Original Article

Pulmonary Implications of Filtering Various Mediators of Morbidity Found in Salvaged Blood

David P. Webb, MSP, CCP; Charles P. Altenbern, BS, CCP; Charles Tritt, PhD; Francis X. Downey, MD; Daniel Minkel, PhD, MD

Milwaukee School of Engineering and St. Luke's Medical Center, Milwaukee, Wisconsin

Keywords: cardiac surgery, blood salvaging, autotransfusion, filtration, leukocyte, lipid

ABSTRACT

Leukocyte reduction of residual circuit blood following cardiopulmonary bypass (CPB) has been demonstrated to improve lung function and reduce the inflammatory response after surgery. In this study, the effect of lipid/leuko-reduction of salvaged blood on pulmonary function and the inflammatory response was examined. Fifteen patients undergoing elective cardiac surgery were randomly assigned to a lipid/leuko-reduced group or a control group. In addition, all residual circuit blood was transferred to the autotransfusion cell-processing device at the end of CPB to contribute a significant portion of the final washed product. In the lipid/leuko-reduced group (N = 10), all processed blood was passed through a lipid globule, C3a, microaggregate pre-filter, followed by a leukocyte removing filter. In the control group (N = 5), all processed blood was filtered using a 40/150 μm dual screen transfusion filter. The lipid/leuko-reduced group showed a significant decrease in pulmonary shunt fraction following reinfusion, whereas the control group did not. The lipid/leuko-reduced group also showed a trend towards decreased pulmonary vascular resistance and a blunting of the leukocytosis that develops following the reinfusion of salvaged blood and CPB, although these trends were not statistically significant. There were no statistical differences between the two groups with respect to oxygenation index or circulating red blood cells following reinfusion. These results suggest that lipid/leuko-reducing salvaged blood improves postoperative lung function and is efficacious.

Address correspondence to:
Charles P. Altenbern, BS, CCP
Milwaukee School of Engineering
EECS/ Perfusion Department
Milwaukee, WI 53202-3109

INTRODUCTION

The potential deleterious effects associated with homologous blood usage has created much concern over recent years (1-5). Methods to reduce these hazards are continually being explored in hopes of improving outcomes for the surgical patient. One method for minimizing homologous blood usage is through intraoperative salvage of shed blood from the operative site. Once collected, the blood can be processed and reinfused, thereby retaining a significant portion of the total blood volume. This particular approach is most advantageous during operative procedures, such as open heart surgery, where surgical blood loss constitutes a significant portion of the transfusion requirement (5). The salvage, processing, and reinfusion of blood that is lost during a surgical procedure is known as autotransfusion, and can be accomplished using a centrifugal cell washing system (6, 7).

While cell washers can reduce patient dependence upon homologous blood usage, the quality of the red cell concentrates produced by cell washers remains questionable (8, 9). The red cell concentrates have been found to contain significant levels of various mediators associated with morbidity (10-15). This calls into question the conventional wisdom that autologous blood is completely safe.

The purpose of this study was to determine the ease of use and clinical benefit associated with the filtration of processed blood during reinfusion. Clinical benefit issues will focus on pulmonary function and the immunosuppressive effects that may help the patient to abate some of the various pathological sequelae associated with post-perfusion syndrome.

MATERIALS AND METHODS

Fifteen patients electively undergoing either coronary artery bypass grafting (CABG) or a combined CABG and heart valve replacement were randomly allocated to a lipid/leuko-reduced group (N = 10) or a control group (N = 5). The investigation was carried out under institutional review board guidelines, and with no exclusion criteria.

ANESTHESIA

Anesthesia was induced and maintained with a combination of narcotics and anxiolytics. Muscle relaxation was achieved by one of the various non-depolarizing steroid derivatives. All patients were treated with antibiotics preoperatively. Anticoagulation was achieved by intravenous administration of bovine lung heparin at a dose of 300 IU/kg prior to initiating cardiopulmonary bypass (CPB).

CARDIOPULMONARY BYPASS

The extracorporeal circuit consisted of a roller pump[a], a microporous polypropylene membrane oxygenator[b], and an arterial line filter[c]. Blood cardioplegia solution (8:1) was infused both antegrade and retrograde at twenty minute intervals, to provide global myocardial preservation. Moderate hypothermia was induced to maintain the esopharyngeal temperature between 27° and 30°C. The mean arterial pressure was maintained at 50 to 70 mmHg with pump flows ranging from 45 to 75 ml/kg.

Anticoagulation during CPB was monitored by celite activated clotting times, and maintained greater than 500 sec. After CPB, protamine sulfate was administered to the patient to neutralize heparin.

CELL WASHER

All residual CPB circuit blood plus blood collected intraoperatively was transferred to a cardiotomy reservoir[d] to be processed by a cell washer[e]. The cell washer separates red blood cells in a spinning polycarbonate bowl, centrifuging and washing aspirated heparinized blood with normal saline. The fill and wash rates were 300 ml/min with a wash volume of 1000 ml normal saline, and a standard centrifuge speed of 5600 rpm.

PRELIMINARY TESTING OF MEDIATORS OF MORBIDITY

Four samples of processed blood prior to filtration were collected to measure the burden of suspected mediators of morbidity associated with the reinfusion of salvaged blood. These samples were sent directly to the medical laboratory where the white blood cell count, lipid count and morphology, and complement C3a were measured. No redo sternotomy samples were obtained during this preliminary testing. These tests, and the testing of debris, were conducted to establish the appropriate filtration system to be implemented.

PRELIMINARY TESTING OF DEBRIS

The burden of debris found in processed blood was assessed by passing a 25 ml aliquot from one of the samples through screen filters. The screen filters were stacked one on top of the other starting at 40 μm down to 10 μm, in increments of 10 μm, for a total of four filters. The blood was gently washed through the filters with normal saline.

FILTRATION SYSTEM

The filtration system used for the lipid/leuko-reduced group consisted of a lipid globule, complement C3a, and microaggregate reduction filter[f] followed by a high efficiency rapid flow leukocyte reduction filter[g]. This filtration system was implemented due to the significant levels of mediators of morbidity

a Model 3 pulsatile flow controller, Stockert/Sorin, Anaheim, CA
b Monolyth hollow fiber with integrated hardshell reservoir, Sorin Biomedical, Irvine, CA
c Gish 40 μm, Anaheim, CA
d CARD3L, Sorin Biomedical, Irvine, CA
e Compact-A, Dideco, Mirandola, Italy
f Lipiguard, Pall Biomedical Products Company, East Hills, NY
g RC400, Pall Biomedical Products Company, East Hills, NY

the control group all processed blood was passed through a 40/150 μm dual screen filter[h] during reinfusion.

PULMONARY FUNCTION

Arterial blood gases were obtained by samples drawn from a radial artery line. Mixed venous blood gases and hemodynamic measurements were obtained through a Swan-Ganz pulmonary artery catheter introduced percutaneously through the right internal jugular vein. The following is a list of formulas commonly used to evaluate pulmonary function (16):

$$\text{Pulmonary Shunt Fraction} = \frac{Qs}{Qt} = \frac{(CcO_2 - CaO_2)}{(CcO_2 - CvO_2)} \quad [\%]$$

$$\text{Oxygenation Index} = \frac{PaO_2}{FiO_2}$$

$$PVR = 80 \left(\frac{PAP - PCWP}{CO}\right) \quad \left[\frac{\text{dyne sec}}{cm^5}\right]$$

LABORATORY AND HEMODYNAMIC MEASUREMENTS

Pre-filtration labs and hemodynamic measurements were taken after the patient was removed from CPB, but prior to reinfusion. Post-filtration samples were taken within one hour after all the processed blood had been reinfused.

STATISTICS

All data was loaded onto a personal computer in a spreadsheet format. Data are expressed as mean plus or minus standard error of the mean (SEM), unless otherwise indicated. Paired t-test was used to compare pre and post filtration values. Unpaired t-test was used to compare lipid/leuko-reduced data with the control data. One sample t-test was used to show a change from zero for both groups of data. A p-value less than 0.05 was considered statistically significant[i].

RESULTS

The demographic data of patients in both groups are summarized in Table 1.

There were no significant differences between the lipid/leuko-reduced group and the control group with respect to CPB duration, aortic cross clamp time, volume of reinfused blood, duration of reinfusion, and latency to reinfusion relative to right ventricular recovery as determined by a pulmonary artery catheter wave form. All patients recovered uneventfully after operation (Table 2).

MEDIATORS OF MORBIDITY

Table 3 shows the results of the preliminary testing of the various mediators of morbidity associated with the reinfusion of salvaged blood.

Table 1: Patient demographic information

	Control (N = 5)	L/L-Reduced (N = 10)
Age (yr)	74 ± 6	72 ± 9
Male	2	9
Female	3	1
Height (cm)	162 ± 9	171 ± 8
Weight (kg)	88 ± 28	80 ± 10
BSA (m^2)	1.90 ± 0.29	1.91 ± 0.11
CABG	4	8
CABG + MVR	1	0
CABG + AVR	0	2
Redo	2 of 5	4 of 10

Values expressed as mean ± standard deviation. CABG = coronary artery bypass graft; AVR = aortic valve replacement; MVR = mitral valve replacement; Redo = redo sternotomy.

Table 2: Perioperative data

	Control (N = 5)	L/L-Reduced (N = 10)
CPB time (min)	121 ± 60	142 ± 65
Crossclamp time (min)	91 ± 44	104 ± 45
Reinfused blood volume (ml)	713 ± 242	772 ± 278
Duration of reinfusion (min)	46 ± 30	70 ± 38
RVR to sample 1 (min)	37 ± 22	42 ± 26
RVR to reinfusion onset (min)	59 ± 14	66 ± 26
RVR to reinfusion end (min)	105 ± 35	137 ± 50
RVR to sample 2 (min)	132 ± 30	179 ± 60

Values expressed as mean ± standard deviation. RVR = right ventricular recovery, or return of blood flow through the lungs.

Table 3: Summary of suspected mediators of morbidity

Date	Volume mL	WBC μL	HCT %	Lipids/mL <10μm	Lipids/mL 10-40 μm	Lipids/mL >40μm	HGB mg/dL	C3a ng/mL
9/16/96	459	11600	49.8	2939	0	0	120.7	0
9/17/96	419	16900	56.7	666	0	0	85.3	17
9/19/96	636	26200	50.2	4677	36	0	99.3	20
9/19/96	954	14700	46	3546	0	0	111.7	26

All blood was collected intraoperatively during cardiac surgery and processed prior to sampling.

h Fenwal transfusion filter, Baxter Healthcare Corp., Irvine, CA

i GraphPad Prism 2.01, Intuitive Software for Science, San Diego, CA

Figure 1: 40 μm screen filter

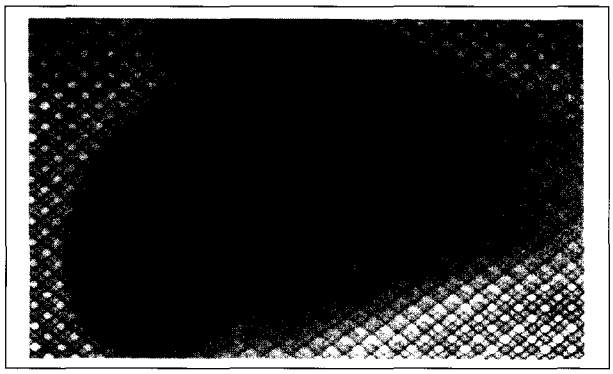


Figure 2: 30 μm screen filter

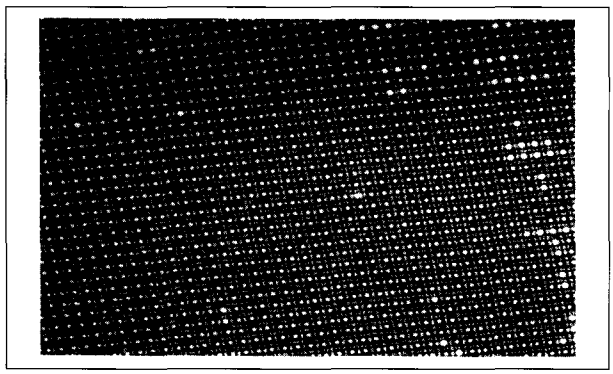


Figure 3: 20 μm screen filter

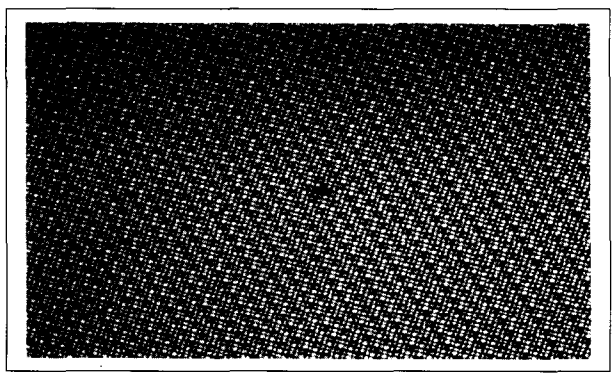
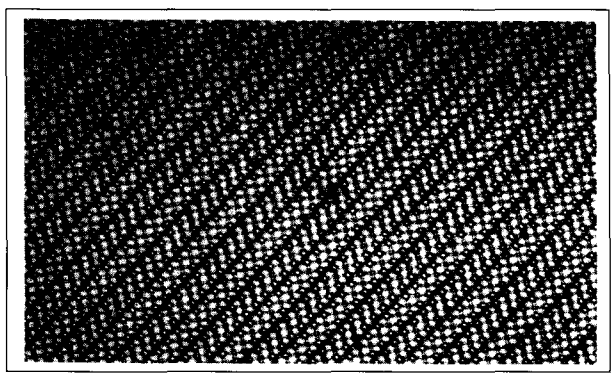


Figure 4: 10 μm screen filter



DEBRIS

Figures 1 through 4 are photographs of the filters at 40X magnification, after the 25 ml blood challenge.

EFFICACY

Circulating red cell counts were similar in both groups prior to and after reinfusion. There was no significant difference in the rise in circulating red blood cells ($p > 0.05$) regardless of the treatment (Figure 5). Lipid/leuko-reducing filters do not blunt the rise in circulating red blood cells following reinfusion.

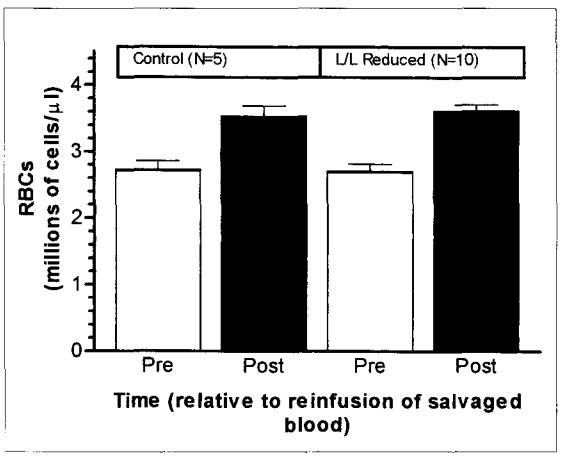
PULMONARY SHUNT FRACTION

The magnitude of the decrease in pulmonary shunt fraction (Qs/Qt) in the treated group was significant ($p = 0.0092$), whereas there was no change in the shunt fraction in the control group (Figure 6).

PULMONARY VASCULAR RESISTANCE

There was not a statistically significant difference between the control group and the treated group with respect to pulmonary vascular resistance ($p > 0.05$). There was, however, a trend toward improved PVR with the lipid/leuko-reduced group, as seen in Figure 7.

Figure 5: Efficacy of salvaged blood reinfusion on circulating red blood cells



OXYGENATION INDEX

There was no significant difference in the control group and the treated group with respect to gas exchange ($p > 0.05$). The graphical representation of this data is shown in Figure 8.

Figure 6: Effect of filtration on pulmonary shunt fraction following reinfusion of intraoperatively salvaged blood

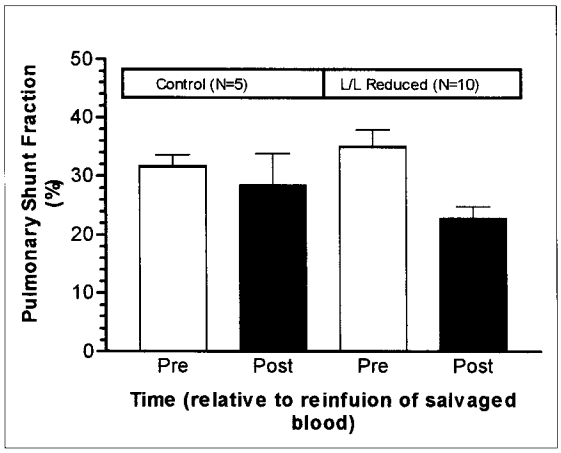


Figure 7: Effect of reinfusion of salvaged blood on pulmonary vascular resistance

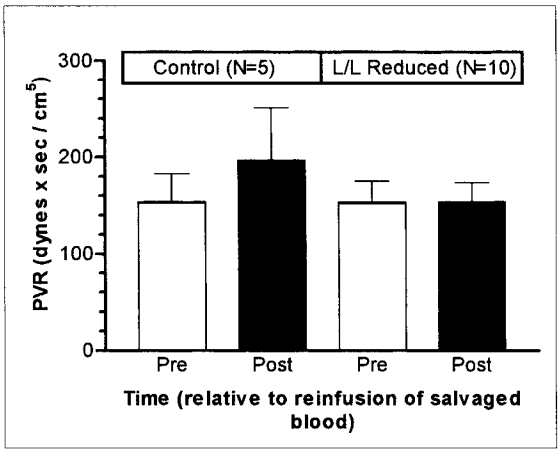


Figure 8: Effect of reinfusion on oxygenation index

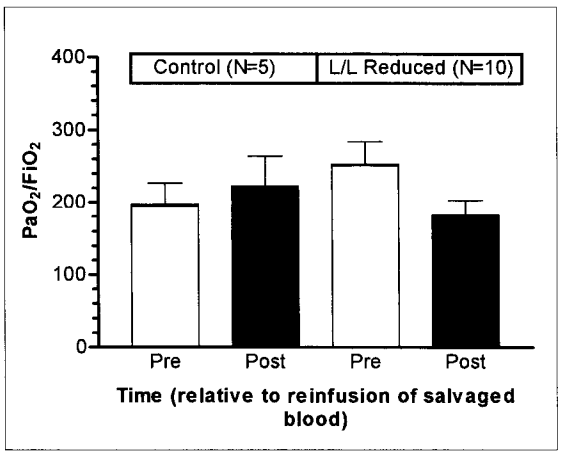
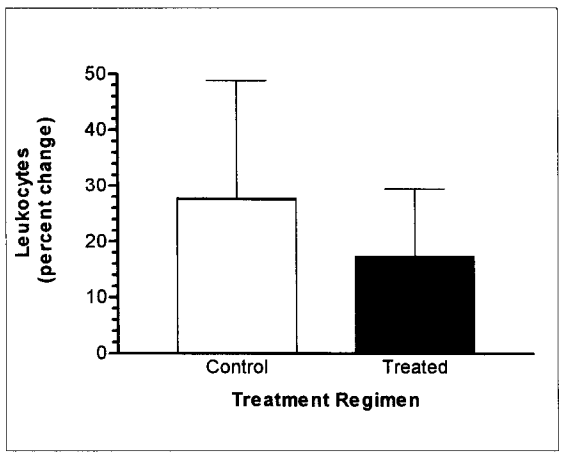


Figure 9: Trend towards a blunting of the leukocytosis that develops following salvaged blood reinfusion



WHITE BLOOD CELL COUNT

While the white blood cell (WBC) counts were higher in the treated group prior to reinfusion, there was not a statistical difference between the groups following reinfusion ($p > 0.05$). There was, however, a greater reduction in the WBCs in the treated group overall, which signifies a trend toward a blunting of the leukocytosis that develops following reinfusion of salvaged blood and CPB (Figure 9).

DISCUSSION

Blood conservation through cell salvaging techniques is practiced in many hospitals that conduct cardiovascular surgery (1, 2). There is a variety of reasons why the implementation of these measures is in the best interest of both the patient and the hospital. While cell washers have the ability to reduce patient exposure to autogeneic blood products and lower the hospital's dependency on banked blood, the red cell concentrates they produce may not be as safe as was once thought.

LARGE DEBRIS

The preliminary testing, which was conducted by passing a 25 ml sample of processed blood through a series of screen filters (from 40 μm to 10 μm in increments of 10 μm), showed that particles in the millimeter size range are actually formed in the cell washer. This is true because all blood collected in the cardiotomy reservoir passed through a clot screen (170 μm), which precludes the possibility that any of the particles seen on the 40 μm screen were originally presented to the centrifugal bowl. Instead, it is possible that millimeter-size particles found on the 40 μm screen could only develop as a result of the centrifugal forces of the bowl coalescing smaller (170 μm or less) particles into larger ones.

SMALLER DEBRIS

While microscopic examination revealed millimeter-sized debris on the 40 μm screen, the 30, 20, and 10 μm screens showed none. This is of particular interest, because one would expect that debris present in the surgical field and aspirated into the cardiotomy reservoir, i.e., stroma, fibrin strands, tissue, bone, and various sized pieces of material, would be found on the smaller sized screens (less than 40 μm). When no particulate debris less than 40 μm was observed, one could argue that the forces generated under centrifugation along with the dynamics of the flow through the centrifugal bowl do, in fact, favor the aggregation of smaller particles into larger ones.

IMPLICATIONS OF DEBRIS

There are two implications that can be drawn from the debris findings. First, a considerable amount of debris would be delivered to the patient if a filter of at least 40 μm were not used during the reinfusion of this blood product. Secondly, if a filter is used for purposes other than microaggregate removal, it should have adequate microaggregate retention capability. In the absence of microaggregate retention capability, a filter designed for a different purpose may clog under the burden of debris presented to it, and therefore require some type of pre-filtration.

LEUKOCYTES PRESENT IN SALVAGED BLOOD

Preliminary testing corroborated the fact that centrifugal cell washing systems actually concentrate leukocytes (15). This data suggests that anything as heavy or heavier than a red cell will be captured and retained in the centrifugal bowl, and is little affected by cell washing.

There is a considerable body of literature showing that neutrophils mediate, at least in part, the lung injury that develops following CPB. These leukocytes are likely activated as a result of the exposure of blood to all the foreign surfaces, and the air to blood interfaces. This blood is then reinfused typically at the end of CPB when the lungs are most sensitive to reperfusion injury.

FAT GLOBULES PRESENT IN SALVAGED BLOOD

While fat embolism syndrome has not been investigated to any appreciable extent as a contributor to post-perfusion syndrome, fat globules (<10 μm) were found in significant amounts (~3000 per ml) during the preliminary testing. These potential mediators of morbidity helped to establish the use of a lipid removal filter as a microaggregate pre-filter in the treated group.

CONCLUSION

Lipid/leuko-reducing cell salvaged blood during reinfusion is a technique that is easy to employ, and has been shown to be efficacious. It improves postoperative lung function by decreasing the shunt fraction (Qs/Qt). It also showed a trend towards decreased PVR and a blunting of the leukocytosis that develops following the reinfusion of salvaged blood and CPB, although neither were statistically significant. Surprisingly, there was not an increase in the oxygenation index, which is often seen with a decrease in shunt fraction. This may be due in part to the fact that the oxygenation index is a gross reflection of pulmonary status, and is not a sensitive measure. Further investigation with a larger sample size should be carried out to determine the overall effectiveness of lipid/leuko-reducing salvaged blood with respect to clinical benefit, cost, and to determine which patient populations can benefit most from lipid/leuko-reducing technology.

ACKNOWLEDGEMENTS

The author would like to thank Charlie Altenburn, BS, CCP; Francis Downey, MD; Tom Brucker, MD; Jerry Ortolano, PhD; the staff at St. Luke's Medical Center; and Pall Corporation for all of their contributions to this project.

REFERENCES

1. Gould, SA, Kruskall MS, Stehling LC, et al. Autologous transfusion: current trends and research interests. Transfusion. 1995; 35: 525-31.
2. Desmond MJ, Thomas MJG, Gillon TH, Fox MA. Consensus conference on autologous transfusion: Perioperative red cell salvage. Transfusion 1995; 36: 644-5.
3. Thomas MJG, Desmond MJ, Gillon J. Consensus conference on autologous tranfusion: General background paper. Transfusion. 1996; 36: 628-32.
4. Bull BS, Bull MH. Hypothesis: Disseminated intravascular inflammation as the inflammatory counterpart to disseminated intravascular coagulation. Proc Nat Acad Sci. 1994; 91: 8190-4.
5. Ovrum E, Holen E, Abdelnoor M, Oystese R. Conventional blood conservation techniques in 500 consecutive coronary artery bypass operations. Ann Thorac Surg. 1991; 52: 500-5.
6. Page P. Perioperative autotransfusion and its correlation to hemostasis and coagulopathies. J Extra-Corpor Technol. 1991; 23(1): 14-21.
7. Page P. Ultrafiltration versus cell washing for blood concentration. J Extra-Corpor Technol. 1990; 22(3): 142-50.
8. Schmidt H, Mortensen PE, Folsgaard SL, Jensen EA. Autotransfusion after coronary artery bypass grafting halves the number of patients needing blood transfusion. Ann Thorac Surg. 1996; 61: 1177-81.
9. Horst HM, Dlugos S, Fath JJ, Sorensen VJ, Obeid FN, Bivin BA. Coagulopathy and intraoperative blood salvage. J Trauma. 1992; 32: 646-53.
10. Bleving FT, Shaw B, Valeri CR, Kasser J, Hall J. Reinfusion of shed blood after orthopedic procedures in children and adolescents. J Bone Joint Surg. 1993; 75: 363-71.
11. Faris PM, Ritter MA, Keating EM, Valeri CR. Unwashed

filtered shed blood collected after knee and hip arthroplasties. J Bone Joint Surg. 1991; 73: 1169-78.
12. Capan LM, Miller SM, Patel KP. Fat embolism: Anesthesiology clinics of North America. Embolism II. 1993; 11: 25-54.
13. Deleuze P, Intrator L, Contremoulins I, Chachera JP, Loisance DY. Complement activation and the use of a cell saver in cardiopulmonary bypass. ASAIO Transactions. 1990; 36: M179-M180.
14. Sieunarine K, Wetherall J, Lawrence-Brown MMD, Goodman MA, Prendergrast FJ, Helling M. Levels of complement factor C3 and its activation product, C3a, in operatively shed blood. Aust NZ J Surg. 1991; 61: 302-5.
15. Perttila J, Leino L, Poyhonen M, Salo M. Leukocyte content in blood processed by autotransfusion devices during open-heart surgery. Acta Anesth Scand. 1995; 39: 443-8.
16. Oakes D. Clinical practitioners' pocket guide to respiratory care. Health Education Publications Inc: Old Town, Maine. 1998.