash with postwash. † $P < 0.02$ for comparison of postwash with postfiltration. ‡ $P < 0.001$ for comparison of prewash with postfiltration.

cfu = colony forming units.

Statistical Analysis

A repeated-measures model was fit with bacterial contamination as the response variable and steps (prewash, postwash, and postfiltration), organism, and the interaction between those two as predictors. $P < 0.05$ was considered statistically significant.

Results

Fifteen units of blood were obtained for each of the four bacterial types. The characteristics of each of these units is shown in table 1. After inoculation, cell salvage washing, and filtration, 99.0%, 99.9%, 100%, and 97.6% of bacterial contaminations were removed from *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. fragilis*, respectively (table 2). From the statistical modeling, the effects of each step of the processing were different across the different organisms.

Discussion

This study found that the combination of cell salvage washing with the use of a leukocyte depletion filter resulted in a significant reduction in bacterial loads of *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. fragilis*. The efficacy of this blood treatment varied depending on the bacterium. Because testing all known bacteria is logistically and financially impossible, selection of a mix of aerobes and anaerobes that are often found in the surgical environment was performed.

Whether these data extrapolate to all other bacteria requires a better understanding of leukocyte depletion filter technology. The leukocyte depletion filter used in this study was a depth filter. A depth of synthetic mate-

Table 3. Relationship of Filtering to Dose of *Bacteroides fragilis*

| Prewash Concentration (cfu/ml) | Postfiltration Concentration \pm SD (cfu/ml) | Mean Reduction \pm SD (%) |
|--------------------------------|---|-----------------------------|
| 1.78×10^3 | $8.3 \times 10^2 \pm 0.6 \times 10^2$ | 53 ± 3.3 |
| 1.54×10^4 | $2.7 \times 10^3 \pm 6.7 \times 10^2$ | 82 ± 4.4 |
| 1.34×10^5 | $3.2 \times 10^4 \pm 3.8 \times 10^3$ | 76 ± 2.8 |
| 1.26×10^6 | $2.3 \times 10^5 \pm 4.0 \times 10^4$ | 82 ± 3.2 |
| 1.71×10^7 | $>3.0 \times 10^5$ (colonies too numerous to count) | NA |

cfu = colony forming units; NA = not applicable.

rial is packed together with increasing density of fibers with incrementally smaller fiber size. The filters work through two mechanisms—sieving and adhesion. With sieving, particles are removed by their size. The size of *E. coli* is 0.5 μm in diameter by 1 to 2 μm in length, whereas an erythrocyte measures 8 μm in diameter by 2 μm in thickness.^{7,8} From these dimensions, it quickly becomes obvious that the mechanism for bacterial removal is more dependent on the adhesive characteristics of the filter. These characteristics would include the surface charge, wettability, and surface structure of the filter material.^{9,10} Therefore, the interaction of a particular bacterium would be dependent on the bacteria and how its cell wall interacts with the filter material.¹¹

Completion of this study left several questions that warranted further investigation, the first of which was, How high would bacterial contamination be if blood from a surgical procedure was frankly contaminated with stool? To study this question, blood samples were taken from blood suction canisters where stool had been suctioned from the surgical field. Twenty samples were collected for quantitative bacterial culture. From these samples, aerobic and anaerobic contamination was measured. For the aerobic bacteria, 1.7×10^4 cfu/ml was found with a 95% confidence interval of 393 cfu/ml, whereas the anaerobic contamination was 5.3×10^5 cfu/ml with a 95% confidence interval of 2.8×10^4 cfu/ml. These concentrations were generally 10-fold greater than bacterial concentrations used in the *in vitro* study.

These data suggested that an understanding of the efficacy of the leukocyte depletion filter was needed when variable quantities of bacteria are passed through it. Therefore, another supplemental study was undertaken to explore how higher concentrations of bacteria might effect the function of these filters. Blood was contaminated with escalating contaminant loads (ranging from 10^3 to 10^7) of the *B. fragilis* cell line and then filtered without washing. Each concentration was filtered and measured three times. *B. fragilis* was chosen because the original study data indicated that it had the least reduction in concentration. Table 3 shows how filtration changes depending on the presenting bacterial

load. It is readily apparent from these data that high loads of bacteria presented to the filter will ultimately result in high loads of bacteria coming out of the filter. The filters by themselves lead to ≈ 10 -fold reduction in bacterial concentration. From these data, it would seem that washing and filtration remove most bacterial contaminants up to a concentration of 10^3 cfu/ml. When blood grossly contaminated with stool is presented, then washing and filtration will most likely result in significant residual bacterial loads.

The next question was, How much blood contamination could a patient tolerate before having clinical symptoms related to readministration of contaminated blood? Blood produced from cell salvage has long been recognized to be bacterially contaminated. Kang *et al.*¹² reported that 9% of the blood returned to liver transplant patients had skin contaminants. In cardiac surgery, Bland *et al.*¹³ found that bacterial contamination of cell salvage blood approaches 30% of the units processed. This contamination was primarily bacteria from skin flora and has been assumed to be inconsequential, but the contaminants of frank stool have been thought to be different than merely skin flora. Several investigators have reported on frank stool contamination of reinfused salvaged blood.¹⁴⁻¹⁶ Despite the gross bacterial contamination, no increased sepsis rates were noted.

Also being addressed by the blood bank community as it relates to bacterial contamination of platelets is: How much bacteria is too much? It is estimated that 500–750 severe reactions or deaths occur per year due to the bacterial contamination of blood products.¹⁷ Many studies report on the incidence of bacterial contamination, but few place quantitative figures on the extent of the contamination. In a report of a prospective surveillance program, Yomtovian *et al.*¹⁸ found an incidence of 0.19% of random donor units to be contaminated. The magnitude of the contamination ranged from 0.5×10^2 to 4×10^{11} cfu/ml. In this surveillance study, eight pools of platelets were administered to patients, of whom five had no symptoms with bacterial loads ranging from 10^2 to 10^{11} cfu/ml; however, patients with symptoms had contamination ranging from 10^6 to 10^8 cfu/ml. From these data and other findings,¹⁹⁻²¹ it would seem that the type of bacteria is more important than the quantity.

The concomitant use of broad-spectrum antibiotics has been advocated by some investigators to decrease the risk of subsequent infectious complications. In a dog model, Smith *et al.*²² demonstrated a significant reduction in dog mortality when contaminated blood administration was accompanied by antibiotics. In a retrospective review of cases of hollow viscera injuries, Timberlake *et al.*¹⁶ concluded that safety could be added through the use of broad-spectrum antibiotics. Wollinsky *et al.*²³ found that antibiotic prophylaxis during hip arthroplasty significantly reduced the bacterial contamination of autotransfusion blood.

The results of this study suggest that it is premature to conclusively state that it is safe to use cell salvage blood when it has been grossly contaminated with stool or other sources of bacteria. In a review of the controversies surrounding cell salvage, Dzik and Sherburne²⁴ pointed out that allogeneic transfusion leads to an increase in infection rate, and that when faced with bacterial contamination of cell-salvaged blood, a clinical decision must be made as to which therapy offers the least risk to the patient. The current study suggests that the use of leukocyte filters may offer an additional degree of safety, but the recommendation of Dzik and Sherburne²⁴ still seems to be sound.

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