The role of cell savers and filters in cardiac surgery
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Chapter 6

Influence of mechanical cell salvage on red blood cell aggregation, deformability, and 2,3-diphosphoglycerate in patients undergoing cardiac surgery with cardiopulmonary bypass

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Abstract

Background: Mechanical cell salvage is increasingly used during cardiac surgery. Although this procedure is considered safe, it is unknown whether it affects the red blood cell (RBC) function, especially the RBC aggregation, deformability, and the contents of 2,3-diphosphoglycerate (2,3-DPG). This study examines the following: (1) whether the cell salvage procedure influences RBC function; and (2) whether retransfusion of the salvaged blood affects RBC function in patients.

Methods: Forty patients undergoing cardiac surgery with cardiopulmonary bypass were randomly allocated to a cell saver group (n=20) or a control group (n=20). In the cell saver group, the blood aspirated from the wound area and the residual blood from the heart-lung machine were processed with a continuous-flow cell saver before retransfusion. In the control group this blood was retransfused without processing. The RBC aggregation and deformability were measured with a laser-assisted optical rotational cell analyzer and 2,3-DPG by conventional laboratory test.

Results: The cell saver procedure did not influence the RBC aggregation but significantly reduced the RBC deformability (p=0.007) and the content of RBC 2,3-DPG (p=0.032). However, in patients receiving the processed blood, their intra-operative and post-operative RBC aggregation, deformability, and 2,3-DPG content did not differ from those of the control patients. Both groups of patients had a post-operative drop of RBC function as a result of haemodilution.

Conclusions: The mechanical cell salvage procedure reduces the RBC deformability and the cell 2,3-DPG content. Retransfusion of the processed blood by cell saver does not further compromise the RBC function in patients undergoing cardiac surgery with cardiopulmonary bypass.
Introduction

Processing the salvaged autologous blood during operation with a mechanical cell salvage device (cell saver) is a well-established blood conservation method to reduce allogeneic blood transfusion during and after cardiac surgery. Recently, the benefit of this method has been further strengthened by reports demonstrating that the cell saver is also associated with the removal of cell-derived micro-particles and a reduction of post-operative neurocognitive complications in patients undergoing cardiac surgery with cardiopulmonary bypass. With a cell saver, the salvaged red blood cells (RBCs) aspirated from the wound area are separated from the plasma through washing and differential centrifugation, providing a high concentration of autologous RBCs to be retransfused to patients during and after operation. Although some safety issues of this cell-saving technique have been well-addressed in the past, such as the effect of the cell processing procedure on haemostasis and complement activation, little is known about its effects on the functional state of salvaged RBCs, especially on the behavior of RBC aggregation, deformability, and their contents of 2,3-diphosphoglycerate (2,3-DPG). It has been demonstrated that RBC aggregation and deformability are affected by blood storage. Moreover, it is also known that the oxygen carrying capacity of the stored blood is reduced along with the drop of 2,3-DPG content in the RBCs. These RBC functional changes may account for the reduced oxygen transport capacity of stored blood after transfusion. If these functional changes are also apparent in the blood collected by the cell saver, it may have clinical implications because cell savers are commonly used in patients with a relatively large amount of blood loss. For these patients, oxygen carrying capacity of the circulating blood is of utmost importance.

The aim of the present study was to examine whether in vitro the cell salvage procedure influences the RBC aggregation and deformability as well as the RBC 2,3-DPG contents. Furthermore, this study was also aimed to observe whether retransfusion of the processed blood salvaged during the operation affects RBC function in patients undergoing cardiac surgery with cardiopulmonary bypass.
Patients and Methods

Patients
This study was approved by the local Institutional Review Board of the University Medical Centre Groningen. After written informed consent was obtained, 40 patients undergoing cardiopulmonary bypass (CPB) for elective coronary artery bypass grafting, single valve replacement, or a combined procedure were prospectively included in the study. Exclusion criteria were patients less than 18 years or over 80 years old and patients presenting for emergency operation. Patients were randomized according to a computer-generated table and allocated to a cell saver group (n = 20) or a control group (n = 20). In the cell saver group, both the shed blood from the wound area during operation and the residual blood from the heart-lung machine were processed with a cell saver, whereas in the control group neither the shed blood from the wound area nor the residual blood from the heart-lung machine was processed with a cell saver. In both groups, all the salvaged blood was returned to patients during and after operation.

Anaesthesia and Cardiopulmonary Bypass
Anaesthesia was induced and maintained by target controlled intravenous infusion of propofol (plasma concentration 1.5 to 2.0 µg/mL) and sufentanil (1.5 µg/kg). Pancuronium (0.1 mg/kg) was used for muscle relaxation. Ventilatory management was aimed at normocapnia throughout the operation and in the intensive care unit, with an inspiratory oxygen fraction of 0.4, a positive end-expiratory pressure of 6 cm H2O and a tidal volume of 6 to 8 mL/kg. Bovine lung heparin (300 IU/kg) was used for anticoagulation, which was monitored by the celite activated clotting time (ACT; International Technidyne, Edison, NJ) and maintained at a level of more than 400 seconds. The extracorporeal circuit consisted of roller pumps (Stöckert, München, Germany), a hollow fiber oxygenator (Sarns Turbo; 3M, St. Paul, MN) and a standard arterial line filter (Affinity 38µ; Medtronic, Minneapolis, MN). The priming consisted of 500mL of 10% hydroxyethylstarch (Haes; Fresenius, Bad Homburg, Germany) and 1,000 mL of lactated Ringer’s solution. Pump flow was adjusted to 2.4 L/m²/min. Nasopharyngeal temperature during CPB was maintained at 32°C. After the termination of CPB, heparin was neutralized by protamine in a 1:1 ratio.
**Clinical Procedures**

In the cell saver group, the wound blood aspirated from both the pericardium and the pleural space from the time of skin incision to wound closure was collected in a cell saver reservoir (ATR120; Fresenius). Conventional cardiotomy suction was not used. The reservoir was primed with 100 mL of normal saline with 30,000 IU/L of heparin. The salvaged blood was then processed with a Continuous Auto Transfusion System cell saver (CATS; Fresenius), which was set up and operated according to the manufacturer’s instructions. The residual blood in the heart-lung machine after CPB was collected in a transfusion bag, transferred to the cell saver reservoir, and processed also by the cell saver. In the control group, conventional cardiotomy suction was applied for the salvage of wound blood that was returned to the CPB circuit without cell saver processing. The residual blood in the heart-lung machine after CPB was collected in a transfusion bag and retransfused through a standard blood transfusion system. The transfusion trigger for allogeneic packed cells was according to institutional guidelines, which included a haemoglobin level of lower than 4 mmol/L during CPB and lower than 5 mmol/L in the post-operative phase.

**Blood Sampling and Assessment**

To study whether the cell salvage process would influence the salvaged RBCs, blood samples were taken, respectively, before cell saver processing in the reservoir and after blood processing in the transfusion bag. To study whether retransfusion of the processed blood would affect patients, blood samples were taken from the arterial line after induction of anaesthesia, at sternal wound closure, 1 hour after arrival in the intensive care unit, and on the morning of the first post-operative day. From each sample, 5 mL of the collected blood was anticoagulated with 0.1 mM ethylenediaminetetraacetic acid (EDTA) and prepared for RBC aggregation and deformability as well as for measuring the sample haematocrit. In addition, 0.5 mL of the collected blood was mixed immediately with 1.5 mL of 8% trichloroacetic acid (TCA). After centrifugation of the mixture at 1,000 g for 10 minutes, the supernatant was stored at -80°C for further analysis of 2,3-DPG and adenosine triphosphate (ATP).
Laboratory Measurements

Both the RBC aggregation and deformability were determined by a laser-assisted optical rotational cell analyzer (Mechatronics, Hoorn, the Netherlands)\(^4\). This machine consists of a laser diode and a thermostated bobcup measuring system. For the in vitro blood samples taken from the salvaged blood, the haematocrit level was adjusted to 40% with the hydroxyethyl starch solution similar to the priming solution for the heart-lung machine. All the blood samples taken from patients were measured without haematocrit correction. During measurement, each sample was sheared under 37°C in a concentric-cylinder system with a gap of 0.3 mm between the cylinders. The time-dependent changes of the reflection, contributed by RBC aggregation, were measured over a period of 2 minutes at a rate of 100 samples per second. The aggregation index was then calculated from the recorded digital data in a syllectogram by the software for Windows (Mechatronics, Hoorn, the Netherlands).

For the measurement of deformability, the RBCs were elongated under various shear rates that lead to shear stresses from 0 to 49.84 Pa. The elongation index was used to estimate the ability of RBC deformation, which was calculated by the ratio of long and short axes of the different patterns of the deformed RBCs at a shear rate of 3.89 Pa. From the stored TCA samples, 2,3-DPG was measured with an ultraviolet test kit (Roche Diagnostics GmbH, Mannheim, Germany) and ATP was determined by a bioluminescence assay kit (Roche Diagnostics GmbH, Penzberg, Germany) following the manufacturer’s instructions.

Statistics

Data processing and statistical analysis were carried out with SPSS 14.0 (SPSS, Chicago, IL). The paired t test was used to compare the difference between the samples obtained before and after cell saver processing, whereas the Student t test was performed to compare the difference between the two groups on post-operative observations. For parameters changing with time, analysis of variance with repeated measures was performed to examine the difference between the two groups. Qualitative variables were examined by the Fisher exact test. Correlation between variables was presented by the Pearson correlation coefficient. A p value of less than 0.05 was considered statistically significant.
Results

Patients and Operation Demographics
There was no significant difference between the cell saver group and the control group with regard to patient’s age, gender, height, weight, and the duration of aortic cross-clamp and CPB (Table 1).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Cell Saver Group (n = 20)</th>
<th>Control Group (n = 20)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68 ± 9</td>
<td>66 ± 11</td>
<td>0.502</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>13/7</td>
<td>13/7</td>
<td>1.000</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>174 ± 10</td>
<td>172 ± 9</td>
<td>0.447</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82 ± 13</td>
<td>78 ± 13</td>
<td>0.271</td>
</tr>
<tr>
<td>CABG</td>
<td>18</td>
<td>16</td>
<td>0.661</td>
</tr>
<tr>
<td>VR</td>
<td>1</td>
<td>3</td>
<td>0.356</td>
</tr>
<tr>
<td>CABG + VR</td>
<td>1</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td>X-clamp time (min)</td>
<td>53 ± 16</td>
<td>62 ± 19</td>
<td>0.282</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>92 ± 24</td>
<td>96 ± 34</td>
<td>0.651</td>
</tr>
</tbody>
</table>

Data = mean ± standard deviation. CABG = coronary artery bypass grafting; CPB = cardiopulmonary bypass; VR = valve replacement; X-clamp = aortic cross-clamp

The collected blood for cell saver processing was 1,646 ± 484 mL from the reservoir and 937 ± 275 mL from the CPB circuit in the cell saver group, yielding 561 ± 189 mL of RBC concentrates for retransfusion, whereas in the control group the 1,160 ± 459 mL residual blood from the CPB circuit was retransfused. The haematocrit level was 17 ± 6% before cell processing and 70 ± 7% after processing.

Effect of Cell Salvage Procedure on RBC Function in Vitro
Before the cell salvage procedure, the RBC aggregation index of the salvaged blood from the cell saver reservoir as 63.0 ± 5.5%, which was within the normal range. After the processing procedure, it did not change (fig 1). However, the RBC elongation index (deformability) dropped significant after the cell salvage procedure (from 0.359 ± 0.018 to 0.305 ± 0.016, p=0.007; fig 1).
Fig 1 (left). Red blood cell (RBC) aggregation index (AI) and elongation index (EI) determined under 3.89 Pa shear stress before and after blood processing in the cell Saver (CS) group in patients undergoing cardiac surgery with cardiopulmonary bypass. Data presented are mean ± SD. (** = p < 0.01 compared with the previous data set by paired t test.)

Fig 2 (right). Red blood cell 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) concentrations before and after blood processing in the cell saver (CS) group in patients undergoing cardiac surgery with cardiopulmonary bypass. Data are presented in mean ± SD. (*) = p < 0.05 compared with the previous data set by paired t test.

The 2,3-DPG concentration from the salvaged RBCs before processing was 7.80 ± 1.28 µmol/g Hb. After processing, it dropped significantly to 0.541 ± 0.77 µmol/g Hb (p = 0.032; fig 2). The ATP concentration from the salvaged RBCs dropped only slightly after the cell processing procedure (from 1.59 µmol/g Hb to 1.44 ± 0.24 µmol/g Hb; fig 2).

RBC Aggregation, Deformability, 2,3-DPG, and ATP in Patients

There was no significant difference between the cell saver group and control group with regard to the baseline RBC aggregation index, elongation index, 2,3-DPG, and ATP concentrations (table 2). The RBC aggregation index dropped more than a half by the end of operation and remained low at the first post-operative day in both patient
groups. Similarly, the elongation index was low at the post-operative day without difference between the two groups. The 2,3-DPG and ATP concentrations dropped significantly during operation and remained low at the post-operative day 1 in both groups (table 2). The post-operative RBC deformability and 2,3-DPG were neither associated with the amount of donor blood transfused (r = 0.129, r = 0.059) nor with the amount of processed blood retransfused (r = 0.349, r = -0.126).

Table 2: RBC aggregation, deformability, 2,3-DPG, and ATP in patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-operation</th>
<th>End-operation</th>
<th>th-ICU</th>
<th>Day 1</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC aggregation index (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell saver (n=20)</td>
<td>50.3 ±14.3</td>
<td>18.6 ±11.8</td>
<td>19.3 ±11.2</td>
<td>30.6 ±13.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>52.9 ±13.2</td>
<td>21.8 ±11.1</td>
<td>24.4 ±14.2</td>
<td>34.1 ±12.7</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>RBC elongation index (3.89 Pa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell saver (n=20)</td>
<td>0.356 ±0.060</td>
<td>0.360 ±0.061</td>
<td>0.350±0.059</td>
<td>0.347±0.059</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>0.321±0.064</td>
<td>0.329 ±0.062</td>
<td>0.323±0.057</td>
<td>0.310±0.059</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>2,3-DPG (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell saver (n=20)</td>
<td>9.2 ±3.5</td>
<td>5.9 ±2.1</td>
<td>8.0 ±4.0</td>
<td>6.2 ±2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>8.2 ±3.1</td>
<td>6.1 ±3.1</td>
<td>6.7 ±3.0</td>
<td>6.4 ±2.5</td>
<td>0.00</td>
<td>0.59</td>
</tr>
<tr>
<td>ATP (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell saver (n=20)</td>
<td>2.9 ±1.8</td>
<td>1.8 ±0.8</td>
<td>2.5 ±2.7</td>
<td>1.8 ±1.1</td>
<td>0.12</td>
<td>0.77</td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>2.3 ±1.6</td>
<td>1.7 ±1.0</td>
<td>1.9 ±1.1</td>
<td>2.7 ±1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell saver (n=20)</td>
<td>35.9 ±3.6</td>
<td>24.8 ±3.0</td>
<td>29.7 ±3.5</td>
<td>31.7 ±3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>35.1 ±3.5</td>
<td>23.3 ±2.7</td>
<td>27.0 ±3.1</td>
<td>28.8 ±3.3</td>
<td>0.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. th-ICU, 1 hour intensive care unit; 2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; P1, p value for interaction between group and time; P2, p value for difference between groups; RBC, red blood cells.

Post-operative Observations

Transfusion of the allogeneic RBCs during the whole intra-operative and post-operative period was 270 ± 455 mL (mean ± standard deviation) in the cell saver group and 375 ± 400 mL in the control group (p = 0.443). However, there were only 6 patients in the cell saver group versus 13 patients in the control group who received allogeneic RBC transfusion during and after the operation. Patients in the cell saver group had a significant higher haemoglobin level on the
first post-operative day than those in the control group \((p = 0.012)\). Post-operative chest drainage was \(460 \pm 347 \text{ mL}\) in the cell saver group and \(400 \pm 222 \text{ mL}\) in the control group \((p = 0.533)\). There was no statistical difference between the two patient groups with regard to the post-operative organ function and hospital stay except for a slightly higher leukocyte count in the cell saver group (table 3).

Table 3: Post-operative Observations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cell saver group (n=20)</th>
<th>Control group (n=20)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes ((x \times 10^9/\text{L}))</td>
<td>17.6 ± 5.9</td>
<td>14.3 ± 3.4</td>
<td>0.043</td>
</tr>
<tr>
<td>Platelets ((x \times 10^9/\text{L}))</td>
<td>190 ± 44</td>
<td>178 ± 54</td>
<td>0.469</td>
</tr>
<tr>
<td>Haemoglobin ((\text{mmol/L}))</td>
<td>6.7 ± 0.65</td>
<td>6.1 ± 0.69</td>
<td>0.012</td>
</tr>
<tr>
<td>Chest Drainage ((\text{ml/24h}))</td>
<td>460 ± 347</td>
<td>400 ± 222</td>
<td>0.533</td>
</tr>
<tr>
<td>RBC transfusion ((\text{ml})^b)</td>
<td>270 ± 455</td>
<td>375 ± 400</td>
<td>0.443</td>
</tr>
<tr>
<td>RBC transfusion(^c)</td>
<td>6/20</td>
<td>13/20</td>
<td>0.056</td>
</tr>
<tr>
<td>CPK ((\text{IU/L}))</td>
<td>397 ± 208</td>
<td>496 ± 376</td>
<td>0.320</td>
</tr>
<tr>
<td>Creatinin ((\mu\text{mol/L}))</td>
<td>76 ± 18</td>
<td>75 ± 29</td>
<td>0.840</td>
</tr>
<tr>
<td>C-reactive protein ((\text{mg/L}))</td>
<td>30.4 ± 58.6</td>
<td>23.3 ± 34.1</td>
<td>0.644</td>
</tr>
<tr>
<td>Hospital stay ((\text{days}))</td>
<td>9.4 ± 5.3</td>
<td>9.0 ± 3.7</td>
<td>0.811</td>
</tr>
</tbody>
</table>

\(^a\) Data recorded on the first post-operative morning. \(^b\) Total intra-operative and post-operative allogeneic blood transfusion. \(^c\) number of patients receiving allogeneic blood transfusion. Data are expressed as mean ± SD.

CPK, creatine phosphokinase; h, hours; RBC, red blood cells.

**Discussion**

The safety issue of blood salvage by the cell saver device during cardiac surgery has been extensively studied in the past \(^7–9\). However, very little attention has been paid to the functional status of salvaged RBCs; especially to the RBC oxygen transport function, which is of particular importance in delivering oxygen to the tissue during the early post-operative period while the haemoglobin concentration is low. In the current study, we found that the elongation index, a measure of the RBC deformability, decreased significantly after the cell saver procedure. Moreover, the content of RBC 2,3-DPG, a crucial biomarker of the RBC oxygen unloading capacity, was also reduced significantly after the cell saver procedure in the salvaged blood, suggesting that the mechanical cell salvage procedure affects the RBC function of the salvaged blood.
The ability of RBCs to aggregate and to deform is one of the key determinants of blood rheology, which contributes to the maintenance of effective microcirculation and organ function. In patients undergoing cardiac surgery with CPB, the RBC deformability decreases as a result of a combined effect of hypothermia, haemodilution, and mechanical stress. In the aspirated blood, as we observed in the current study, the RBC aggregation was within the normal range and it was not affected by the cell saver processing procedure. However, the cell saver procedure significantly reduced the RBC deformability. The mechanism by which the cell saver procedure reduces the RBC deformability is not quite clear. It is conceivable that the shear force applied during the cell saver procedure may directly affect the deformability as well as the shape of RBCs. However, it is uncertain whether the washing solution used during the cell saving procedure would have influenced the RBC deformability. As a routine, normal saline was used in the present study because this solution was regarded as an easy and simple solution for washing RBCs during the cell saver procedure. It remains to be determined whether a better preservation solution, as any of those used for blood storage, should be developed for the cell saver processing of the salvaged blood during cardiac surgery.

The RBC 2,3-DPG content plays an important role in keeping the oxygen dissociation curve within normal range as 2,3-DPG lowers the affinity of haemoglobin for oxygen. A reduced 2,3-DPG content functionally limits the ability of RBCs to unload oxygen in the peripheral circulation, which in turn leads to a reduced capacity of oxygen transport. In patients undergoing cardiac surgery, an early report by Schmidt and colleagues revealed that the plasma level of 2,3-DPG did not change significantly in patients whose shed mediastinal blood was processed by a cell saver. However, because the content of 2,3-DPG in the salvaged RBCs was not measured in that study it was unable to judge whether or not the mechanical cell salvage procedure per se would have any detrimental effects on 2,3-DPG of the salvaged blood. In the current study, we took samples especially for this purpose and found that the 2,3-DPG content of the salvaged RBCs dropped significantly after the cell salvage procedure, which suggests that the high shear stress generated during the cell saver procedure may have resulted in the damage of cell membrane, and in turn causing 2,3-DPG depletion in the salvaged RBCs.
Despite the fact that our results have shown a reduction of RBC deformability and 2,3-DPG contents by the cell saver procedure, retransfusion of this processed blood does not seem to lead to a systemic reduction of RBC function in patients. Furthermore, with a small sample size this study does not power enough to address any connection between the changes in RBC deformability and 2,3-DPG concentration and clinical outcome such as transfusion or blood loss. As we observed in this study, patients in both the cell saver and control groups had a similar drop of RBC aggregation during operation, which is caused largely by haemodilution of the plasma factors that are necessary for the RBC aggregation. However, RBC deformability observed in the current study was kept stable during operation. On post-operative day one, the RBC deformability dropped in both groups, which was probably a result of a general inflammatory response known to be associated with a reduced RBC deformability.

In conclusion, both the RBC deformability and RBC 2,3-DPG contents dropped significantly in vitro after the cell saver procedure of the salvaged blood during cardiac surgery. For patients who received salvaged blood processed by the cell saver, their in vivo RBC function does not seem to be affected. Thus, intra-operative cell processing with the continuous-flow cell saver is safe and it does not contribute to significant RBC dysfunction after CPB. A general adverse effect of cardiopulmonary bypass on RBC aggregation, deformability, and depletion of 2,3-DPG was equally observed in patients with and without the mechanical cell saving procedure.
References


