Amniotic Fluid Removal during Cell Salvage in the Cesarean Section Patient

Jonathan H. Waters M.D.,* Charles Biscotti, M.D.,† Paul S. Potter M.D.,‡ Eliot Phillipson M.D.§

Background: Cell salvage has been used in obstetrics to a limited degree because of a fear of amniotic fluid embolism. In this study, cell salvage was combined with blood filtration using a leukocyte depletion filter. A comparison of this washed, filtered product was then made with maternal central venous blood.

Methods: The squamous cell concentration, lamellar body count, quantitative bacterial colonization, potassium level, and fetal hemoglobin concentration were measured in four sequential blood samples collected from 15 women undergoing elective cesarean section. The blood samples collected included (1) unwashed blood from the surgical field (prewash), (2) washed blood (postwash), (3) washed and filtered blood (postfiltration), and (4) maternal central venous blood drawn from a femoral catheter at the time of placental separation.

Results: Significant reductions in the following parameters were seen when the postfiltration samples were compared to the prewash samples (median [25th-75th percentile]): squamous cell concentration (0.0 [0.0-0.1 counts/high-powered field (HPF)] vs. 8.3 counts/HPF [4.0-10.5 counts/HPF], , P < 0.05); bacterial contamination (0.1 [0.0-0.2] vs. 3.0 [0.6-7.7] colony-forming units (CFU)/ml, P < 0.01); and lamellar body concentration (0.0 [0.0-1.0] vs. 22.0 [18.5-29.5] thousands/μl, P < 0.01). No significant differences existed between the postfiltration and maternal samples for each of these parameters. Fetal hemoglobin was in higher concentrations in the postfiltration sample when compared with maternal blood (1.9 [1.1-2.5] vs. 0.5% [0.3-0.7]). Potassium levels were significantly less in the postfiltration sample when compared with maternal (1.4 [1.0-1.5] vs. 3.8 mEq/l [3.7-4.0]).

Conclusions: Leukocyte depletion filtering of cell-salvaged blood obtained from cesarean section significantly reduces particulate contaminants to a concentration equivalent to maternal venous blood. (Key words: Autologous blood; autotransfusion; embolism; obstetrics.)

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tion, the influence of filtering the washed blood product with leukocyte reduction filters was assessed. The washed, filtered product was then compared to that of a maternal blood sample drawn from a catheter placed slightly above the uterine veins in the vena cava. The catheter tip placement was intended to capture any amniotic fluid contaminants as they occurred at the time of placental separation and should reflect levels of contaminants to which the mother normally is exposed.

Materials and Methods

After institutional review board approval from the Cleveland Clinic Foundation, with written and verbal consent, 15 nonlaboring patients undergoing elective cesarean section were enrolled in the study. Exclusion criteria included maternal fever, infection, or antibiotic administration.

At arrival in the operating room, all patients received a spinal anesthetic using 1.5 ml hyperbaric bupivacaine, 0.75%, combined with 0.25 mg preservative-free morphine sulfate. At completion of the spinal anesthetic, all patients underwent placement of a 20-cm, 16-gauge femoral venous catheter during sterile conditions using a catheter-over-a-wire technique. To capture blood contaminated with amniotic fluid, the catheter was placed so that the catheter tip was above the level of the uterine veins as they flow into the common iliac vein. After placental separation, 45 ml maternal blood was aspirated through this line (maternal sample).

During the surgical procedure, all blood and amniotic fluid was suctioned directly into a cell-salvage reservoir that had a 120-μm filter. In addition, all sponges were rinsed with 1,000 U/ml heparin solution before the introduction of the blood sample to prevent clotting. Electronic resonance detection enhanced by hydrodynamic focusing (Sysmex SE9500; Roche Diagnostics, Indianapolis, IN) was used for lamellar body counting.

1. Seven milliliters of blood for lamellar body count was placed in a plastic specimen tube at their acquisition and placed in an envelope to prevent light degradation of the lamellar bodies. The specimen tube in which the maternal blood sample was placed was rinsed with 1,000 U/ml heparin solution before the introduction of the blood sample to prevent clotting. Electronic resonance detection enhanced by hydrodynamic focusing (Sysmex SE9500; Roche Diagnostics, Indianapolis, IN) was used for lamellar body counting.

2. Five milliliters of blood was placed into a purple-top tube for quantitative fetal hemoglobin using the Kleihauer-Betke test.

3. Five milliliters of blood was placed into a red-top tube for potassium measurement. Potassium concentration was determined using ion-selective electrode technology (Beckman CX3; Beckman Instruments, Berea, CA).

4. Seven milliliters of blood was placed into a blood isolator tube for quantitative blood culture.

5. Ten milliliters of blood was placed into CytoLyt solution (Cytyc Corporation, Boxborough, MA) for evaluation of squamous cells. The vials of CytoLyt solution were centrifuged and the supernatant discarded. The sample was resuspended in Preservcyt (Cytyc Corporation), yielding 1 alcohol-fixed slide/sample. Subsequently, the slide was stained using the Papanicolaou technique. Each slide was screened for the presence of squamous epithelial cells by a pathologist blinded to the origin of the sample. If squamous cells were present, counts were performed by starting on a representative high-magnification field, which contained squamous epithelial cells, and counting 10 consecutive fields. Counts were repeated four times and then averaged. Nucleated and anucleated squamous cells were counted. Anucleated squamous cells were defined as folded, cosinophilic, angular structures closely resembling their nucleated counterparts.
### Table 1. Concentrations of Amniotic Fluid Markers

<table>
<thead>
<tr>
<th>Test</th>
<th>Maternal (n = 14)</th>
<th>Prewash (n = 14)</th>
<th>Postwash (n = 14)</th>
<th>Postfiltration (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (mEq/l)</td>
<td>3.8 (3.7–4.0)</td>
<td>3.8 (2.4–4.0)</td>
<td>1.5 (1.1–1.5)*</td>
<td>1.4 (1.0–1.6)*</td>
</tr>
<tr>
<td>Lamellar body count (K/μl)</td>
<td>31.0 (24.0–49.9)</td>
<td>22.0 (18.5–29.5)</td>
<td>3.0 (2.0–3.5)*</td>
<td>0.0 (0.0–1.0)*</td>
</tr>
<tr>
<td>Squamous cell count (count/HPF)</td>
<td>0.0 (0.0–0.1)†</td>
<td>8.3 (4.0–10.5)</td>
<td>4.4 (3.0–7.6)</td>
<td>0.0 (0.0–0.1)†</td>
</tr>
<tr>
<td>Quantitative bacterial culture (CFU/ml)</td>
<td>0.0 (0.0–0.1)†</td>
<td>3.0 (0.6–7.7)</td>
<td>1.3 (0.4–6.1)</td>
<td>0.1 (0.0–0.2)†</td>
</tr>
<tr>
<td>Fetal hemoglobin (%)</td>
<td>0.5 (0.3–0.7)</td>
<td>1.1 (0.7–1.5)‡</td>
<td>1.7 (1.0–2.1)‡</td>
<td>1.9 (1.1–2.5)‡</td>
</tr>
</tbody>
</table>

Values expressed as median (twenty-fifth to seventy-fifth percentile).

* P < 0.05 compared with prewash and maternal.
† P < 0.05 compared with prewash and postwash.
‡ P < 0.05 compared with maternal.
$ K = $ thousands; HPF = high powered field, CFU = colony-forming units.

### Statistical Analysis

The data were analyzed using standard descriptive techniques. Statistical significance between prewash, postwash, postfiltration, and maternal samples was assessed using a Kruskal-Wallis test with a Dunn post hoc test. P < 0.05 was considered statistically significant.

### Results

Fifteen patients were enrolled in the study. The average patient age was 33 ± 6 yr. The average gestational age of the fetus at cesarean section was 38 ± 2 weeks. The average estimated blood loss was 800 ± 250 ml. All cesarean sections were performed through a low transverse incision with manual removal of the placenta. One patient was excluded from the study after a cesarean section that resulted in such a small amount of blood loss that blood salvage and washing could not be performed. No patient showed evidence of amniotic fluid embolism during childbirth. Table 1 shows the results from the measured parameters. Figure 1 shows a typical Papanicolaou stain of blood from a prewash sample. Figure 2 shows a typical postwash sample. Figure 3 shows a typical postfiltration blood sample. Bacterial contamination was significantly less for the postfiltration and maternal samples when compared with the prewash sample. No statistical difference was found between these samples and the postwash, though the trend would suggest that with a larger sample size a difference would have been found. All prewash samples contained bacteria, primarily *Staphylococcus*. In addition, one sample contained gram-negative bacilli and one sample contained diphtheroid bacilli. In the postwash sample, 93% of the samples grew only *Staphylococcus* species. The postfiltration sample grew *Staphylococcus* species in 50% of the samples. The maternal sample grew only staphylococci in 25% of the samples.

Fig. 1. Squamous cells in a typical prewash sample are shown with numerous white blood cells. Red cells are visible but difficult to differentiate from the other contaminants (Papanicolaou stain).
Discussion

The mechanism of amniotic fluid embolism is not certain; therefore, the effectiveness of washing and filtering amniotic fluid-contaminated blood was evaluated using several different components. Because amniotic fluid is an electrolyte solution up until late pregnancy, at which time phospholipids from lung maturation and particulate matter, such as squamous cells, start to accumulate, markers for each of these components of amniotic fluid contamination were measured. Potassium was chosen as a marker for electrolyte removal. Lamellar bodies were chosen as a marker of phospholipids. (Lamellar bodies are composed of phospholipids, such as lecithin and sphingomyelin, and are derived from the type II epithelial cells of the fetal lung.8) Lastly, squamous cell contamination was chosen as a marker of particulate contamination. In addition, a previous investigator7 found bacterial contamination and fetal hemoglobin in his cell-salvage product, so these markers were also measured.

Unlike all but one previous study, an additional step was added to the cell-salvage processing. This additional step was filtering the cell-salvage product with leukocyte reduction filters. Leukocyte reduction filters are used to remove leukocytes from donated units of blood. The filters work through the use of a small-pore microfiber web and a negative surface charge.9 Several studies have shown that these filters are effective in filtration of tumor cells in cell salvage,10-13 so it would be reasonable to postulate that these filters might be effective in removing the particulate components of amniotic fluid.

In the only other study of cell-salvaged blood contaminated with amniotic fluid in which leukocyte reduction filters were used, Catling et al.14 found that half of their postfiltration samples were contaminated with fetal squamous cells. This result is significantly different than the findings of this study. This difference relates to a difference in the type of leukocyte reduction filters used among studies. Catling et al.14 used the older generation,
Pall RC 100 filter; whereas, in this study, a Pall RS filter was used. According to the manufacturer, there are significant differences in the media (fiber, fiber diameter, spacing between fibers, composition, and charge) between the two filters, which would account for differences in filtering capability (personal oral communication, Girolomo Ortolano, Ph.D., Senior Scientist, Pall Biomedical Products Co., February 2000).

Because the process of washing and filtering was not expected to obtain a perfect product, a washed, filtered, salvaged blood product was compared with a maternal central venous blood sample. We found that after cell-salvage processing and filtration, squamous cell and bacterial contaminations were similar to those in maternal blood. Potassium and lamellar body concentrations were significantly less than maternal concentrations, whereas fetal hemoglobin concentrations were significantly higher. Thus, a combination of cell-salvage washing and filtration appears to produce a blood product comparable with maternal blood, with the exception of the fetal hemoglobin contamination.

The significance of fetal red cell contamination is understudied. Fetal blood is routinely entrained into the maternal circulation during delivery; therefore, our supposition is that the fetal red cell contamination is insignificant, except when there is a red cell antigen incompatibility between mother and infant. Isoimmunization can occur from this exposure, leading to erythroblastosis in subsequent pregnancies. Blood group system (ABO) incompatibility tends to be a minor problem when compared with Rh factor (Rh) incompatibility. To prevent isoimmunization, anti-D immune globulin (Rhogam; Ortho-Clinical Diagnostics, Inc., Raritan, NJ) is administered to the mother, neutralizing this immune response. The immune globulin is administered based on a calculation of the volume of fetal red cells transferred to the mother (Kleihauer-Betke test). If cell-salvaged blood was administered in the circumstances of an Rh incompatibility, it would seem logical to measure the maternal fetal hemoglobin concentration after cell-salvaged blood has been administered, so that the immune globulin dose is adequate to neutralize these additional cells.

Measurement of bacterial contamination was performed to determine the effectiveness of the leukocyte reduction filters in reducing bacterial contamination. Compared with the prewash sample, a 24-fold reduction of the bacterial colony counts was obtained through washing and filtering. Bacterial contamination of cell-salvaged blood has been recognized before and it has been deemed to be clinically insignificant. This insignificance relates to a number of factors. First, during the course of most operations, bacteremia is present. Second, homologous blood obtained from the blood bank is not bacteria free. Lastly, postoperative infection rates are lower in patients receiving autologous blood when compared with homologous blood because of immunomodulation that occurs after homologous blood exposure.

Unlike the methods of the current study, additional safety in the application of cell salvage might be achieved through the use of a double-suction setup. In this setup, one suction line should be connected to the cell-salvage reservoir and used for suctioning of maternal blood, whereas the other should be connected to the regular wall suction and used for aspiration of amniotic fluid. By using separate suction devices, the contamination of the salvaged blood could be further minimized. The smaller the overall contamination of the salvaged erythrocytes, the lower the resultant concentration in the washed product. Although this recommendation seems logical and intuitive, it is unproven.

Support for the use of cell salvage in obstetric hemorrhage encompasses 390 reported cases in which blood contaminated with amniotic fluid has been washed and readministered without filtration. Discussion with anesthesiologists from several different institutions would suggest that this practice has been established for a decade and has gone markedly underreported. From the results of the current study, use of a leukocyte reduction filter appears to reduce amniotic fluid contaminants to a level approaching those levels found in maternal blood. This study provides further evidence for the use of cell salvage in obstetrics. Because the incidence of amniotic fluid embolism is rare, the safety of cell salvage in obstetrics will most likely never be firmly established.

**References**


