Original Article

Ultrafiltration of the Waste Plasma Effluent from Cardiopulmonary Bypass Circuit Contents Processed with a Cell-Washing Device

Alfred H. Stammers, MSA, CCP; John F. Morrow, BS, CCP; Chad P. Brady, BS; Joseph J. Deptula, BS; Suzanne M. Huffman, BS; Andrew S. Bonness, BS, MT; Timothy A. Galbraith, MD; Anselmo Alonso, MD

University of Nebraska Medical Center, Division of Clinical Perfusion Sciences, Omaha, Nebraska

Presented as a poster at the AmSECT 34th International Conference, Dallas, Texas, March 8-11, 1996

Keywords: ultrafiltration, autotransfusion, blood conservation, autologous blood, cardiopulmonary bypass

ABSTRACT

Blood conservation methods are commonly practiced throughout most hospitals that conduct cardiothoracic surgery. In an effort to reduce patients' exposure to homologous blood products and due to cost effectiveness of blood conservation techniques, this present study combines autotransfusion of the remaining blood in the extracorporeal circuit and ultrafiltration of the plasma effluent, and describes the resulting product.

Seven patients, greater than 19 years of age, requiring cardiopulmonary bypass (CPB) were incorporated into this study. Exclusion criteria included age limitation. At termination of CPB, the remaining blood in the circuit was transferred to an autotransfusion machine and processed. Plasma (1054 ± 206 ml) effluent was collected directly from the centrifugal bowl and processed through a ultrafiltrator, with a constant flow rate and negative pressure, until the plasma effluent concentrated down to an end processed volume of approximately 150 ml. The following variables were either measured or calculated: plasma-concentrate volumes per three minute interval, inlet/outlet pressures of an ultrafiltrator, transmembrane pressure (TMP), plasma free hemoglobin, fibrinogen, total protein, and colloid osmotic pressure.

The average ultrafiltrate volume taken off from the plasma effluent was 828 ± 237 ml, with an average ultrafiltrate volume of 115 ml in every three minute interval. The TMP did not change over the first 15 minutes of processing but became significantly elevated at the 18th minute interval and continued to increase and reach a maximum TMP of 286.5 ± 2.1 mmHg at the end of concentration. Fibrinogen levels increased from pre-concentration values of 118.2 ± 64 to 317 ± 177 mg/dl (p=.03) along with increases in plasma free hemoglobin from 97.7 ± 46 to 402.1 ± 180 mg/dl (p=.0002). The total protein concentration increased by over 330% from baseline values.

Ultrafiltrating plasma effluent from autotransfused cell salvaged CPB circuit contents could prove beneficial, but further study is required to discover ways to separate unfavorable products, such as activated platelet-leukocyte products and reduced plasma free hemoglobin, and to lower heparin concentrations of the plasma-concentrate.

Address correspondence to:
Chad P. Brady, B.S.
University of Nebraska Medical Center
Division of Clinical Perfusion Education
600 South 42nd Street
Omaha, NE 68198-5155

INTRODUCTION

Since cardiopulmonary bypass (CPB) induces a variety of hemostatic derangements (1) that result from the extracorporealization of blood, conservation techniques have evolved into standards of care for most cardiac surgical centers. Methods to reduce dependency on autogeneic blood include autotransfusion (2,3), ultrafiltration (both modified and conventional techniques) (4), plasmapheresis (5), preoperative autologous blood donation (6), asanguineous priming of the heart-lung machine, and pharmacologic measures such as the use of aprotinin (2), erythropoietin, lysine analog antifibrinolytic agents (epsilon aminocaproic acid and tranexamic acid), desmopressin, and phytonidione (Vitamin K).

In the present study we chose to examine the combination of two techniques of blood conservation that have been shown, independently, to reduce the risk of exposing patients to autogeneic blood transfusions: autotransfusion with cell washing and ultrafiltration. Autotransfusion/cell separation is the use of centrifugal force to separate the blood components according to their relative size. Since blood is a heterogeneous mixture of components with differing densities, the application of a centrifugal force in a constrained environment will result in the separation of components according to their respective size. The heavier, more dense, red blood cells separate from the lighter plasma components which contain the platelets and coagulation factors. The packed red cells are then washed until all traces of effluent containing the plasma, anticoagulants, cellular debris, plasma free hemoglobin, bone chips, fat, and activated clotting factors are removed. The major disadvantage with this technique is that platelets, plasma, and its constituents are removed in the process (2,7,8-10).

Ultrafiltration devices are semi-permeable hollow fiber membranes which remove excess fluids from the blood, including: plasma water, electrolytes, crystalloid, and other small molecular weight substances (11,12). They have recently gained popularity with the application of modified ultrafiltration techniques primarily in pediatric patients (13). Ultrafiltration is accomplished as a result of a hydrostatic pressure gradient applied across a semi-permeable membrane created by a positive blood pressure phase and negative filtrate pressure achieved by a either siphon drainage or vacuum suction. In contrast to autotransfusion, ultrafiltration has the advantage of conserving the plasma effluent containing proteins such as albumin and clotting factors (2,3,7,8). During ultrafiltration, any blood component whose molecular weight is greater than 50,000 Daltons (sieving maximum) will be saved. The major disadvantage with ultrafiltration is that plasma free hemoglobin has a molecular weight similar to albumin and can be transfused along with the rest of the ultrafiltrated blood (9). Furthermore, ultrafiltration does not remove any biochemical debris such as activated coagulation factors, platelet leukocyte aggregates, activated complement protein, or high molecular weight heparin (10).

Studies of blood loss in patients undergoing various surgical procedures have shown that as many as 80-90% of all patients received some form of blood product transfusion (3,6). Most of those cases were coronary artery bypass patients; autotransfusion was used with 65% of these patients as a source of transfusion (3). Following CPB, autotransfusion may be used to process shed blood aspirated from the field, along with the volume of blood remaining in the extracorporeal circuit. During autotransfusion, the plasma effluent is discarded due to the cell washing technique, which results in the loss of albumin, clotting factors, and immunoglobulins in the plasma effluent.

This study was designed to investigate the effectiveness of separating plasma proteins and fibrinogen from the plasma effluent of autotransfused extracorporeal circuit contents utilizing an ultrafiltrator to create a concentrated source of plasma (plasma-concentrate).

MATERIALS AND METHODS

After Institutional Review Board approval, patients requiring CPB were enrolled in this study. The investigation was carried out on seven patients and there were no exclusion criteria from this study except age limitation (greater than 19 years of age). Informed consent was not required for this project and no patient identifiers were used. Data collected on blood components were identified by sequential sample numbering 001, 002, 003, etc. The subjects did not undergo any additional procedures where blood was drawn directly from them.

Anesthesia was similar in all patients with weight related dosages of midazolam, pancuronium bromide, and fentanyl or sufentanyl. Following intubation, patients were ventilated with 100% oxygen. Anticoagulation consisted of administering heparin five minutes prior to initiation of CPB in a dose of 300 IU/kg to achieve an ACT greater than 480 seconds.

The extracorporeal circuit consisted of a membrane oxygenator and a centrifugal pump. The prime solution included 2000 ml balanced electrolyte solution, 100 ml 25% mannitol, 100 ml 25% albumin, and 10,000 IU of heparin. Patients were perfused at a pump flow rate of 2.4 l/min/m^2. Moderate hypothermia was maintained at an esophageal temperature of 32°C, and mean arterial pressure was kept between 85-100 mmHg when esophageal temperature was greater than 34°C, and 65-85 mmHg when the esophageal temperature was below 34°C. Cardioplegia consisted of a 8:1 blood set with the crystalloid portion containing a high dose of 110 mEq KCl in 500 mL D5W, 0.25 NaCl and the low dose at 30 mEq KCl in 500 mL D5W, 0.25 NaCl.

PLASMA EFFLUENT COLLECTION

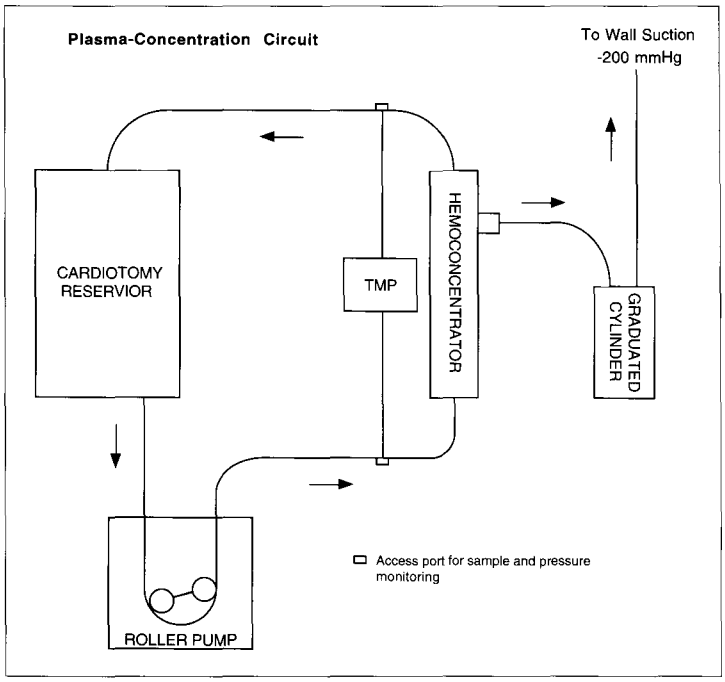
After termination of CPB, the remaining volume in the circuit was collected, packed and washed through the use of an autotransfusion system[a]. The autotransfusion processing cycle

a Shiley Stat P, Sorin Biomedical, Irvine,CA

Table 1: Patient demographic and operative data

Parameter	Mean ± SD
Number	7
Age (years)	62.1 + 1.2
Height (cm)	166.3 + 7.5
Weight (kg)	81.0 + 12.9
BSA (m^2)	1.9 + 0.2
Bypass Time (min)	68.3 + 32.3
Heparin (IU)	37400 + 21500

Figure 1: Plasma effluent collection circuit



was completed as follows: centrifuge speed = 5600 RPM, prime fill rate = 300 ml, wash rate = 300 ml, wash volume = 150 ml. The plasma effluent was directed away from the waste bag through a high flow 4-way stopcock and directed into collection bags.

Once all of the plasma effluent from the bowl was collected, it was transferred to a cardiotomy reservoir. A 3/8-1/4 straight connector with 1/4 inch tubing preceded the inflow portion of the heart-lung machine roller pump. The outflow portion of the roller pump had an ultrafiltrator[b] in line with 1/4-1/4 straight luer connectors with 3-way stopcocks placed on either side of the ultrafiltrator. Inlet/outlet pressures and samples were taken from these luer connectors. The outflow line of the ultrafiltrator was connected to the top of the cardiotomy reservoir (Figure 1).

The plasma flow rate was maintained at 200 ml/minute, while a constant negative pressure of 200 mmHg was applied to the ultrafiltrate port of the device. The plasma effluent was pumped through the ultrafiltrator and recirculated back to the cardiotomy reservoir. The following variables were either measured or calculated: plasma-concentrate volumes per 3 minute interval, inlet/outlet pressures of the ultrafiltrator, transmembrane pressure (TMP), plasma free hemoglobin, fibrinogen, total protein, and colloid osmotic pressure (COP). The plasma-concentrate was processed until an end volume of approximately 150 ml.

LABORATORY STUDIES

Blood samples for plasma free hemoglobin were taken from the extracorporeal circuit at three times: prior to autotransfusion, pre-ultrafiltrator, and post-ultrafiltrator. Fibrinogen samples were also taken pre-ultrafiltrator and post-ultrafiltrator. Total protein/COP samples were drawn from the extracorporeal circuit before autotransfusion, and post-ultrafiltrator at scheduled 3 minute intervals until the end-processed volume was concentrated to 150 ml. Total protein analysis was performed by centrifuging the samples at 10,200 RPMs for four minutes and determining the total protein content with a refractometer. Colloid osmotic pressure was determined using the formula of Beshere and Dearing (14): [Total Protein x 3.32] - 2. Transmembrane pressure was calculated by the following formula (15):

$$\frac{P\ inlet + P\ outlet}{2} + [P\ suction]$$

where P inlet = inlet pressure (mmHg) of the ultrafiltrator; P outlet = outlet pressure (mmHg) of the ultrafiltrator; and P suction = vacuum pressure (mmHg).

STATISTICS

All data was loaded onto a personal computer in a spread sheet format. One way analysis of variance was performed on intragroup data, and when significant F ratios were achieved, an additional Fisher's Least Significant Difference Test was performed. Statistical significance was accepted at a $p<.05$. All data were reported as a mean ± standard deviation of the mean.

RESULTS

Patient demographic and operative data are shown in Table 1. The average plasma effluent processed from the CPB circuit was 1053 ± 206 ml. The total average ultrafiltrate volume taken off from the plasma effluent was 828 ± 237 ml and ranged from 550 ml to 1200 ml. Ultrafiltrate volume stayed fairly constant through each three minute interval until 150 ml plasma effluent concentration was reached.

The TMP increased gradually over time. When comparing

b Minntech Corporation, Minneapolis MN

the TMP of the early intervals to the end intervals, statistical significance was found. The TMP was held below the manufacturer's recommendation of 500 mmHg with a range of 209 mmHg to 288 mmHg (Figure 2).

Laboratory analysis of plasma free hemoglobin showed statistical significance from the CPB circuit and pre-ultrafiltrator samples compared to the post-hemoconcentrator sample (p=.003). The average plasma free hemoglobin prior to autotransfusion was 97.7 ± 46.1 mg/dl and increased to 402 ± 180 mg/dl after ultrafiltration (Figure 3). Fibrinogen pre-ultrafiltrator versus the post-hemoconcentrator values were statistically significant (p=.03). Pre-ultrafiltrator fibrinogen level was 118.2 ± 63.9 mg/dl and increased to 317.2 ± 176.5 mg/dl.

Refractometer analysis of total protein was statistically significant when compared to circuit samples prior to autotransfusion to the end-data points. The average total protein from the circuit was 3.06 ± 1.1 mg/dl and increased to 10.4 ± 1.8 mg/dl with completion of final sample collection (Figure 5).

Figure 2: Transmembrane pressure of the hemoconcentrator

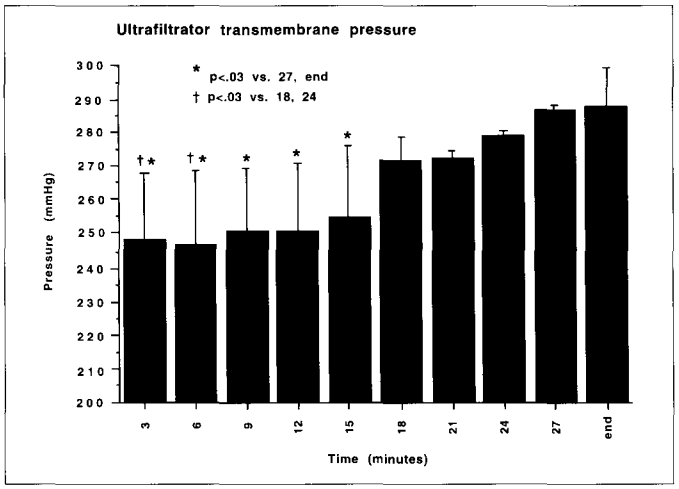
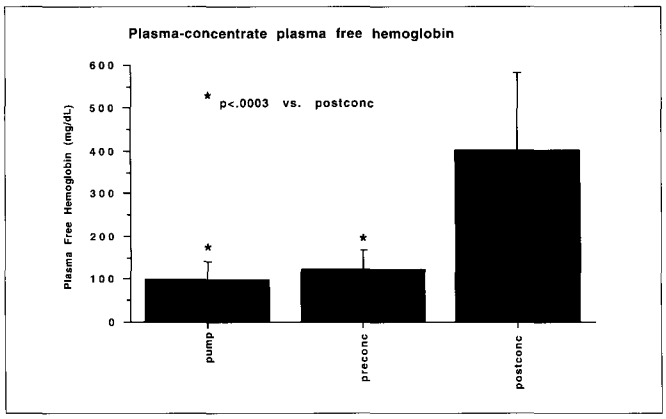


Figure 3: Plasma free hemoglobin concentration at various times during the experiment



DISCUSSION

Blood conservation methods are commonly practiced in most hospitals that conduct cardiothoracic surgery (2,3,7,16). There are a variety of reasons why the implementation of these measures are in the best interest of both the patient and the hospital, as well as society as a whole. A well managed blood conservation program reduces patient exposure to autogeneic blood products and lowers the hospital's dependency on banked blood. This study examined the processing of the plasma waste effluent, normally discarded from autotransfusion devices following CPB, with a commercially available ultrafiltrator. This study was the first phase of a two part examination of autologous plasma collection: first, to examine the processing technique of plasma-concentrate, and secondly, to compare this concentrate with that of a comparable product of fresh frozen plasma.

The measurement of a timed collection of ultrafiltrate volume was expected to show a decreased yield over time, yet stayed statistically equivalent throughout the process period. Since the circuit was a closed system, and the total protein content of the plasma-concentrate increased steadily, the ultrafiltrate rate became dependent upon the transmembrane pressure, which continued to rise despite maintenance of constant flow and negative pressure. This was undoubtedly related to the increase in viscosity of the plasma-concentrate due to the large molecular weight of fibrinogen and other proteins.

Plasma free hemoglobin samples were drawn at several times to examine the contribution of each process in promoting the generation of this marker of red blood cell destruction. This study showed no actual difference between the CPB circuit and pre-ultrafiltrator plasma free hemoglobin levels, but a dramatic increase from pre- to post-ultrafiltrator levels as the plasma water was removed. Red blood cell hemolysis during extracorporeal flow has a number of etiologies, such as the occlusive action of roller pumps (with either under or over occlusion leading to increased hemolysis), stasis within the ultrafiltrator (especially with negative pressure applied to the plasma water side), and excessive transmembrane negative pressure (17). The increase in plasma free hemoglobin in the post-ultrafiltrator sample may also have been largely due to the concentration effect of removing plasma water, since plasma free hemoglobin does not pass readily across the pores of the ultrafiltrator (17,18). The mean effect of plasma-concentration on fibrinogen and total protein was approximately a 300% increase in concentration. By taking this change into consideration for plasma free hemoglobin, the total contribution of the ultrafiltrator towards increased hemolysis would decrease to approximately 113%.

The effectiveness of ultrafiltrating plasma effluent from

autotransfused CPB circuit contents could prove beneficial, but further study is required in considering a way to separate unfavorable products that include plasma free hemoglobin, heparin, and activated complement/coagulation proteins. One technique would be the utilization of a high centrifuge speed to pack the plasma layer and then process the plasma with a cell washing device to separate unfavorable products of the plasma-concentrate. We examined rewashing the plasma-concentrate at 5600 RPM with a fill and wash rate of 100 ml/min. We were able to reduce plasma free hemoglobin by 200%, but the residual total protein content was less 1 mg/dl. Commercial production of a heparin capturing device may offer some hope in removing the residual heparin that is known to concentrate in the plasma-concentrate.

In conclusion, the present study has shown that processing of the waste effluent from an autotransfusion device resulted in a highly concentrated source of autologous plasma with fibrinogen levels approximately twice that contained in units of autogeneic fresh frozen plasma. The second phase of this study is to examine methods of removing the activated platelet-leukocyte products, reduce plasma free hemoglobin, and lower heparin concentrations of the plasma-concentrate.

Figure 4: Fibrinogen concentration at various times during the experiment

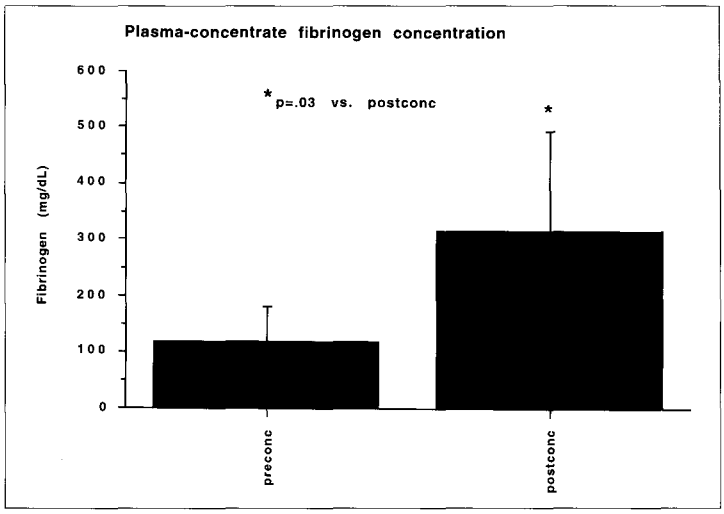
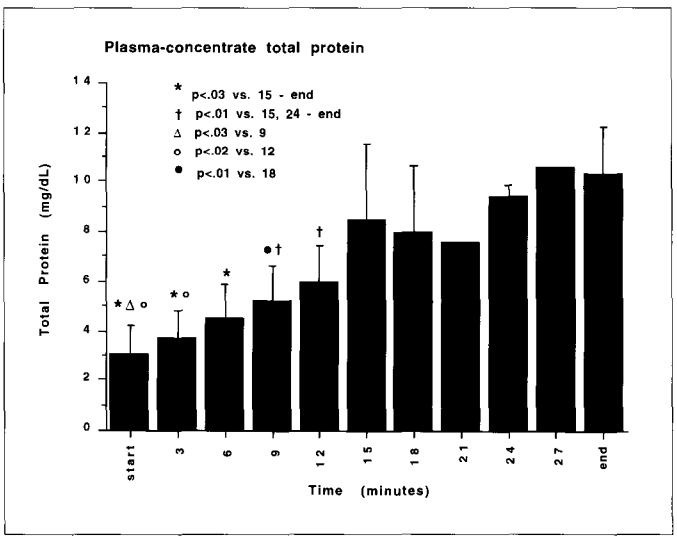


Figure 5: Total protein concentration at various times during the experiment



ACKNOWLEDGEMENT

This work was completed in part by a continuing education grant from the Minntech Corporation, to whom the authors express their gratitude.

REFERENCES

1. Page P. Perioperative autotransfusion and its correlation to hemostasis and coagulopathies. J Extra-Corpor Technol. 1989; 23: 14-24.
2. Boldt J, Zickmann B, Czeke A, et al. Blood conservation techniques and platelet function in cardiac surgery. Anesthesia. 1991;75:426-432.
3. Keeling MM, Gray JA, Brink MA, et al. Intraoperative autotransfusion. Ann Surg. 1983;197: 536-541.
4. Boldt J, Zickmann B, Fedderson B, et al. Six different hemofiltration devices for blood conservation in cardiac surgery. Ann Thorac Surg. 1991;51: 747-753.
5. Stammers AH, Kratz J, Johnson T, Crumbley J, Merrill J. Hematological assessment of patients undergoing plasmapheresis during cardiac surgery. J Extra-Corpor Technol. 1993; 25: 6-14.
6. Chambers LA, Kruskall MS. Preoperative autologous blood donation. Transfusion Med. 1990; 4:35-46.
7. Boldt J, Kling D, von Bormann B, et al. Blood conservation in cardiac operations. J Thorac Cardiovasc Surg. 1989; 97: 832-840.
8. Brickley JM, Kalshoven JD, Wilds SL, et al. A comparison of two methods of post-bypass ultrafiltration. J Extra-Corpor Technol. 1987; 19: 281-286.
9. Nakamura Y, Masuda M, Toshima Y, et al. Comparative study of cell saver and ultrafiltration nontransfusion cardiac surgery. Ann Thorac Surg. 1990;49: 973-978.
10. Page PA. Ultrafiltration versus cell washing for blood conservation. J Extra-Corpor Technol. 1990; 22: 142-150.
11. Walpoth BH, von Albertini. Ultrafiltration in cardiac surgery. J Extra-Corpor Technol. 1984; 16: 68-72.
12. Magilligan DJ. Indications for ultrafiltration in cardiac sur-

gery patients. J Thorac Cardiovasc Surg. 1985; 89: 183-189.
13. Naik SK, Elliot MJ. Ultrafiltration and paediatric cardiopulmonary bypass. Perfusion. 1993;8: 101-112.
14. Beshere G, Camerlengo LJ, Dearing JP. Estimation of colloid osmotic pressure during hemodilutional cardiopulmonary bypass. J Extra-Corpor Technol. 1982;14: 145-148.
15. Moore RA, Laub GW. Hemofiltration, dialysis, and blood salvage techniques during cardiopulmonary bypass. In: Gravlee GP, Davis RF, Utley JR, eds. Cardiopulmonary Bypass: Principles and Practices. Baltimore: Williams & Wilkins; 1993: 93-123.
16. Walton RM. Prospective study indicating the advantages of ultrafiltration of the post bypass perfusate in patients undergoing elective coronary artery bypass. Proceedings: Am Acad Cardiovasc Perf. 1986;7: 69-72.
17. Tamari Y, Nelson RL, Levy RS, et al. Effects of the hemoconcentrator on blood. J Extra-Corpor Technol. 1984;16: 89-94.
18. Traynor L, Sutton R, Riley F, Crawford FA, Jr., Sade RM. Comparing ultrafiltration and centrifugation during and after pediatric cardiopulmonary bypass. Proceed Am Soc Extra-Corpor Technol. 1992; 23:140-151.