Detection and removal of fat particles from postoperative salvaged blood in orthopedic surgery

Gemma Ramírez, Adolfo Romero, Juan Jesús García-Vallejo, and Manuel Muñoz

BACKGROUND: Although transfusion or return of salvaged shed blood has become popular in major orthopedic procedures, this blood-saving method is still controversial because shed blood may be contaminated with chemical and tissular debris, such as fat particles, which may increase the risk of fat embolism after bone surgery.

STUDY DESIGN AND METHODS: In an effort to find an easy, reliable method for determination of both fat particle content and removal from shed blood, analyses of perioperative blood samples were performed with a cell counter (Technicon H3 [H3]) in orthopedic patients undergoing spinal fusion in which postoperative shed blood was collected and returned with a blood collection canister. A screen or surface filter was intercalated in the return line to eliminate microaggregates, fat particles, and/or WBCs.

RESULTS: Fat particles in shed blood are clearly detected as a condensed, sigmoidal-shaped area at the right-hand side of the PMN zone in the channel in which the H3 measures particles according to their degree of lobularity. This signal can be reproduced by the addition of animal or vegetable fat to venous blood, but not by the addition of activated platelets or RBC membranes. Fat particles, together with WBCs and microaggregates, in shed blood were effectively removed by surface filters, whereas screen filters were not effective.

CONCLUSION: The use of the TH3 seems to be an easy, reliable, and low-cost approach for monitoring fat particle content and removal from postoperative salvaged shed blood in orthopedic procedures.

The increased awareness of the potential hazards of allogenic blood transfusion, such as compatibility reactions and metabolic and immunologic disorders or transmission of viral diseases,1-4 has led to an emphasis on allogenic blood alternatives.5-7

For orthopedic surgery, several autologous transfusion modalities have emerged as alternatives to allogenic blood transfusion, avoiding its immunomodulatory effects. Preoperative autologous blood donation (PABD) has been reputed as one of the most safe and effective transfusion therapies,8-10 but it may have problems of overcollection, overtransfusion, and allogenic transfusion may still be required.7,10 Moreover, because the role of blood storage on transfusion-induced immunomodulation is still unclear,11 PABD might be accomplished as close to the operation date as possible, hence demanding a tight surgical program and limiting the number of units to be collected. Intraoperative cell salvage and postoperative autologous transfusion completely avoid the problem of blood storage. However, intraoperative cell

ABBREVIATIONS: BASO = channel in which particles are measured according to their degree of lobularity; PABD = preoperative autologous blood donation; SCAD = small capillary and arteriolar dilation; PEROX = channel in which particles are measured according to their size and number.

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salvage is cost-effective for only those operations with high intraoperative blood loss (>1000-1500 mL), and is not useful in others, such as total knee replacement, in which postoperative autologous transfusion with or without PABD seems to be the best alternative to allogeneic blood.

On the other hand, although the effectiveness of the return of unwashed shed blood after orthopedic operations is well documented, several authors have questioned the safety of this blood-saving method because unwashed shed blood may be contaminated with fat particles, bone fragments, methylmethacrylate monomers, activated coagulation factors, fibrin degradation products, or inflammatory mediators.

Return of fat probably increases the risk of fat embolism syndrome, which is mostly associated with acute lung injury but may also result in postoperative neurological deficit. If salvaged blood is returned through an arterial line, as in cardiac surgery under cardiopulmonary bypass, neurologic complications could be relatively common. However, contamination of shed blood with fat particles leads to two unsolved problems: that is, the lack of easy and reliable methods for both detecting and removing these particles. Usually, fat content in shed blood is measured in Nile red-stained samples with flow cytometry or fluorescence microscopy, which is an expensive and complex methodology, whereas fat embolism has been detected by magnetic resonance imaging, transcranial doppler, or histologic methods (small capillary and arteriolar dilation [SCAD]). On the other hand, fat particle removal has been accomplished by either filtration or the use of cell salvage devices that wash and concentrate autologous RBCs.

Accordingly, this study was initiated to examine the ability of an automated cell counter to detect fat particles in shed blood collected in the postoperative period from patients undergoing elective orthopedic surgery and to assess the effectiveness of different filter devices in removing these fat particles from shed blood.

MATERIALS AND METHODS

Patients
The study group consisted of 35 orthopedic patients who were receiving postoperative shed blood after instrumented lumbar spinal fusion (Allospine, Sulzer, Winterthur, Switzerland), comprising two to four intervertebral spaces, with autologous bone graft from the iliac crest (Table 1).

Postoperative blood salvage and return of shed blood
At the end of surgery, the blood collection canister (ConstaVac CBC II, Stryker, Kalamazoo, MI) was connected to both wound and autologous bone donor site, and shed blood was collected without anticoagulant, at a negative pressure of 25 mmHg. Salvaged shed blood was returned without washing during the first 6 postoperative hours. A screen or surface filter was intercalated in the return line to eliminate microaggregates, fat particles, and/or WBCs (the manufacturer recommended use of a 20- to 40-μm microaggregate filter).

Blood samples
Blood samples (3 mL) were drawn from the patients (Sample 1) and from the shed blood return bag at the 6th hour of the postoperative period before blood filtration (Sample 2). The postfiltration sample (Sample 3) was a pooled blood sample resulting from mixing three to four aliquots of filtered blood obtained during blood return, the first aliquot being taken 10 minutes after filtration was started.

Hematologic variables
RBC counts, Hct levels, Hb levels, WBC counts, platelet counts, and fat particle counts were determined using an automated cell counter (Technicon H3 [H3], Bayer, Tarrytown, NY) in blood samples collected in K2 EDTA tubes (Venoject II, Terumo, Leuven, Belgium).

In the TH3, cells are counted by using a system in which a photodetector cell detects light that is refracted, diffracted, or scattered by cells passing through a small illuminated area in the optical system. RBCs are counted in diluted samples that also contain WBCs and platelets. WBCs are counted along with RBCs; however, because the number of RBCs exceeds that of WBCs by a factor of 500 or more, the error is usually negligible. Platelets are so small that they do not usually induce errors in the RBC count. To detect platelets, TH3 uses a laser and increased gain on the high-angle detector.

WBC counts are performed on blood samples appropriately diluted with a solution that causes lysis of the RBCs. The TH3 performs a WBC differential count by using a flow system. The instrument incorporates a tungsten halogen light source and cytometer for WBC peroxidase analysis, with the addition of a helium-neon red laser for RBC and platelet and basophil counts. Two WBC

<table>
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<tr>
<th>TABLE 1. Characteristics of patients (n = 35)</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Sex (men/women)</td>
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<tr>
<td>Operation duration (hours)</td>
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<tr>
<td>Hb level (g/dL)</td>
</tr>
<tr>
<td>Preoperative</td>
</tr>
<tr>
<td>Postoperative</td>
</tr>
<tr>
<td>Discharge</td>
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<tr>
<td>Hospitalization (days)</td>
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<td>Infectious complications (%)</td>
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scattergrams are produced. In the peroxidase (PEROX) channel, cells are plotted according to their OD (peroxidase staining) on the x axis and light scatter (size) on the y axis. In this channel (Fig. 1A, upper panel), lymphocytes and basophils are small and devoid of peroxidase activity. Monocytes have intermediate size and staining, and neutrophils are large and stain heavily. Eosinophils have the most intense peroxidase activity but appear smaller because they absorb some of the light scatter. In the second channel (BASO), the cells are plotted according the nuclear complexity (degree of lobularity) on the x axis and light scatter (size) on the y axis (Fig. 1A, lower panel). The cytoplasm is stripped from all WBCs except basophils, which therefore appear larger. Below them, MNCs are on the left, and PMNs are on the right of the vertical threshold.

Protocol for validation of the detection method
To validate the detection method, the following steps were accomplished:

**Cross mixture of plasma and cells.** In brief, blood samples from the patient (Sample 1) and from the return blood bag (Sample 2) were obtained at the 6th hour of the postoperative period and were subsequently analyzed in the TH3. After that, samples were centrifuged (10 min at 3000 rpm), and plasma and blood cells were analyzed separately. Then each blood cell sample was made up to a 3-mL volume with the other blood plasma (i.e., shed blood cells plus peripheral blood plasma and peripheral blood cells plus shed blood plasma) and was analyzed in the TH3.

**Supplementation of venous blood samples.** To examine the possible origin of the signal registered in the scattergrams for shed blood, activated platelet-rich plasma (200 μL), animal fat (lard, 50 μL), vegetable fat (olive oil, 50 μL), or RBC membranes (200 μL) were added to different venous blood samples (3 mL) and then analyzed in the TH3.

**Centrifugation of shed blood samples.** In addition, after the first analysis in the TH3, some shed blood samples were centrifuged (10 min at 3000 rpm), and then the upper (plasma plus fat), middle (plasma), and lower (cells) parts of each sample were sequentially analyzed in the TH3.

**Examination of blood film.** Duplicated thin blood films were prepared from each blood sample that was analyzed in the TH3. Films were stained by the polychrome method of May-Grünwald-Giemsa and were examined visually and under low-power magnification (×100-250) to assess the relative amount of fat particles (unstained) and cells.

**Blood filters**
Standard filters (Sangofix, Braun, Melsungen, Germany [pore size 200 μm]; SQ40SJ, [pore size 40 μm], RC100KLE and LeukoGuard, all: Pall Biomedical, Portsmouth, UK; and BIO R, BIOM40 [pore size 40 μm] and BIO R Plus, all: Fresenius AG, Bad Homburg, Germany; Sepacell R, Asahi Medical, Tokyo, Japan; Immugard III-RC, Terumo, Milan, Italy) were used. Except for the Sangofix, which was only tested in vitro, each kind of filter was used in at least three different patients.

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**Fig. 1. Blood count and WBC plots on the TH3.** A) Blood count and WBC plots from a venous blood sample drawn from the patient at the 6th postoperative hour (Sample 1). Two WBC scattergrams were obtained. In the PEROX channel, cells are plotted according to their OD (peroxidase staining) on the x axis and light scatter (size) on the y axis (L = lymphocytes; B = basophils; LUC = large, unstained cells; M = monocytes; N = neutrophils; E = eosinophils) In the BASO channel, the cytoplasm is stripped from all WBCs except basophils, and the cells are plotted according to nuclear complexity (degree of lobularity) on the x axis and light scatter (size) on the y axis. Basophils (B) appear in the upper part of the plot. Below them, mononuclear cells (MNC) are on the left, and PMNs are on the right of the vertical threshold. B) Blood count and WBC plots from a shed blood sample drawn from the return bag at the 6th postoperative hour (Sample 2). In the PEROX channel, fat particles appear as a rotated “∼”-shaped area spreading from the left-hand bottom side of the lymphocyte (L) zone to the right-hand top side of the neutrophil (N) zone (dotted line). In the BASO channel, fat particles are clearly detected as a condensed, sigmoidal-shaped area at the right-hand side of the PMN zone (dotted line).
RESULTS

Shed blood collection and return

Shed blood was collected without anticoagulant, at a negative pressure of 25 mmHg, with the cell salvage device (ConstaVac CBC II, Stryker) up to the 6th postoperative hour. During this time, fat particles rose from the bottom and accumulated in the upper part of the reservoir, forming a grossly visible fat layer. To return the salvaged blood, a vacuum pump was switched off, and shed blood was transferred from the bottom part of the reservoir to the blood return bag, discarding the last 50 to 100 mL to diminish fat and debris contamination. A mean volume of 418 ± 26 mL of shed blood with a mean Hct of 28 percent was returned to the patients (74% of postoperative blood lost) (Table 2). Allogeneic blood requirements were 1.56 ± 0.15 units per patient (Table 2).

Detection of fat particles in shed blood

As depicted in Fig. 1, the TH3 provided the WBC count by means of two different analyses. In the PEROX channel, the equipment measured the OD after peroxidase staining, determining particle size and number (Fig. 1A, upper panel). In this channel, fat particles appeared as a rotated "∼"-shaped area spreading from the left-hand bottom of lymphocyte zone to right-hand top of the neutrophil zone (Fig. 1B, upper panel). In the BASO channel, the TH3 measured the complexity, size, and number of PMN

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TABLE 2. Blood lost and returned and hematologic characteristics of salvaged shed blood before filtration

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<tr>
<th>Blood lost</th>
<th>Volume (mL)</th>
<th>1235 ± 66</th>
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<tr>
<td>Intraoperative</td>
<td>853 ± 45</td>
<td></td>
</tr>
<tr>
<td>Postoperative</td>
<td>579 ± 43</td>
<td></td>
</tr>
<tr>
<td>Salvaged shed blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>418 ± 26</td>
<td></td>
</tr>
<tr>
<td>RBCs (x10⁶/µL)</td>
<td>3.3 ± 0.2</td>
<td></td>
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<tr>
<td>Hb (g/dL)</td>
<td>9.9 ± 0.5</td>
<td></td>
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<tr>
<td>Hct (%)</td>
<td>29.4 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>WBCs (x10³/µL)</td>
<td>5.9 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Platelets (x10³/µL)</td>
<td>83 ± 15</td>
<td></td>
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<tr>
<td>Allogeneic blood (units/patient)</td>
<td>1.56 ± 0.15</td>
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Fig. 2. WBC plots (BASO) on the TH3. A, B, and C were obtained with whole blood, plasma, and blood cells from venous blood, respectively (Sample 1). E, F, and G were obtained with whole blood, plasma, and blood cells from postoperative shed blood, respectively (Sample 2). D and H were obtained when each blood cell sample was made up to a volume with the other blood plasma: that is, shed blood cells plus peripheral blood plasma (H) and peripheral blood cells plus shed blood plasma (D).
WBC nuclei (Fig. 1A, lower panel). Here, fat particles are clearly detected as a condensed, sigmoidal-shaped area at the right-hand side of the PMN zone (Fig. 1B, lower panel). Because the image in BASO is clearer than that in PEROX, the former will be used hereafter.

Validation of fat particle detection method

To validate the specificity of blood fat particle detection with the TH3, the steps described in the Methods section were followed, and the results are depicted in Figures 2-4, in which only the BASO channel scattergrams are shown. Figures 2A, 2B, and 2C were obtained with whole blood, plasma, and blood cells from venous blood, respectively. Figures 2E, 2F, and 2G were obtained with whole blood, plasma, and blood cells from postoperative shed blood, respectively. Interestingly, full analysis of shed blood plasma sample also allows an estimation of fat particle concentration and plasma-free Hb (data not shown). Figure 2D and 2H were obtained when each blood cell sample was made up to a 3-mL volume with the other blood plasma, indicating that the elements responsible for the signal are in the plasma fraction.

To investigate further the possible origin of the signals shown in Figures 1B, 2D, 2E, and 2G, activated platelet-rich plasma (Fig. 3A), animal fat (Fig. 3B), vegetable fat (Fig. 3C), or RBC membranes (Fig. 3D) were added to different venous blood samples and then analyzed in the TH3. Interestingly, the sigmoidal signal was only present in the scattergrams obtained for the blood samples to which fat was added (Fig. 3F and 3G). In addition, after the first analysis in the TH3 showing the signal (Fig. 4B), some shed blood samples were centrifuged (10 min at 3000 rpm), and then the upper, middle, and lower parts of each sample were sequentially analyzed. Again, the signal was intense in the upper part (plasma + fat; Fig. 4D), very weak in the middle (mostly plasma; Fig. 4E), and absent in the lower (cells; Fig. 4F).

Examination of blood films showed grossly visible unstained fat globules in shed blood samples (Fig. 5B), as well as in those blood samples to which lard (Fig. 5D) or...
olive oil (Fig. 5E) was added. These fat globules did not appear in venous blood samples (Fig. 5A) or in those to which activated plasma-rich plasma (Fig. 5C) or RBC membranes (Fig. 5F) were added. Examination under low-power magnification did not produce additional information.

**Effectiveness of blood filters in fat particle removal**

As shown in Figures 1B, 2E, and 4B, shed blood contains fat particles. Some filters (Sangofix, Braun, SQ40SJKL, Pall Biomedical and BIOM40, Fresenius AG) were totally ineffective in removing fat particles from shed blood (Fig. 6A-C).

On the other hand, the specifically designed fat-removal filter (LeukoGuard RS, Pall Biomedical, Fig. 6D) removed some but not all fat particles and virtually all WBCs, while the WBC-reduction filters BIO R, BIO R Plus, all: Fresenius, RC100KLE, Pall Biomedical, and Sepacell, Asahi Medical; (Fig. 6E-H) removed almost all of the particles, as well as the WBCs.

**DISCUSSION**

The perioperative collection and return of autologous blood is beneficial for the patient, as it may decrease alloegenic blood requirements, thus reducing or avoiding the risk of transfusion-associated complications. In this series of 35 consecutive patients undergoing elective lumbar spinal fusion, a mean of 400 mL per patient of postoperative salvaged shed blood was returned. The hematologic characteristics of salvaged shed blood (Table 2) were quite similar to those previously published. According to patient preoperative Hb level (14 g/dL) and to the Hb content of the returned blood (10 g/dL), this volume is equivalent to 0.65 units of standard whole blood and may contribute to a reduction in postoperative blood requirements, as already reported. Moreover, because the last 50 to 100 mL of shed blood were discarded and a filter was intercalated in the line, no reactions were seen during or after shed blood return.

A point of controversy regarding this practice is that
Fat infusion probably increases the risk of fat embolism syndrome, and certainly, even when no adverse effects have been clearly reported, there is potential toxicity of these particles. Hence, return of fat should be minimized, or, even better, it should be avoided. However, there is little information about fat contamination of shed blood or about easy and reliable methods for detecting and removing fat particles. Blevins et al., with Nile red staining and flow cytometry, have measured fat particle number and size in postoperative shed blood from orthopedic patients and found the majority of them to be <9 μm (23,643 ± 56,965 particles/mL), very few between 9 and 40 μm (24 ± 36 particles/mL), and none >40 μm. Others have reported similar results with the same methodology. However, because flow cytometry is too sophisticated, expensive, and time consuming to be routinely used in the clinical setting, we have tried to validate a simple method for detection of fat particles in shed blood using the TH3.

As shown in Figures 1B, 2E, and 4B in the BASO scattergram for shed blood, there is a clearly defined, condensed, sigmoidal-shaped area at the right-hand side of the PMN zone that is not present in patient venous blood scattergrams (Figs. 1A, 2A, and 4A). After centrifugation, this condensed area is also present in shed blood plasma (Fig. 2G), but not in venous blood plasma (Fig. 2C). Moreover, it is present in the scattergram when the venous blood cells fraction was made up to a 3-mL volume with venous blood plasma (Fig. 2D). Hence, this sigmoidal area corresponds to particles of 1- to 10-μm in shed blood plasma, and several pieces of evidence indicate that they are mostly, if not exclusively, composed of fat. First, we can reproduce the signal by adding lard or olive oil to venous blood, but not by adding activated platelets or RBCs membranes (Fig. 3). Second, only the blood films corresponding to shed blood and fat-added blood showed unstained fat globules (nonemulsified fat) (Fig. 5). Third, as expected, after centrifugation, the signal is only clearly present in the upper plasma fraction because of the lower density of fat (Fig. 4D). In addition, because a portion of salvaged shed blood comes from the open iliac crest (the autologous bone donor site), it may contain bone marrow and, hence, fat. Scattergrams of bone marrow produced by the TH3 are very similar to those of shed blood (Fig. 4C). However, because a portion of these particles is retained in the cellular fraction or adhered to the tube wall, an accurate counting cannot be accomplished.

Once validated, the TH3 can be used to evaluate the effectiveness of two methods to eliminate fat from salvaged blood: filtration or cell washing devices. In this work, we have tested the effectiveness of different filtration systems to remove fat particles from shed blood. As expected, standard screen filters (Sangofix, Braun; Fig. 6A) and microaggregate screen filters (SQ40SJKL, Pall Biomedical and BIOM40, Fresenius AG; Fig. 6B and C) were totally ineffective because fat particle diameters were smaller than filter pore diameters. The Sangofix was used only in vitro; that is, once the filtered shed blood sample was obtained, the Sangofix was replaced by a microaggregate filter (SQ40SJKL, Pall Biomedical) before shed blood was returned to the patient.

Thus, fat particles smaller than 40 μm will be returned to the patient when a microaggregate filter is used. Hence, more effective filtration devices are needed to remove smaller fat particles and, as universal WBC reduction is being introduced for all blood components, we have tested the effectiveness of some commercially available, high-efficiency, WBC-reduction filters (BIO R, BIO R Plus, all: Fresenius AG; RC100KLE, Pall Biomedical; Immugard III-RC, Terumo), together with the one filter designed to include fat and WBC removal (LeukoGuard RS, Pall Biomedical), in fat particle removal from shed blood collected during the first 6 hours of the postoperative period. As shown in Figures 6E-H, WBC-reduction
filters almost completely removed all WBCs and fat particles, whereas the LeukoGuard RS was a little less effective (Fig. 6D). (According to the manufacturer, LeukoGuard RS is made for filtering up to 1000 mL of unwashed blood to produce WBC reduction >99% and fat particle retention >82%.) These results are in agreement with a recently published experimental study that established that another filter (Purecell RC400, Pall) removed 99 percent of the fat particles, whereas the LipidGuard filter (no longer available and similar to LeukoGuard RS) was less effective.22 Moreover, they are also in agreement with other work demonstrating that WBC reduction by filtration with another RBC filter (RCXL-1, Pall) is highly effective when performed after 7 to 8 hours of storage at room temperature.34

Adverse cerebral outcomes after coronary bypass surgery are relatively frequent (6%),35 and a portion of them are caused by cerebral fat embolization,23 with cardiomyotomy suction being the major source of brain lipid emboli in this surgery.36 Kincaid et al.28 have reported that processing salvaged blood with a cell saver has been reported to reduce SCAD. Although it has been reported that continuous autologous transfusion systems are more effective than intermittent autologous transfusion systems in fat removal,22 the difference in SCAD decrease was not significant.29 In contrast, arterial line filters (Du-raflo II AF-1025D, Bentley, Irvine, CA; LeukoGuard AL, Pall; StatPrime, Pall) were ineffective in reducing SCAD, most likely because they are 25- to 40-μm filters and most of the fat particles are smaller than 9 μm. Also, it may relate to overloading of the filtering capabilities of the arterial line filter through which hundreds of liters of blood pass during the cardiopulmonary bypass. Obviously, this is not the case in orthopedic surgery, where only a discrete volume (usually <1000 mL) of shed blood is passed through the transfusion filter.

In conclusion, our results suggest that the use of the TH3 for monitoring fat particle content of salvaged shed blood from orthopedic procedures seems to be an easy, reliable, and low-cost method and that WBC-reduction
filters are also effective in filtering fat particles from this blood. However, more studies are needed to apply these results to other types of surgery, hematologic cell counters, shed blood collection devices, and blood filtration systems.

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REFERENCES