

A Cytokine Analysis of the Effect of Cell Saver on Blood in Coronary Bypass Surgery

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ABSTRACT

Background: Increasing concern about the transmission of viral disease has generated greater interest in the use of salvaged blood as a means of alleviating the demand for homologous blood and expediting resuscitation during massive hemorrhage. Autologous blood processed by autotransfusion devices has become increasingly common in major surgery and is now largely viewed as safe and efficacious. However, there may be serious complications and sequelae associated with the use of processed blood, such as adult respiratory distress syndrome (ARDS) and renal failure. Complement cascade activation resulting from blood coming into contact with autotransfusion equipment leads to enrollment of leukocytes and release of large concentrations of cytokines, which may contribute to the development of organ failure. Our study evaluated cytokine release during cell saver (CS) blood salvage in the course of coronary artery bypass grafting (CABG) surgery.

Materials and Methods: Forty-five patients randomly selected for CABG were evaluated. All had received at least one unit of autotransfused blood by means of the Haemonetics Cell Saver® System 5 (Haemonetics Corp., Braintree, MA). Each patient had four blood samples taken (pre-operative, CS container, autotransfusion from the blood bag, and one hour post-transfusion). These samples were then centrifuged and the sera were collected. An enzyme linked immunosorbent assay (ELISA) test, using the Biosource

Cytoscreen solid phase "sandwich" ELISA kit (Biosource International, Camarillo, CA) was conducted to determine levels of the cytokines Interleukin (IL) 1, 2, 4, 6, 8, and 10, tumor necrosis factor (TNF), intracellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM).

Results: Significantly increased concentrations of the pro-inflammatory cytokines IL-1, 2, 4, 6, and 8, TNF, ICAM, and VCAM were noted throughout all time periods studied. The same effect was observed for the anti-inflammatory cytokine IL-10.

Conclusion: Statistically significant increases in both the circulating levels of the pro-inflammatory and anti-inflammatory cytokines studied were recorded. It is our contention that the presence of IL-10, a down-regulator of inflammation, is responsible for attenuating the possible deleterious effects of the pro-inflammatory cytokines observed. However, morbidity and mortality, as well as the future patency of the bypass grafts, have not been correlated with the use of the autologous method of transfusion.

INTRODUCTION

Autologous blood transfusion, or autotransfusion, may be defined as the collection of a patient's own blood shed at operation or from the surgical wound in such a way as to allow its re-infusion into the patient [Tawes 1994]. The technique for autotransfusion has been developed to avoid the risks and complications of homologous transfusions [Tawes 1996].

The initial motivation for blood salvage was to alleviate the demand for homologous blood and expedite resuscitation during massive hemorrhage. Increasing concern over transmission of viral diseases, especially hepatitis and human immunodeficiency virus, has generated even greater interest in the use of salvaged blood [Stehling 1991]. Although intra-operative blood salvage and autotransfusion are now largely viewed as safe and efficacious, there may be

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serious complications and sequelae associated with the use of processed blood, such as adult respiratory distress syndrome (ARDS) and renal failure [Casey 1993, Reents 1999]. Studies have shown that changes in shed blood will occur in association with the use of autotransfusion equipment [Tawes 1996]. When blood comes in contact with the foreign materials of the equipment, the complement cascade is activated. Activation of blood with large concentrations of cytokines may contribute to the development of organ failure. It may also affect the patency of the autologous graft used in coronary artery bypass grafting (CABG) surgery.

Cytokines are proteins of low molecular mass produced by leukocytes. Cytokine production occurs in short bursts during the effector phase of the immune or inflammatory response. The cytokines examined in our study were pro-inflammatory cytokine mediators (those that induce adhesion of leukocytes by up-regulation of the antigen-presenting function of macrophages). The cytokines studied were Interleukin-1 (IL-1), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-8 (IL-8), intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), and the anti-inflammatory cytokine mediator Interleukin-10 (IL-10), which inhibits production of cytokines by macrophages and down-regulates the antigen-presenting function of macrophages).

IL-1 is a lymphokine and polypeptide hormone synthesized by monocytes that acts on the hypothalamus to induce fever and directly on skeletal muscle to promote protein catabolism necessary for efficient helper T cell activation. At high levels it is associated with shock, intravascular coagulation, and leucopenia [Devita 1997]. IL-2, also known as T cell growth factor, stimulates helper T cells to multiply into a clone of antigen-specific helper T cells. The major function of IL-2 is the activation of a variety of cells in the immune system, including helper T cells, cytotoxic T cells, B cells, macrophages, and natural killer (NK) cells. IL-2 is also known to produce "vascular leak syndrome" in humans by induction of cytokines that activate endothelial cells [Devita 1997]. IL-4, a pro-inflammatory cytokine, promotes eosinophil adhesion to and migration through the endothelium. It is believed to have a role similar to that of histamine in the pathogenesis of chronic allergic inflammation and is an important correlate of immediate hypersensitivity [Devita 1997]. IL-6, a multifunctional cytokine-differentiation factor for B lymphocytes, is a potent inducer of acute phase protein genes in the liver. IL-6 enhances proliferation of hematopoietic progenitor cells, particularly megakaryocyte, and Ig secretion by B cells. It induces B cell differentiation and augments human cytotoxicity [Devita 1997]. IL-8 is strongly chemotactic for neutrophils, T and B cells, and monocytes. It enhances or inhibits growth of hematopoietic progenitors (depending on co-factor) [Devita 1997]. On the other hand, IL-10 regulates the production of Th-1 (helper) T cells that mediate delayed hypersensitivity. IL-10 is produced by Th-2 (helper) T cells and inhibits the development of Th-1 cells by limiting interferon gamma (IFN) production [Devita 1997].

Adhesion molecules, including ICAM-1, which is a cellular membrane glycoprotein that serves as a ligand for leukocyte integrins, mediates cell-to-cell adhesion, and induces the effector functions in the immune response [Devita 1997], and VCAM-1, a serum glycoprotein responsible for adhesion of monocytes to vascular endothelium [Devita 1997], have been found to play an important role in the physiological progression of acute inflammatory response and, hence, in graft patency following CABG surgery [Alrawi 2000].

Tumor necrosis factor (TNF) stimulates T cell proliferation, enhances natural killer (NK) cell activity, induces systemic acute phase responses, stimulates production of other cytokines, and mediates catabolic processes, including septic shock [Devita 1997].

The aim of this study is to evaluate the expression of various inflammatory mediators, both pro- and anti-inflammatory, in the blood salvaged by new cell saver (CS) machines during the early phase of saphenous vein harvesting and CABG surgery.

MATERIALS AND METHODS

Forty-five patients undergoing elective coronary artery bypass at Maimonides Medical Center from October 1999 to January 2000 were studied. All enrolled patients were operated on with the on-pump procedure, which requires cardiopulmonary bypass. After institutional review board approval, consent was obtained for blood collection and study. The Haemonetics Cell Saver® System 5 (Haemonetics Corp., Braintree, MA) was used for intraoperative blood salvage. Intraoperative blood loss was collected through a suction catheter that was anticoagulated with heparin (30,000u/L). A 10cc sample of blood from each patient was obtained at the following time points: (1) before operation and prior to induction of anesthesia (venous sample); (2) before filtration (from the CS collection device); (3) before autotransfusion (collected blood filtered, centrifuged, and washed with saline); and (4) one hour after transfusion, from patient (central venous access site).

For analysis of cytokine levels, the Biosource Cytoscreen solid phase "sandwich" Enzyme Linked Immunosorbent Assay (ELISA) kit (Biosource International, Camarillo, CA) was used for all time periods of collection. An antibody specific for the cytokine to be studied was coated onto the wells of the microtiter strips. Samples, including standards of known cytokine content, control specimens, and unknowns, were pipetted into these wells, followed by the addition of a biotinylated second antibody. During the first incubation, the cytokine antigen bound simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, streptavidin-peroxidase (enzyme) was added. This enzyme bound to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove the entire unbound enzyme, a substrate solution

Table 1. Expression of various cytokines included in the study in various periods

	A1	B2	C3	D4	A-B-C-D5	A-B6	B-C7	C-D8
IL-1	0.54250	0.343500	0.39275	0.537750	<0.0001	<0.0001	0.0026	0.0013
IL-2	0.2757	10.01	10.93	15.81	<0.0001	<0.0001	0.1700	<0.0001
IL-4	0.07065	0.09625	0.10055	0.20825	<0.0001	<0.0001	0.1370	0.0006
IL-6	0.1076	0.333	0.8102	1.8661	<0.0001	0.0006	0.0007	0.0001
IL-8	1.68925	12.88125	15.51825	11.026	<0.0001	<0.0001	0.5065	0.3011
IL-10	2.625	1.907	5.5266	33.4398	0.0077	0.5920	0.2263	0.01
ICAM-1	0.04745	14.82	16.77	27.84	<0.0001	<0.0001	<0.0001	<0.0001
VCAM-1	0.22025	0.78775	1.62	2.80	<0.0001	0.0004	<0.0001	<0.0001
TNF	0.0338	0.139625	0.1478	0.193425	<0.0001	<0.0001	0.0129	0.0063

1A = pre-operative concentration (pg/ml).

2B = pre-filtration concentration (pg/ml).

3C = post-filtration concentration (pg/ml).

4D = post-operative concentration (pg/ml).

5A-B-C-D = p value for the change in cytokine concentration over the four stages of the experiment.

6A-B = p value for the difference between pre-operative and pre-filtration cytokine concentration.

7B-C = p value for the difference between pre-filtration and post-filtration cytokine concentration.

8C-D = p value for the difference between post-filtration and post-operative cytokine concentration.

was added, which was acted upon by the bound enzyme to produce color. The intensity of this colored product was directly proportional to the concentration of cytokine present in the original specimen. All results were analyzed using a mixed linear model with a repeated measure program developed for Lotus software with significance set at $p < 0.05$.

RESULTS

Expression of various cytokines included in the study is illustrated graphically in Table 1 (●). The following results were observed:

Interleukin-1 (IL-1): Mean levels of IL-1 were seen to increase from time point 1 through time point 4, with concentrations of 0.0542 pg/ml, 0.3435 pg/ml, 0.3927 pg/ml and 0.5377 pg/ml, respectively, with the trend being statistically significant at $p < 0.0001$. Statistical significance was seen between all the time points, pre-operative and pre-filtration ($p < 0.0001$), pre-filtration and post-filtration ($p = 0.0026$) and post-filtration and post-operative ($p = 0.0013$).

Interleukin-2 (IL-2): Mean IL-2 concentrations began at pre-operative levels of 0.2757 pg/ml and increased throughout the time periods, with results of 10.01 pg/ml, 10.93 pg/ml, and 15.81 pg/ml, respectively. Statistical significance was seen between pre-operative and post-transfusion levels ($p < 0.0001$), pre-operative and pre-filtration levels ($p < 0.0001$), and post-filtration and post-transfusion levels ($p < 0.0001$). However, there was no statistical significance between pre-filtration and post-filtration values ($p = 0.1700$).

Interleukin-4 (IL-4): Mean IL-4 concentrations were 0.07065 pg/ml, 0.09625 pg/ml, 0.10055 pg/ml, and 0.20825 pg/ml from time points 1 through 4, respectively.

Statistical significance was seen between time points 1 and 4 ($p < 0.0001$), 1 and 2 ($p < 0.0001$), and 3 and 4 ($p = 0.0006$). However, there was no statistical significance between time points 2 and 3 ($p = 0.1370$).

Interleukin-6 (IL-6): Mean IL-6 concentrations increased steadily throughout the experiment (0.1076 pg/ml, 0.333 pg/ml, 0.8102 pg/ml, and 1.8661 pg/ml, respectively). This trend was extremely significant ($p < 0.0001$). Differences in the mean concentrations of IL-6 between the pre-operative and pre-filtration samples, the pre-filtration and post-filtration samples, and the post-filtration and post-operative samples were also significant ($p = 0.0006$, 0.0007, 0.0001, respectively).

Interleukin-8 (IL-8): Mean IL-8 concentrations showed a generally increasing trend (1.689 pg/ml, 12.88 pg/ml, 15.51 pg/ml, and 11.026 pg/ml, respectively). However, the changes were not statistically significant between pre- and post-filtration values ($p = 0.5065$) and between post-filtration and post-transfusion values ($p = 0.3011$). The differences between the pre-operative and post-transfusion levels and the pre-operative and pre-filtration levels were statistically significant, both at $p < 0.0001$.

Interleukin-10 (IL-10): Mean IL-10 concentrations increased steeply, especially for the post-operative samples (2.625 pg/ml, 1.91 pg/ml, 5.53 pg/ml, and 33.44 pg/ml, respectively), with the trend being statistically significant ($p = 0.0077$). However, with the exception of the differences between post-filtration and post-operative samples ($p = 0.01$), the differences in mean concentrations in the other samples were not statistically significant ($p = 0.592$ and 0.2263).

Adhesion Molecules (ICAM-1): Mean ICAM levels increased markedly throughout the study, with levels of 0.04745 pg/ml, 14.82 pg/ml, 16.77 pg/ml, and 27.84 pg/ml, respectively. All of the time points studied showed

statistical significance ($p < 0.0001$, < 0.0001 , < 0.0009 , and < 0.0001 , respectively).

Vascular Adhesion Molecules (VCAM-1): Mean VCAM-1 concentrations increased throughout the study (0.22 pg/ml, 0.79 pg/ml, 1.62 pg/ml, and 2.80 pg/ml, respectively). The trend, as well as the difference between pre- and post-filtration values and the post-filtration and post-transfusion values, was considered extremely significant at $p < 0.0001$. The pre-operative and pre-filtration value differences were also significant at $p = 0.0004$.

Tumor Necrosis Factor (TNF): Mean TNF levels through the four time periods were 0.0338 pg/ml, 0.1396 pg/ml, 0.1478 pg/ml, and 0.1934 pg/ml, respectively. Statistical significance was seen between pre-operative and post-transfusion levels ($p < 0.0001$), pre-operative and pre-filtration levels (< 0.0001), and post-filtration and post-transfusion levels ($p = 0.0063$). However pre- and post-filtration levels were not statistically significant ($p = 0.0129$).

DISCUSSION

For the pro-inflammatory cytokines IL-1, 2, 4, 6, 8, ICAM, VCAM, and TNF, statistically significant levels are seen from time point 1 through 4. Filtration by the cell saver device did not statistically change the levels of the circulating cytokines IL-2, 4, and TNF, while IL-1, 6, ICAM, and VCAM did show statistically significant increases in their circulating levels despite filtration. The post-filtration/post-transfusion level changes for IL-8 were also not statistically significant.

The anti-inflammatory cytokine, IL-10, also showed a statistically significant increase at time points 1 through 4. However there were no statistically significant changes seen due to filtration by the cell saver, and likewise no statistically significant pre-operative/pre-filtration increase was seen.

It was the aim of our study to examine the progression of cytokines elaborated during surgical stress and to determine if any change in the level of the cytokines occurred as a result of contact with the cell saver blood salvage equipment. Our study found evidence of elevation in both the pro- and anti-inflammatory cytokines in blood salvaged with the use of cell saver equipment, and all elevations were statistically significant. In the case of the anti-inflammatory IL-10, the process of filtration by the cell saver device resulted in no statistically significant change, thereby eliminating the problematical effects that might result from removal of this mediator.

For the pro-inflammatory cytokines IL-1 IL-6, ICAM, and VCAM, significant changes in circulating levels were seen after filtration. This may have resulted either from physical trauma to the formed and unformed elements present in the scavenged blood due to filtration or from hemoconcentration of the salvaged blood.

These results point to a relationship between the stress the patient experiences during and immediately after CABG when an autotransfusion device is used and increased concentration of cytokines, which in turn may

be a prognosticator of postoperative course, morbidity, and mortality.

On the other hand, this study did not take into consideration co-morbid conditions of the patients or the medications they used, both of which might have a significant effect on cytokine expression at various points in the study. Likewise, despite the uniformity of the procedures used in the CABG operation, the duration of each operation, the number of vessels bypassed, and the use of the bypass pump all have a significant effect on cytokine expression, especially in the case of IL-10, a precursor of nitric oxide.

The observed increase of cytokine expression needs to be correlated with future graft patency using various modes of patient follow-up, including magnetic resonance coronary imaging, which is less invasive than standard Angiogram which will be the focus of our future projects.

CONCLUSION

The goal of our study was to examine the contention that suction collection and filtration by a cell saver device causes sufficient trauma to the circulating formed elements of blood to elaborate cytokine inflammatory mediators. Our study showed an increase in all circulating cytokines, with filtration by the cell saver being associated with a statistical increase in the circulating levels of four pro-inflammatory cytokines (IL-2, IL-6, ICAM, and VCAM). It was also noted that a statistically significant increase was seen with the inflammatory mediating cytokine IL-10, possibly exerting a protective effect. The pro-inflammatory cytokines have previously been described as inducers of end organ dysfunction. Because of this potential for increased morbidity and mortality, we will attempt in future studies at our facility to correlate cytokine elaboration and patient outcome, most specifically the degree of cytokine release and its relationship to end organ dysfunction. In addition, the patient subset we examined in our study, those undergoing coronary artery bypass grafting, allows us to correlate the degree of cytokine expression with another point of comparison—the patency of the bypass vessel. We will attempt in future studies to evaluate this outcome using magnetic resonance Angiography.

Acknowledgments

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REVIEW AND COMMENTARY

1. Editorial Board Member SC389 writes:

I am assuming that these patients were operated upon on-pump, but could only find one reference to the use of the bypass machine in the discussion. It would be very interesting to repeat this study on patients done off-pump.

Authors' Response by Steven Sandoval, MD:

All of the patients in our study group were operated upon on-pump, but we do have a patient population at our facility undergoing bypass off-pump. We agree that a study that includes this group should yield fascinating results, and we will pursue this in the future.

2. Editorial Board Member NC124 writes:

It would be interesting to know if Trasylol, because of its anti-inflammatory effect, was used.

Authors' Response by Steven Sandoval, MD:

Trasylol was not used in this study but is being considered as a variable to be included in further study of cytokine release and its attenuation in bypass procedures.

3. Editorial Board Member AR11 writes:

- (a) There are major problems with the statistical analysis, particularly in the Table at Column "A/B/C/D5"—How can statistical analysis be applied across these four data points in the form described?
- (b) It would appear from the data that the greatest, most consistent changes in data are found in those samples obtained immediately after aspirating the shed blood, prior to processing and/or storage in the cell saving equipment. This would suggest that the recovery process, rather than the storage or processing of the recovered blood, is the instigator of the inflammatory process, or that additives given during this stage (saline and heparin) are causative agents. This should be investigated and discussed in more detail.

Authors' Response by Steven Sandoval, MD:

- (a) For the column A/B/C/D5 data, analysis was conducted using a mixed linear model with repeated measures.

This method was chosen over repeated measures ANOVA due to its ability to deal with considerable heterogeneity of variance across the four repeat conditions.

- (b) The suggestion that the significant cytokine release seen from time point 1 to time point 2 may be the "instigator" of the inflammatory process raises a valid question. However, the increase in cytokine expression along all time points studied could be related to variable traumatic events inflicted during aspirating, processing, and bypass surgery.

4. Editorial Board Member SG14 writes:

- (a) If the samples were collected before instituting CPB, dosage of heparin is very important.
- (b) Were there any patients with unstable angina? (very important for adhesion molecules: ICAM, VCAM).
- (c) Was correction for hemodilution performed? Even if CPB was not used at the time or at all, CS use is always combined with hemodilution.
- (d) As there was no control group, the authors cannot imply that cytokine increase is connected with the use of CS. In fact, there are no papers to show that even in off-pump surgery without the use of CS there is an increase of several inflammatory parameters related to surgical trauma or even anesthetic drugs.

Authors' Response by Steven Sandoval, MD:

- (a) Samples at time point 1, (pre-operative), were drawn prior to heparinization. However the Cell Saver device does have a degree of heparin in its use: a 30,000u/L is in a ratio of 1:8 with saline as the fluid also present in the collection bag.
- (b) A careful review of the charts for our study group shows that no patient had a documented case of unstable angina.
- (c) Hemodilution is only a factor at time point 2 when blood is suctioned and combined with saline and heparin. Time point 1 is prior to any procedure so hemodilution is not present, and time point 3 is a period of hemoconcentration, with hematocrits of the collection approaching 80. Time point 4 is after delivery of one unit of transfusion and is therefore not a period of dilution.
- (d) You are correct that there was no control group for this study. The off-pump patients are to be included in future research. Future projects should be designed more thoroughly dividing the variables including each proinflammatory, anti-inflammatory cytokines, with pump and off-pump in direct correlation rather all these variable in a single pattern.

5. Editorial Board Member XA5 writes:

- (a) I would love to see the concentrations of these cytokines in the pericardial fluid itself. I bet they're high even before the blood enters the cell saver apparatus. I would also like to have compared the blood in the cardiotomy reservoir with the cell saver blood.
- (b) The authors don't need to have the concentrations of the cytokines recorded to 4+ decimal places. This sug-

gests a level of precision that I doubt their tests are capable of delivering. This level of precision is certainly not needed to interpret the data.

- (c) I believe that the authors should provide more detail about how the cell saver they used works. There are many different protocols for aspirating, anticoagulating, washing, concentrating, and delivering the saved blood back to the patient. All of these details are necessary for the report, at the very least so that anyone in the future studying this phenomenon could repeat the study or parts of it.

Authors' Response by Steven Sandoval, MD:

- (a) Pericardial fluid is among the fluids collected by the suctioning device, and time point 2 is when we obtained a sample of material collected by the cell saver prior to any further trauma other than suctioning.
- (b) As mentioned in the article, the Biosource Cytoscreen solid phase sandwich ELISA test was performed on all sera. This utilizes optical density information plotted on a standard curve. The intensity of this colored product is directly proportional to the concentration of cytokine present in the specimen. I agree that we may not need to express the data to the degree of precision that we have, but this was the information provided by computer analysis of the optical data.
- (c) The Haemonetics Cell Saver 5 device is mostly automatic in function. The suction device collects wound material in addition to saline and heparin (30,000u/L) in a ratio of 8:1. When the device collects 800ml of salvage (determined by weight), it is spun at 5280 rpm for one minute. Using optical sensors for detection of RBCs, the salvage is moved to a storage bag. Subsequent collection is processed when the collection bag is at 400ml. The company does not recommend changing the default settings without prior discussion with their technical support staff. The full description of how the device works since it has been standardized is available to any researcher through Haemonetics Corp.

6. Editorial Board Member MY17 writes:

It is difficult to understand why the levels of cytokines in filtered/centrifuged/washed blood were even higher than those observed for prefiltered blood?

Authors' Response by Steven Sandoval, MD:

That information would correspond to Column C3 in the Table. The material in that column is post-filtration, washed, and centrifuged. We postulated that it was the mechanical trauma of processing that caused the increase of cytokine release beyond the level of release observed in the pre-filtration state. However, statistically significant increases were seen only for IL-1, IL-6, ICAM, and VCAM.

There were few previous, similar investigations to lend support to the conclusions of our study. Extensive literature searches have revealed no data recorded on cytokine elaboration and the use of the Haemonetics Cell Saver Model 5 in CABG. One study (Reents W, Babin-Ebell J, Misoph MR, Schwarzkopf A, Elert O. "Influence of differ-

ent autotransfusion devices on the quality of salvaged blood," *Ann Thorac Surg* 68(1):58-62, 1999), conducted at the Department of Cardiothoracic Surgery of University Hospital, Wurzburg, Germany, arrived at the following conclusion: "Cardiotomy suction additionally contributes to the release of proinflammatory cytokines, activation of coagulation, and hemolysis. Because blood salvage with a Haemonetics cell-saving device led to normalization of some, but not all, parameters and bacterial contamination was common, the alternative use seems at least questionable."

There is also data from the field of orthopedics(as the only form of surgery having data related to our topic) indicating that mechanical devices cause an increase in TNF ("Mechanical autotransfusion procedures: The effect of cytokines and leukocytes on washed erythrocyte concentrate," *Anaesthesia* 49(6):505-10, 2000). The latter study, although useful, was not included in our references because of the difference in protocols and mediators studied, and the fact that it was published after our study was submitted.

7. Editorial Board Member L023 writes:

The authors conclude that "our study showed an increase in all circulating cytokines, with filtration by the cell saver being associated with a statistical increase in the circulating levels of four pro-inflammatory cytokines". The authors have shown an increase in cytokines but not the entire source of the increase because they only measured pre-anesthetic levels in the patient and not systemic patient levels simultaneous with "pre-filtration levels" and prior to retransfusion.

It is mere speculation that the increase in the pre-filtration readings was due to suction, filtration, or other trauma. It is more likely that similar levels were also present in "non-suctioned" systemic blood as a result of other operative changes or exposures. Similarly, without knowing the patient systemic values immediately prior to retransfusion, one cannot imply that the retransfusion produced the increased "post-transfusion" results.

Authors' Response by Steven Sandoval, MD:

As for your first point, our aim was to follow the path of salvaged blood and any subsequent increase in mediators released, and, as can be seen in the Table, Columns A1 and B2 show a statistically significant increase for IL-2, 4, 6, 8, ICAM, VCAM, and TNF. You make a valid point that samples obtained simultaneously from the patient may clarify whether suction alone is responsible for some of the elaboration. That suggestion will be incorporated into our future research on this subject.

8. Editorial Board Member L023 writes:

Could you explain why you stated that you observed an increase in circulating cytokines in your preoperative samples? I did not see a control value to support that statement. I would like to see a bleeding time or something similar to see if the shed blood returned made a difference in that factor.

Authors' Response by Steven Sandoval, MD:

Our statement that significant levels were seen from time point 1 to 4 was meant to convey an increase or change from time point 1 to 4, not a significant value at the preoperative time point. However, future studies could

include samples at a time period further in advance of surgery to determine if the stress of being in an operating theatre could increase mediator levels. Although bleeding time was not one of the parameters included in this study, it may be valuable to include it in future studies.