Retrograde Autologous Priming (RAP) increases transfusion requirement in the late postoperative period: a pilot study.

Master thesis in cardiovascular technology  
– with special reference to cardiopulmonary bypass

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Abstract

Background: Hemodilution associated with cardiopulmonary bypass (CPB) exerts the risk for blood transfusion and volume overload. Priming of the CPB circuit with patients’ own blood – Retrograde Autologous Priming (RAP) limits hemodilution and has potential of reducing transfusion requirements and decreasing perioperative volume load. The aim of our study was to investigate if RAP reduced the need for Red Blood Cell (RBC) transfusion during and after cardiac surgery with CPB and if the decreased hemodilution during bypass affected the total postoperative fluid administration.

Method: Thirty patients undergoing coronary artery bypass grafting and/or aortic valve replacement were randomly allocated to standard priming (STP) or RAP. Volume removed in the RAP group amounted to 600 ml. A benchmark for RBC transfusion was a hemoglobin concentration < 80 g/L. Homologous RBC transfusion, fluid administration and hemoglobin values were evaluated both peri and postoperatively.

Result: No difference was demonstrated between the groups regarding number of patients transfused during operation or in the early postoperative period. In the late postoperative period a significant increase in transfusion rate (p 0,005) was detected in the RAP group for unknown reasons. A trend towards reduced intraoperative fluid balance in the RAP group was noted. Postoperative fluid administration until 06:00 the first postoperative morning did not differ between the groups, nor did the postoperative weight gain measured at the same point in time. A higher hemoglobin concentration was demonstrated in the STP group the first postoperative morning (p 0,004).

Conclusion: RAP has potential of reducing hemodilution during CPB but appears to have no favorable impact on transfusion rate, hematocrit value or fluid balance during the subsequent postoperative process.
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Abbreviations

RAP – Retrograde Autologous Priming
CPB – Cardiopulmonary Bypass
CABG – Coronary Artery Bypass Grafting
BSA – Body Surface Area
RBC – Red Blood Cell
AVR – Aortic Valve Replacement
STP group – Standard Priming Group
RAP group – Retrograde Autologous Priming group
ACT – Activated Clotting Time
ICU – Intensive Care Unit
SD – Standard Deviation
OR – Operating Room
FPM – First Postoperative morning
Introduction

Transfusion of homologous blood components increases both postoperative morbidity and mortality in patients undergoing cardiac surgery \(^1\)–\(^3\). Crystalloid priming in the cardiopulmonary bypass (CPB) circuit has been used as standard practice in adult cardiac surgery to reduce the need for blood transfusion. Open heart surgery with CPB is associated with hemodilution and the priming volume of the CPB circuit is normally between 1500-2000 ml. Decreased blood viscosity, improved microcirculation and enhanced venous return with a following increase in cardiac output are known benefits of moderate hemodilution \(^4\).

However, severe hemodilution with subsequent anemia may compromise oxygen delivery at tissue level, resulting in ischemic organ injury \(^5\)\(^,\)\(^6\) and furthermore increase the risk for homologous blood transfusion contrary to the intent \(^7\). Perioperative administration of large amounts of crystalloid fluid has been associated with postoperative elevation of extravascular lung water due to a reduction in colloid osmotic pressure \(^8\) and identified as a risk factor for postoperative delirium in elderly patients \(^9\).

The balance between giving homologous blood transfusions and accepting lower hematocrit levels with potentially detrimental effects to the patient is delicate. No lowest safe hematocrit level on CPB has been determined. Studies suggest that hematocrit levels below 22-23% is associated with increased morbidity and mortality with a higher risk of developing myocardial infarction, renal failure, sepsis and multi organ failure \(^5\)\(^,\)\(^6\)\).

Several perfusion techniques and strategies are available to reduce homologous blood transfusion and excessive hemodilution during CPB. Miniaturized CPB circuits decrease priming volume, prevent immoderate activation of blood and reduce intraoperative transfusion rate \(^10\)\(^,\)\(^11\). Cell salvage \(^12\) and ultrafiltration are other established blood conservation techniques commonly used. An advantage of both cell saving and ultrafiltration is the removal of inflammatory markers and cytokines \(^12\)\(^,\)\(^13\).
Retrograde autologous priming (RAP) is an easy, safe and effective way of reducing hemodilution during CPB. During RAP the priming volume of the CPB circuit is displaced with the patient’s own blood after the patient is heparinized and cannulated. The technique was first described in 1960 and regained new interest after a study conducted by Rosengart et al in 1998.

Studies on RAP show varying results regarding its efficacy in preventing perioperative blood transfusion. In a prospectively randomized study of sixty coronary artery bypass grafting (CABG) patients the authors were able to conclude that RAP significantly decreased hemodilution and reduced Red Blood Cell (RBC) transfusion. One study, investigating only patients with low body surface area (BSA), demonstrated a pronounced decrease in transfusion rate whereas another failed to show any benefit of RAP regarding transfusion requirements.

**Hypothesis and aim**

RAP decreases hemodilution during CPB. We hypothesized that a higher hematocrit level on bypass reduces peri- and postoperative need for RBC transfusion and that the maintenance of blood oncotic pressure reduces vascular leakage, resulting in a decreased total fluid balance. The aim of our study was to investigate if RAP reduced the need for RBC transfusion during and after cardiac surgery with CPB and if the decreased hemodilution during bypass affected the total postoperative fluid administration.
**Material and methods**

*Patients*

The study was approved by the ethics committee in Lund Sweden 09/01/2014, Dnr 2013/860. Forty patients scheduled for elective coronary artery bypass grafting (CABG) or aortic valve replacement (AVR), as single or combined procedure and with a preoperative hemoglobin $\leq 140$ g/L, were asked to participate in the study. All patients received both oral and written information and signed a written informed consent.

Patients with poor left ventricular function (ejection fraction $<30\%$), hemodynamic instability (systolic blood pressure $<85$ mmHg after induction of anesthesia despite support with phenylephrine/norepinephrine), use of cell saver technique, leukocyte filtration, emergency or redo-operations and/or patients who were reinstminated back on bypass which necessitated re-priming of the CPB circuit were excluded from the study. Ten of the original forty patients were excluded from the study due to high levels of hemoglobin after induction of anesthesia (n=4), reinstitution of CPB (n=5) or the use of cell saver (n=1).

Thirty patients were randomly allocated, either to the RAP group or to the standard priming group (STP group).

*Endpoints*

The primary endpoints were number of patients receiving peri- and postoperative Red Blood Cell (RBC) transfusion and total administration of fluid after CPB until 06:00 the first postoperative morning. Secondary endpoints were hemoglobin levels at various time points, peri- and postoperative bleeding, fluid balance during and after perfusion as well as postoperative weight gain as a marker for interstitial fluid sequestration. All patients were treated according to local routines.
Anesthesia

Anesthesia was induced using Propofol Lipuro (Braun, Melsungen AG, Germany) and 0.3 – 0.5 mg/kg Fentanyl (Braun, Melsungen AG, Germany). Muscle relaxation was achieved with 0.6 mg/kg Rocuronium (Fresenius Kabi, Uppsala, Sweden). Anesthesia was maintained with Sevoflurane (Abbvie AB, Solna, Sweden) before, during and after CPB.

The initial dose of heparin given before CPB was 350 IU/kg bodyweight to achieve an activated clotting time (ACT) of 480 seconds or above. An additional dose of heparin was administrated if the target was not reached. During bypass the ACT level was kept > 480 seconds with additional doses of heparin. After CPB Heparin anticoagulation was reversed with Protamine (Leo pharma, A/S, Ballerup, Denmark). The Protamine dose was calculated based on the total initial dose of Heparin with 1 mg of Protamine for every 100 IU of Heparin. Additional doses of Protamine were given if the ACT target of 130 seconds or less was not achieved and according to the attending physician.

CPB circuit

A S5 heart-lung machine with a roller pump (Sorin Group Deutschland GmbH, München) was used during the procedures. The CPB-circuit contained a membrane oxygenator with an integrated arterial filter (Quadrox-i HMO 71000) and a hard-shell venous reservoir (VHK 2001, Maquet, Getinge Group AB, Germany, Rastatt). The tubing diameters of the perfusion set were ½ inch for the venous line and 3/8 inch for the arterial line with ½ inch in the pump housing. The institutional standard priming of the bypass circuit consisted of 1500 ml Ringer's solution, 100 ml mannitol, 160 mmol (40 ml) sodium chloride, and 10 000 IU (2 ml) of heparin. After aortic cross-clamping, cardioplegic arrest was induced with cold blood cardioplegia (4:1), administrated in to the aortic root, using Plegiox cardioplegia set (Maquet, Getinge Group AB, Germany, Rastatt).
Retrograde Autologous Priming procedure (RAP procedure)

After the disconnection of the pre-bypass filter from the CPB circuit, an infusion bag was connected to a pre-oxygenator purge line. A cross clamp was placed on the arterial tubing distal to the oxygenator. The arterial pump was slowly rotated, draining the fluid in the venous reservoir into the infusion bag through the purge line. When the level of fluid in the reservoir reached 50 milliliter, the pump was stopped. The purge line was then closed and the cross clamp reassigned to the arterial tubing proximal to the oxygenator. After arterial cannulation the retrograde drainage of the arterial line was achieved by reopening the purge line. The arterial blood pressure pushed the crystalloid fluid in the arterial line and oxygenator into the infusion bag. The RAP procedure was terminated when blood reached the purge line, reducing the priming volume by 600 ml.

Figure 1. RAP technique.
**Transfusion criteria**

A benchmark for RBC transfusion was a hemoglobin concentration < 80 g/L before weaning from CPB. After CPB and during the remaining time of the hospital stay the threshold for RBC transfusion was a hemoglobin concentration < 80 g/L according to local routines. Deviations from these routines were decided by the attending physician.

**Measurements and blood analysis**

Hemoglobin concentrations were obtained and measured at six time points: after induction of anesthesia, at initiation of CPB, 3 minutes after protamine administration, at arrival in the Intensive Care Unit (ICU), in the first postoperative morning and five days after the operation. All blood samples during operation and in the ICU, were drawn from an existing arterial catheter. Five days after surgery a venous puncture was performed.

The arterial blood samples were analyzed with a blood gas analyzer (ABL 800, Radiometer, Copenhagen, Denmark). Other blood samples were analyzed in the department of Clinical chemistry, Blekinge Hospital, Karlskrona.

Fluid balance and total amount of fluids administrated during the day of surgery was measured. RBC transfusion during operation and until discharge was noted. Bleeding was recorded during operation and until the first postoperative morning.

**Time plan**

The scientific protocol was carried out during October/November 2013 and approved by the ethics committee in January 2014. Data was collected between February and June 2014. Interpretation of data, writing and compilation of the thesis was conducted between June and August 2014.
Statistical analysis

Results are presented as mean ± SD unless otherwise stated. Non-parametric test has been used to evaluate the result in IBM SPSS, version 22. Mann-Whitney U test was used to compare continuous variables between groups. P <0,05 was considered significant.

Result

No differences with regards to baseline characteristics, coexisting disease or duration of CPB and aortic cross clamping were found between the groups (table 1).

Table 1. Patient characteristics and risk factors.

<table>
<thead>
<tr>
<th></th>
<th>RAP group (n=15)</th>
<th>STP group (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>12/3</td>
<td>11/4</td>
<td>ns</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70 ± 7</td>
<td>72 ± 9</td>
<td>ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81 ± 13</td>
<td>81 ± 15</td>
<td>ns</td>
</tr>
<tr>
<td>BSA (m2)</td>
<td>1,92 ± 0,17</td>
<td>1,96 ± 0,21</td>
<td>ns</td>
</tr>
<tr>
<td>Red cell mass (ml)</td>
<td>2081 ± 265</td>
<td>2125 ± 298</td>
<td>ns</td>
</tr>
<tr>
<td>Preoperative hemoglobin (g/dl)</td>
<td>12,2 ± 1,2</td>
<td>12,5 ± 1,2</td>
<td>ns</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>104 ± 46</td>
<td>95 ± 57</td>
<td>ns</td>
</tr>
<tr>
<td>Aortic cross clamp time (min)</td>
<td>63 ± 38</td>
<td>61 ± 44</td>
<td>ns</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (20%)</td>
<td>3 (20%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>8 (53,3%)</td>
<td>7 (46,7%)</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>0 (0%)</td>
<td>1 (6,7%)</td>
<td></td>
</tr>
<tr>
<td>Increased preoperative creatinine</td>
<td>4 (26,7%)</td>
<td>2 (13,3%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. All values are presented as mean ± SD unless otherwise stated. BSA=Body surface area. NS=Not significant. COPD=Chronic Obstructive Pulmonary Disease. RAP=Retrograde Autologous Priming. STP=Standard Priming Group.

Transfusions and blood loss

Two of 15 patients received an intraoperative RBC transfusion in both groups (figure 1).

During the ICU stay, three patients in the RAP group and two patients in the STP group received RBC transfusion. During the remaining of the stay in the hospital significantly fewer patients (p 0,005) received RBC transfusion in the STP compared to the RAP group (3 vs. 11). Intraoperative blood loss as well as losses from chest tube drainage the first postoperative morning revealed no difference between groups (figure 2).
**Figure 2.** Transfusion incidence during hospital stay.

**Figure 2**: Number of patients receiving RBC transfusion. RBC=Red blood cell. OR=Operation Room. ICU=Intensive Care Unit. RAP=Retrograde Autologous Priming. STP=Standard Priming Group.

* = p0.005

**Figure 3.** Peri- and postoperative blood loss.

**Figure 3**: Blood loss during the day of surgery presented as mean values. FPM= first postoperative morning. RAP=Retrograde Autologous Priming. STP=Standard Priming Group.


**Fluid administration**

The fluid volume administrated before CPB was equal in both groups. The priming volume in the STP group was 1640 ml and the volume removed in the RAP group was 600 ml, representing 37.5% of the total priming volume (table 2).

**Table 2. Fluid administration and fluid balance.**

<table>
<thead>
<tr>
<th></th>
<th>RAP group (n=15)</th>
<th>STP group (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluids administrated before CPB (ml)</td>
<td>637 ± 209</td>
<td>707 ± 164</td>
<td>0.652</td>
</tr>
<tr>
<td>Priming volume removed (ml / %)</td>
<td>600 ml / 37.5%</td>
<td>0 ml / 0%</td>
<td>na</td>
</tr>
<tr>
<td>Fluid balance during perfusion (ml)</td>
<td>1830 ± 484</td>
<td>2176 ± 604</td>
<td>0.146</td>
</tr>
<tr>
<td>Fluid balance during operation (ml)</td>
<td>2341 ± 708</td>
<td>2640 ± 651</td>
<td>0.306</td>
</tr>
<tr>
<td>Postoperative fluid administration until first postoperative morning (ml)</td>
<td>2987 ± 819</td>
<td>2887 ± 815</td>
<td>0.648</td>
</tr>
<tr>
<td>Total fluid balance during day of surgery (ml)</td>
<td>4431 ± 1165</td>
<td>4567 ± 1153</td>
<td>0.781</td>
</tr>
<tr>
<td>Postoperative weight gain (% increase from preoperative body weight)</td>
<td>4.1 ± 1.4</td>
<td>3.8 ± 1.7</td>
<td>0.713</td>
</tr>
</tbody>
</table>

Table 2: All values are presented as mean ± SD unless otherwise stated. Postoperative weight gain, measured the first morning after surgery. RAP=Retrograde Autologous Priming. STP=Standard Priming Group. na = not applicable.

The average difference between the groups regarding fluid balance during CPB and total perioperative fluid balance represented approximately 50% of the amount removed during RAP.

Postoperative fluid administration from end of CPB until 06:00 the first morning after surgery as well as total fluid balance during the day of surgery did not reveal any difference between groups (p 0.648).

Postoperative weight gain the morning after surgery was measured as an indication of leakage from vascular to interstitial space. Presented as a relative weight gain in percentage from baseline values, no difference regarding extravascular fluid sequestration could be demonstrated.
Baseline hemoglobin values were similar between the two groups (Figure 3). The hemoglobin concentration after onset of bypass in the RAP group compared to the STP group demonstrated a significant less reduction from baseline values in the RAP group (25% vs. 31.8%, p 0.001). Three minutes after protamine administration and at arrival in the thoracic ICU the hemoglobin values were once again similar between groups. The first postoperative morning after surgery the patients subjected to RAP paradoxically demonstrated a significantly lower hemoglobin concentration compared with the STP group (p 0.004).
Discussion

This pilot study revealed no benefits of RAP regarding RBC transfusion during operation or postoperative in the ICU. In fact, a significant increase of RBC transfusions in the late postoperative period was demonstrated in the RAP group.

Similar to previous studies, maintenance of higher hemoglobin concentrations in the RAP group were noticed at initiation of CPB \(7,16\), however this effect was not persistent and at the time of protamine administration no discrepancies between the groups could be detected, despite similar blood loss during surgery.

Bleeding was measured in the chest tube drainage from end of surgery until the first postoperative morning. No difference in bleeding between the groups could be detected; unexpectedly however we found a significant higher hemoglobin concentration in the STP group (\(p 0.004\)). Interpretation of this somewhat contradictory result is not easily done. One argument for improved outcome in the STP group might be attributed to a protective effect of hemodilution, reducing the loss of actual blood cells during surgery.

When investigating blood conservation techniques and utilization of blood, transfusion thresholds appear to play an important role. No lowest safe hematocrit has been established either on CPB nor postoperative. Hematocrit transfusion thresholds ranges from 16-24\% during CPB and from 22-27\% after termination from bypass in different studies \(7,15,16\).

In this study, the transfusion incidence during operation was 13 \% in both groups. The benchmark for transfusion was a hemoglobin < 80 g/L (hematocrit of approximately 24\%), but was finally determined by the attending physician. RBC transfusion was considered at higher hematocrit values if signs of clinical symptomatic anemia did occur. Compared to existing studies \(15–18\), this appears to be an overall modest intraoperative transfusion frequency. In fact only one study has demonstrated a lower transfusion incidence \(7\) for a RAP group, although this clinical trial used hypothermic CPB with a hematocrit transfusion trigger of 16\%. The overall low intraoperative transfusion incidence in our study may explain the lack of RAP efficacy in reducing perioperative RBC transfusion in this small material. A larger sample size would perhaps be able to reveal a difference between the groups concerning perioperative transfusion incidence.

In the late postoperative period we were able to demonstrate an apparent difference in transfusion requirements between the groups. In the STP group the transfusion rate was 20\%
which is similar to other studies. However in the RAP group the incidence amounted to 73% which is remarkably high compared to other clinical trial. This pronounced difference between the groups seems somewhat difficult to interpret. The groups appeared closely matched with regard to preoperative transfusion risk factors. Predictors of RBC transfusion in cardiac surgery include increased age (> 70-75 years), small size (BSA < 1,7 m²) and preoperative anemia (hematocrit <32-34%). In addition a prolonged CPB time (>120 min) has been identified as a risk factor for blood utilization. Subsequent data analysis indicates slightly more perioperative bleeding and an increased number of patients with a prolonged CPB time (> 120 minutes) in the RAP group. Furthermore an analysis of postoperative transfusion thresholds indicates a trend towards more restrictive transfusion triggers in the STP group. Altogether, these factors could have accounted for some impact on the study outcome.

In our study the RAP volume removed from the CPB circuit amounted to 600 ml whereas some studies that reported clinical benefits of RAP, had a mean volume withdrawal of 880 ml and 1200 ml respectively. In addition to RAP, intraoperative autologous donation was performed in one of these studies with a mean volume of 780 ml whole blood donated in the RAP group. Assumptions can be made that the removal of 600 ml priming volume may not be sufficient to reduce the incidence of transfusion. In a study by Nanjappa et al, investigating 201 patients, only 300 ml was removed, demonstrating no reduction in RBC transfusion. On the other hand a large retrospective study by Vandewiele et al including 753 patients, identified an overall optimal RAP volume of 475 ml to significantly decrease transfusion rate.

In some patients, the removal of 600 ml priming volume caused certain difficulties. We noticed that patients with a small intravascular volume, for instance patients with low body surface area (BSA), could pose a problem regarding the ability to obtain adequate venous return and run a sufficient pump flow. Unfortunately these patients, who would achieve a proportionally greater benefit from RAP, also proved to be more difficult to implement and sometimes required reinfusion of the RAP volume.

Despite larger RAP volume removal in some studies, none of these addresses difficulties regarding venous return. Even though a decreased venous reservoir volume in the RAP group is described by Rosengart et al, the ability to maintain adequate flows were not described as an issue. One reason for this may be attributed to the fact that two of these studies were
conducted using moderate hypothermia during bypass, reducing the CPB flow requirements. One of these trials\textsuperscript{16} was performed without active cooling but substantial amounts of crystalloid infusion were administrated by the anesthetic personnel during the intraoperative period, which is likely to have facilitated venous return.

The amount of fluid administrated before CPB was equal between the groups. When looking at the total perioperative fluid balance in both groups we could notice a trend towards lesser fluid balance in the RAP group. The average perioperative difference between the groups was about half the amount removed during RAP.

During the early postoperative phase, we identified a substantial volume demand. Both groups required almost 3000 ml of crystalloid fluid from the end of operation until the first postoperative morning, yielding a final fluid balance of approximately 4500 ml during the day of surgery. This corresponds well to the weight gain, measured at the same time point. If the considerable postoperative volume infusion is a genuine requirement or linked to institutional routines was not investigated in this study.

Currently, postoperative fluid therapy using colloids is not preferred\textsuperscript{21}, which may be one reason for the considerable volume demands. Seen in this context, the removal of 600 ml of priming volume seems to decrease hemodilution during CPB, but appears to be of minor importance when looking at the entire operative process.

\textit{Limitations}

Our study was limited by a small sample size. The trial was not restricted to a single surgeon, making variability in surgical techniques inevitable. Inconsistent transfusion thresholds may have influenced the outcome regarding transfusion incidence in the late postoperative period.

\textit{Conclusion}

We found no perioperative or early postoperative reduction in RBC transfusion when RAP was applied but an increased transfusion incidence was revealed in the RAP group for unknown reasons in the late postoperative period. A trend towards reduced perioperative fluid
balance was noticed in the RAP group. Differences regarding postoperative fluid requirement could not be demonstrated.

RAP has potential of reducing hemodilution during CPB but appears to have no favorable impact on transfusion rate, hematocrit value or fluid balance during the subsequent postoperative process. Further studies, preferably with larger sample sizes are needed to evaluate the efficiency of RAP in cardiac surgery with CPB.
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References


