Qualitative Assessment of Shed Mediastinal Blood

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Abstract

In a randomized prospective study of patients undergoing coronary bypass surgery, autologous blood from chest drainage was collected and retransfused into 10 patients (RTX) whereas 10 patients without retransfusion served as controls. There was no significant difference referring to age, weight, height, perfusion time, and number of distal anastomosis. Our main regard was directed to a qualitative analysis of the retransfused autologous blood. Results (mean values ± 1 SD/patient): one; there was no bacterial contamination in the shed mediastinal blood just prior to retransfusion, two; elevated levels of free plasma hemoglobin (otolidin-method) were encountered after one hour on cardiopulmonary bypass (CPB) and a six-fold increase in the shed mediastinal blood from the cardiotomy reservoir (211 ± 44 mg/dl), three; the potassium value in the cardiotomy reservoir was slightly increased (5.8 ± 2.9 mmol/l), four; in no case was hemoglobinuria or coagulation disfunction noted (the activated clotting time (ACT) after retransfusion did not change significantly (117 ± 15 sec) as compared to preoperative values (112 ± 16 sec)) five; no evidence for further complement activation (determined by C3a and C5b-9), induced by CPB, was seen, and six; red cell adenosine-triphosphate (ATP) in the shed mediastinal blood was lowered to 2.56 ± 0.78 umol/gHb whereas red cell 2,3diphosphoglycerate (2,3DPG) remained normal.

On the basis of these results we consider the retransfusion of shed mediastinal blood following cardiac surgery a simple and safe method of blood salvage.

Introduction

The increasing concern about transmission of infectious diseases with transfusion of homologous blood and its labile components (1, 2, 3) led to develop various procedures to avoid homologous transfusion. In a recent survey, the risk of transmission of the human immunodeficiency virus (HV-1) was 0.003 percent per unit (4). Procedures to inactivate virus in plasma derivatives such as albumin, immunoglobulins and clotting factors by pasteurization and detergent/solvent inactivation are used (5). Pasteurization and irradiation, are currently evaluated for fresh frozen plasma. In addition to transmission of viral diseases, the immunological consequences of homologous blood transfusion are known with more precision. Alloimmunization with a number of antigens to the transfused blood occurs more frequently than it is clinically perceived but probably remains irrelevant for most recipients. However, with immunocompromised recipients and patients on prolonged anesthesia (6), and with patients needing repeated transfusions such as patients suffering from cancer, leukemia, and thalassemia inappropriate HLA constellations between donor and recipient may be a deleterious consequence of the presence of immunocompetent lymphocytes in transfused red cell or platelet concentrates (7). In a prospective randomized study of patients undergoing heart surgery we assessed the quality of shed mediastinal blood before retransfusion. Our principal aim was to examine the functional integrity of the red cells through measurements of organic adenosine-triphosphate (ATP) and 2,3diphosphoglycerate (2,3DPG), extent of activation of the coagulation and complement systems, as well as red cell damage by hemolysis and bacterial contamination.

Materials and Methods

Twenty consecutive patients subjected to coronary bypass surgery were assessed and divided in a group receiving transfusion of shed mediastinal blood (retransfusion group = RTX, n=10) and a control group consisting of patients whose mediastinal blood was discarded (control, n=10). There were no significant differences concerning age, height and weight. Myocardial revascularisation was predominantly performed by using internal mammaria, arteria and vein grafts. The number of distal anastomosis performed amounted to 2.4 ± 0.8 per patient in the RTX vs. 3.1 ± 0.7 per patient in the control (ns). Likewise the time of perfusion, ischemia and duration of surgery were not significantly different.

The cardiotomy reservoir (Jostra Medizintechnik, Hechingen, FRG) with a 20 um filter was installed in the cardiopulmonary bypass (CPB) prior to shunting the patient. We used two different membrane oxygenators (CML2, Cobe and Maxima, Medtronic, USA) with an arterial blood filter (Bentley AF 1040, USA), filled routinely with Ringer's lactate and 0.9% NaCl. In three cases (1 RTX, 2 control) we supplemented the filling with homologous packed red cells because of a hemoglobin
concentration in circulatory blood below 8 g/dl during CPB. As soon as the patient was weaned from CPB, the mediastinal tubes were connected to the cardiotomy reservoir and fixed to a low pressure wall suction (20 cm H2O). Reinfusion was executed by a piston pump system (Inmed 962, Inmed Corp., San Diego, USA) that consisted of a suction from the cardiotomy reservoir, a piston pump and an infusion unit to a peripheral patient vein. The system was closed and featured an air trap. According to our protocol we reinfused the shed mediastinal blood within 3 to 6 hours after CPB. Volume and velocity of retransfusion were adapted to the patient’s requirements.

Laboratory measurements in the patient's venous blood were done in both groups preoperatively, one hour on CPB and six hours after CPB; further measurements were done in the RTX group three hours after CPB in the patients' venous blood as well as in the blood collected in the cardiotomy reservoir. The assessment of quality of shed mediastinal blood is based on the following parameters:

Hematology: hemoglobin, hematocrit and platelet counts (Coulter counter Model S, Coultronics France).

Clinical Chemistry: serum sodium and potassium (Ciba Corning 614), total protein, albumin and calcium (Hitachi).

Coagulation: Activated clotting time (HemoTec), free heparin (Hepcon System Four, HemoTec, Inc. Englewood, USA), fibrinogen (Clauss).

Bacteriology: Aerobic and anerobic cultures (Bactec 600).

Quantitative measurements of hemolysis (O-tolidin method), immunoglobulins (radial immunodiffusion, Behring AG, Marburg; FRG), complement activation (C3a: Upjohn Kit, CSB-9; ELISA according to Mollnes (8)), and organic phosphates of the erythrocytes (ATP: Sigma Kit 366- W, 2,3DPG: Sigma Kit 665, Sigma Diagnostics, St. Louis, USA) completed our qualitative assessment. Morphologic alterations of red cells were identified under the light microscope.

Unless otherwise stated results are expressed as mean ± 1 SD per patient. Statistical comparisons were made by the paired and unpaired student t-test.

Results

Hematology: The hemoglobin concentration are listed in Table 1. The preoperative value in the RTX group dropped from 13.2 ± 1.8 g/dl (hematocrit 0.39) after one hour on CPB to 8.3 ± 0.9 g/dl (0.24) (control: 13.2 ± 0.9 g/dl [0.39] to 8.3 ± 1.3 g/dl (0.23)). Before retransfusion the RTX group showed a mean concentration of 10.9 ± 0.9 g/dl (0.31), while the hemoglobin concentration in shed mediastinal blood was 9.6 ± 1.5 g/dl (0.27). Six hours after CPB the values reached in the RTX group 11.1 ± 1.2 g/dl (0.33) vs. 12.3 ± 1.9 g/dl (0.36). On the first postoperative day hemoglobin concentration fell slightly in both groups, but increased again prior to discharge (RTX: 12.3 ± 2.1 (0.38) compared to control with 12.1 ± 2.1 g/dl (0.39)). All differences were not statistically significant.

The mean platelet counts revealed a preoperative value of 199 ± 55 x 109/l in RTX group (control: 188 ± 36 x 109/l), with a reduction six hours after CPB to 143 ± 50 x 109/l (resp. 141 ± 25 x 109/l). In the shed mediastinal blood we found a mean concentration of 71 ± 37 x 109/l. During hospitalization the platelets recovered and mean values prior to discharge in the patient blood were 347 ± 155 x 109/l (RTX, n=7) and 283 ± 219 x 109/l in the control respectively (ns).

Clinical chemistry serum sodium remained within normal range in both groups during the entire observation period whereas mean serum potassium was elevated from preoperative 3.9 ± 0.3 mmol/l to 5.8 ± 2.9 mmol/l three hours after CPB in the RTX group. In shed mediastinal blood the mean potassium level was 5.7 ± 0.7 mmol/l. Immediately after surgery all patients received an infusion with 20-40 mmol/l potassium to avoid early postoperative arrythmia. Six hours after CPB serum potassium decreased to normal values again (RTX: 4.9 ± 0.8 mmol/l; control: 4.8 ± 0.6 mmol/l). Total serum calcium showed a decrease after one hour on CPB in the RTX group from preoperative 2.21 ± 0.08 mmol/l to 1.98 ± 0.18 mmol/l (control: 2.14 ± 0.24 mmol/l to 2.00 ± 0.14 mmol/l). Thirty minutes after weaning from CPB, 2g calcium gluconate 10% were dispensed to all patients routinely. Serum calcium reached normal range again six hours after CPB. None of the changes was significant between the two groups. Bicarbonate concentrations in the patient blood were in the normal range (21.1 to 25.6 mmol/l), whereas shed mediastinal blood showed a low mean of 16.6 ± 1.5 mmol/l with a pH of 7.4. Total protein and albumin concentrations were diminished during CPB and three hours after CPB. If corrected for hemodilution, six hours after CPB the concentrations of total protein and albumin were in the normal range, namely 65.6 ± 15.1 g/l and 32.4 ± 10.9 g/l in the RTX group compared to 58.3 ± 8.7 g/l and 28.1 ± 4.2 g/l in the control respectively (ns).

Evaluation of the coagulation system The patient's coagulation status was controlled by measuring the activated clotting time (ACT), especially before, during and after full heparinization on CPB. No significant differences were noted between both groups at any time (figure 1). Protamine was given intravenously at the end of the extracorporeal circulation in order to bring the ACT back to normal values. Though shed mediastinal blood did not clot there was no increase of the ACT in the patients' venous blood after retransfusion (RTX group 118

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TABLE 1: Hemoglobin profile (hematocrit) in the patients' venous blood and the cardiotomy reservoir g/dl ± 1SD

<table>
<thead>
<tr>
<th></th>
<th>RTX</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>preoperative</td>
<td>13.2 ± 1.8</td>
<td>13.2 ± 0.9</td>
<td>ns</td>
</tr>
<tr>
<td>1h on CPB*</td>
<td>8.3 ± 0.9</td>
<td>8.3 ± 1.3</td>
<td>ns</td>
</tr>
<tr>
<td>3hrs post CPB</td>
<td>10.9 ± 0.9</td>
<td>0.31</td>
<td>no value</td>
</tr>
<tr>
<td>6hrs post CPB</td>
<td>11.1 ± 1.2</td>
<td>12.3 ± 1.9</td>
<td>ns</td>
</tr>
<tr>
<td>1 day postop</td>
<td>10.9 ± 1.7</td>
<td>11.0 ± 0.8</td>
<td>ns</td>
</tr>
<tr>
<td>discharge</td>
<td>12.3 ± 2.1</td>
<td>12.1 ± 2.1</td>
<td>ns</td>
</tr>
<tr>
<td>Shed mediastinal</td>
<td>9.6 ± 1.5</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>blood (before retransfusion)</td>
<td></td>
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*CPB = cardiopulmonary bypass
± 15 sec vs. control 114 ± 17 sec). Clinical course gave no evidence for any other coagulation disorder. Despite that small quantities of free heparin were present in the shed mediastinal blood, heparin measurements after retransfusion in the patients' venous blood revealed values below the detection limit. Preoperatively the concentrations of fibrinogen in the patients were 2.69 ± 0.56 g/1, but decreased to 1.53 ± 0.31 g/1 three hours after CPB. Only five out of 10 samples of shed mediastinal blood showed minimal amounts of fibrinogen (range 0.1-0.3 g/l) while in the other five samples no fibrinogen could be detected.

**Bacteriology**

All aerobic and anaerobic cultures taken from the cardiotomy reservoir just before retransfusion remained sterile during seven days.

**Hemolysis**

The preoperative value of free plasma hemoglobin was 9.4 ± 13.0 mg/dl in the RTX group vs. 14.3 ± 17.2 mg/dl in the control. After 1 hour on CPB these values were 33.7 ± 13.0 mg/dl (RTX) and 33.7 ± 12.5 mg/dl (control) respectively, without macroscopically visible hemoglobinuria. The shed mediastinal blood yielded substantial hemolysis (mean free hemoglobin 211.15 ± 44.29 mg/dl). Overall, a mean of 696.1 mg free hemoglobin per patient was retransfused to the patients in the RTX group (retransfused volume [ml] x free hemoglobin concentration [mg/dl]). With respect to each patient's blood volume, an additional free hemoglobin concentration of 12.2 mg/dl was added to his baseline. Despite this, hemoglobinuria after retransfusion of shed mediastinal blood was noted in no case; hemolysis in circulatory blood was decreased within 6 hours after CPB to 19.97 ± 12.64 mg/dl in the RTX group (control: 12.10 ± 12.87 mg/dl ns).

**Immunoglobulins**

In the patient blood the concentration of immunoglobulins (expressed in % of total protein) did not change significantly. In the shed mediastinal blood the absolute amounts figured up to 6.10 ± 2.71 g/l for IgG, 0.84 ± 0.25 g/l for IgM and 1.53 ± 0.88 g/l for IgA. Thus IgG ranged below the normal limits (indicated by Behring AG, Marburg for NOR-Partigen), whereas IgM and IgA clearly fell within normal range.

**Complement activation**

The main activation of the complement system is well known to occur during extracorporeal circulation. In our patients an increase of C3a levels was noted from preoperative 0.64 ± 0.64 to 13.99 ± 27.63 ug/ml (Figure 2). As for C5b-9, we encountered the same relations, namely 1.02 ± 1.17 to 14.7 ± 15.47 U/l (ns). Comparing the values in venous blood after one hour on CPB with the concentrations in shed mediastinal blood three hours after CPB, we could not detect a further activation (C3a: 11.26 ± 5.63 ug/ml, C5b-9: 16.05 ± 4.66 U/l).

**Energy rich phosphates of the erythrocyte**

The mean preoperative concentrations of erythrocyte ATP was 3.91 ± 0.61 umol/gHb (n=9), compared to 2.56 ± 0.78 umol/gHb (p<0.05) in the collected shed blood. As for 2,3DPG, the concentrations were not significantly different in the patient's venous blood preoperative as compared to the shed blood (17.86 ± 6.31 and 14.77 ± 4.23 umol/gHb respectively) (Figure 3).

**Blood smears**

A moderate poikilocytosis in the stained blood smears of shed mediastinal blood was observed; the proportion of damaged red cells was < 15%.
Discussion

The cardiomyotomy reservoir, postoperatively converted to a mediastinal drainage system and combined with an infusion pump allows to retransfuse shed mediastinal blood in fractions. The maintenance of a closed system reduces the risk of contamination (9,10).

The shed mediastinal blood revealed an acceptable hemoglobin concentration of 9.6 g/dl and a hematocrit of 0.27, the latter was significantly lower than the patient's hematocrit taken at the same time (p<0.05) (table 1). This may be due to exsudative pleural and pericardial fluid, cellular loss by intrathoracic wound clots and/or remaining cardioplegic solution (9). The plateau counts were surprisingly high (71 x 109/1) and seemed to be a quantitative as well as a qualitative advantage compared to stored bank blood, however, we did not assess the functional integrity of the platelets. Clotting functions after reinfusion of shed mediastinal blood did not indicate any measurable coagulation disorders or extended postoperative bleeding tendency as compared to the control. After CPB further complement activation did not occur, probably due to the fact that the cardiomyotomy reservoir is already an integral part of the extracorporeal circulation. Our findings showed a nearly complete defibrination of the shed mediastinal blood caused by the previous intrathoracic surface contact (9). The decreased fibrinogen level in the patient after surgery can be related to the infusion of protamine (11) at the end of CPB. Hemolysis was induced by CPB regardless to the use of two different membrane oxygenators (12). Free plasma hemoglobin and elevated serum potassium in shed mediastinal blood indicated a substantial hemolysis, but still below the values reported in literature for CPDA-1 packed cells at expiration date (13). Despite the hemolysis retransfused shed blood seemed to be well tolerated (10). The elevated serum potassium requires frequent controls after retransfusion of larger amounts, in particular when potassium infusions or homologous packed cells are dispensed concomitantly.

Energy rich phosphates such as ATP and 2,3DPG are responsible for membrane stability, rheological attributes and functional integrity of the erythrocyte. 2,3DPG decreases the affinity of hemoglobin for O2, thus allowing the maintenance of efficient oxygen delivery to tissue (14,15). The measurements of ATP in shed mediastinal blood compared to the preoperative patient value revealed a loss of 30%, which might indicate a decreased deformability. In comparison, ATP concentration in homologous packed cells at expiration date may be reduced to as much as 50% (13). Further investigations on erythrocyte function in preservation solutions showed a marked loss in deformability comprising a volume loss and an increase in viscosity (16,17). The literature is controversial on the survival rate of retransfused erythrocytes. However, there is obvious agreement on a correlation between the 24 hour survival rate and the ATP concentration of the erythrocyte. The oxygenation capacity in shed blood represented by 2,3 DPG was preserved in the normal range, thus maintaining a sufficient delivery of oxygen to the tissue. Similar to ATP there may be a marked decrease in 2,3DPG concentration in homologous packed cells at expiration date (13,18). This may not be of further relevance because transfused cells regain normal levels of 2,3DPG and PaO2 (PO2 associated with 50% O2 saturation of hemoglobin at 37°C and pH 7.4) but this may take several days (19,20).

Conclusions

Autotransfusion of salvaged mediastinal blood in cardiac surgery may help to reduce homologous blood requirements. The shed mediastinal blood is of satisfactory quality as regards erythrocytes' function, platelets counts, content of proteins and sterility. We consider the reinfusion of shed mediastinal blood as an integral method of a blood salvage concept, safe, easily applicable and readily accepted in the intensive care unit.

We owe special gratitude to the team of perfusionists, to the laboratories for hematology and clinical chemistry and to the nursing staff in the intensive care unit for their invaluable help.

References

Questions and Answers

Dick Klausen, Riverside, PA

Q. Did you have any problem post-operatively reinfusing the blood? Did you have any problem with the blood clotting off in the cardiotomy before it was able to be reinfused into the patient?

A. No we never did. We followed up 300 patients and never detected any problems in retransfusing the blood.

Q. Was there a reduction in the use of homologous blood?

A. Yes, there was. If we only retransfused the shed mediastinal blood usually one unit packed cells or less. It was not significant, but we used it normally in combination with retransfusion of centrifuged oxygenated blood, the remaining blood in the machine that we recentrifugated and then we can reduce the homologous blood about 50%.

Gordon Malone, Kingsport, TN

Q. Did you compare using other types of retransfusion bags versus cardiotomies?

A. No, we didn't.

Jim McMillan, Melbourne Australia

Q. What is the lowest hemoglobin levels you accept in post-operative phase?

A. Well, when I did this study it was in the intensive care unit—about 9 gm% but we always consider the status of the patient so it might be that a patient was 10 gm% had already had a transfusion of blood but we also had a patient with 7.5 gm% who was doing very well, so we didn't retransfuse anything.