Comparative Effects of Hemodilutional Anemia and Transfusion during Cardiopulmonary Bypass on Acute Kidney Injury: A Prospective Randomized Study

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ABSTRACT

Aim: Acute kidney injury after cardiopulmonary bypass has been associated with dilutional anemia during surgery. We aimed both to explore if this relation is modulated by blood transfusion and to understand the postoperative contribution of protein oxidation.

Methods: In this randomized prospective study, after ethics committee approval and informed consent, 30 patients undergoing first-time elective coronary artery bypass grafting (CABG) with hematocrit between 21% and 25% at any time during extracorporeal circulation (ECC) were randomly and equally allocated into two groups. Group I consisted of patients who received red blood cells (RBC) during ECC, while in Group II, patients did not receive any RBCs. Besides routine hemodynamic and biochemical parameters, markers of renal injury such as neutrophil gelatinase-associated lipocalin (NGAL), creatinine clearance, and protein oxidation parameters (advanced oxidative protein products [AOPP], total thiol [T-SH]) were determined in both groups.

Results: (1) Both cardiovascular parameters (MAP, HR) and the hospitalization period of the transfused group were not significantly different compared to the non-transfused group (P > .05); (2) While urine NGAL level (P < .05) and GFR (P < .01) decreased in the transfused group compared to the preoperative period, there were no significant changes in respective parameters of the non-transfused group compared to preoperative period; (3) AOPP concentrations did not change compared to postoperative periods in both groups (P > .05). However, T-SH concentration showed a transient increase at postoperative hour 6 (P < .001 vs preoperative period) but normalized at postoperative hour 24 (P > .05 versus preoperative period).

Conclusion: These findings suggest that a hematocrit value over 21% during ECC is safe for renal functions. RBC transfusion just to increase hematocrit may be deleterious.

INTRODUCTION

Acute kidney injury (AKI), previously called acute renal failure [Bellomo 2004; Moore 2010], has been reported to occur in approximately 36% of critically ill patients and is common after major surgery such as open heart surgery (OHS) [Bagshaw 2008; Ostermann 2007].

In spite of advances in OHS, AKI remains one of the most common postoperative complications with a high incidence of mortality (7-30%) and morbidity. AKI after OHS may be defined as an abrupt reduction in kidney function characterized by a 50% increase in serum creatinine or a 25% decrease in glomerular filtration rate (GFR) [Bellomo 2004; Mehta 2007; Ricci 2011; Karkouti 2012; Wijeysundera 2003]. After OHS, 30-50% of patients may develop postoperative AKI, 1-5% of whom may eventually progress to having severe AKI that requires permanent hemodialysis [Ricci 2011; Karkouti 2012].

It is well known that both the dilutional anemia during OHS and perioperative transfusion of blood and blood products given in order to treat anemia are deleterious and may increase the risk of postoperative AKI in OHS [Mehta 2007; Karkouti 2012].

The changes in serum creatinine level, which is the most common marker used to diagnose AKI after OHS, may not always be related to renal injury. Factors like age, gender, nutrition, muscle mass, muscle metabolism, drug therapies, and hydration may influence the changes in serum creatinine levels [Karkouti 2012; Mangano 1998; Ryckwaert 2002], and early postoperative hemodilution and hypothermia in patients who undergo OHS may result in a decrease in serum creatinine and renal injury may unfortunately go unnoticed [Karkouti 2012; Chertow 1998].

The aim of the current study was to compare the effects of blood transfusion and anemia on the kidney during OHS. Therefore we measured protein oxidation markers (advanced oxidation protein products and total thiols) and neutrophil gelatinase-associated lipocalin (NGAL) to investigate the effects of transfusion and anemia on the complex relationship between oxidative stress and kidney functions.

MATERIALS AND METHODS

Patient Selection

After approval from the Ethics Committee of Acibadem University for this randomized prospective study, informed consent was preoperatively taken from patients. These patients had undergone elective coronary artery bypass graft surgery under cardiopulmonary bypass (CPB). The patients who had diabetes mellitus, preoperative dialysis-dependent renal failure, and serum creatinine over 1.2 mg/dL were...
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1.79 ± 0.13
9/6
2
Group II
39 ± 9
1.74 ± 0.15
71.6 ± 9.5
-
-
158 ± 8
64 ± 5
39 ± 9
68 ± 13

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Dynamically stable, ECC was discontinued and heparin was reversed with protamine sulfate. Infusions of both midazolam and vecuronium were restored to 80 μg/kg/h during rewarming and were reduced to 50 μg/kg/h after termination of ECC. They were discontinued at skin closure.

Data Collection and Blood Sampling
Continuous measurements of heart rate (HR) and mean arterial pressure (MAP) were performed throughout surgery. Htc, hemoglobin (Hb), and lactate concentrations were determined at seven particular time points as follows: (T1) before anesthesia induction; (T2) after anesthesia induction; (T3) 10th minute of ECC; (T4) 20th minute of ECC; (T5) after end of ECC; (T6) second postoperative hour; (T7) fourth postoperative hour.

Assessment of Renal Injury and Renal Function
Urine neutrophil gelatinase-associated lipocalin (NGAL), urea, GFR, and fractional sodium excretion (EFNa) were measured preoperatively, at postoperative 6th hour, and at postoperative 24th hour time points. Urinary NGAL was determined by ELISA assay using a commercially available ELISA kit (Antibodyshop, Gentofte, Denmark). The limit of detection for this assay is 0.5 to 4.0 pg/mL, with an intrasassay variation in urine of 2.1%.

Determination of Protein Oxidation
For measurement of advanced oxidative protein products (AOPP), a modified method of Hanasand et al [Hanasand 2012] was performed for spectrophotometric determination of AOPP levels. According to the procedure, serum samples were diluted with citric acid, 10 μL of 1.16 M KI was added to diluted solution, 2 minutes later followed by 20 μL acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against the blank solution. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

Measurement of Total Thiol (T-SH)
Spectrophotometric methods were performed for determination of T-SH groups [Sedlak 1968]. 50 μL of plasma

Table 1. Demographic Data of the Patients*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body surface area, m²</td>
<td>1.74 ± 0.15</td>
<td>1.79 ± 0.13</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158 ± 8</td>
<td>163 ± 8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.5 ± 11.9</td>
<td>71.6 ± 9.5</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>9 / 6</td>
<td>8 / 7</td>
</tr>
<tr>
<td>Age, y</td>
<td>64 ± 5</td>
<td>66 ± 4</td>
</tr>
<tr>
<td>Cross clamp time, min</td>
<td>39 ± 9</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>CPB duration, min</td>
<td>68 ± 13</td>
<td>66 ± 12</td>
</tr>
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</table>

Data are given as the mean ± standard deviation. Only sex is defined as number. CPB indicates cardiopulmonary bypass.

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was mixed in test tubes with 150 μL of 0.2 M Tris buffer, pH 8.2, and 10 μL of 0.01 M 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). The mixture was brought to 1 mL with absolute methanol. Additionally, reagent blank and sample blank were prepared in a similar manner. All the test tubes were incubated for 15 minutes and the mixtures were centrifuged at 3,000 g at room temperature for 15 minutes. The absorbance of supernatant fractions was read in a spectrophotometer at 412 nm.

Both AOPP and total thiol levels were determined preoperatively, at postoperative 6th hour, and at postoperative 24th hour.

**Statistical Analysis**

Values are reported as the mean ± SEM. Statistical analysis was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). The two groups were compared using unpaired-t test and one-way ANOVA for repeated measurements with a Bonferroni post hoc test. A P value of <.05 was considered statistically significant.

**RESULTS**

The demographic data are given in Table 1 and there were no statistically significant differences regarding demographic data (P > .05). The hemodynamic parameters and lactate can be seen in Table 2. All variables were similar between groups and there were no statistically significant differences regarding hemodynamic parameters (P > .05). At T5 Htc and Hb were found to be statistically significantly lower in group II than in group I at T5 (P < .05). However, no significant differences were found in other time points (P > .05). Also, no significant differences were found between the groups regarding the overall fluid balance (Figure).

GFR at postoperative hour 24 was lower than the other time points in group I (P < .05). NGAL was measured as 9.3 ± 4.0 ng/mL and 26.2 ± 6.0 ng/mL in group I; and 9.0 ± 3.2 ng/mL and 22.4 ± 8.8 ng/mL in group II, respectively, in the preoperative time point and at postoperative hour 6 (Table 3). In group I, NGAL was statistically significantly higher compared to postoperative hour 6 measurements (P < .05). In group I, total thiol at postoperative hour 6 (69.7 ± 7.3 μmol/L) was statistically significantly higher compared to preoperative (44.0 ± 6.7 μmol/L) and postoperative hour 24 (34.8 ± 8.9 μmol/L) measurements (P < .05) (Table 4).

Duration of intubation, intensive care unit stay, and hospital stay showed no significant difference between groups (Table 5).

**DISCUSSION**

In the present study, we examined the effects of blood transfusion and anemia on renal function and injury in OHS. We tested if blood transfusion would be superior in maintaining kidney function and redox balance after OHS compared to dilutional anemia. Our main findings were that: (1) OHS was associated with acute dilutional anemia stress; (2) perioperative strategies with blood transfusion and without blood transfusion mostly resulted in similar outcomes; (3) the blood transfusion acutely changes the redox balance to favor...
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Table 3. Renal Function Markers

<table>
<thead>
<tr>
<th></th>
<th>Preoperation</th>
<th>Postoperative 6th hour</th>
<th>Postoperative 24th hour</th>
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</thead>
<tbody>
<tr>
<td><strong>Glomerular filtration rate, mL/min/1.73 m²</strong></td>
<td></td>
<td></td>
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<tr>
<td>Gl</td>
<td>70.9 ± 5.0</td>
<td>65.4 ± 5.0</td>
<td>62.2 ± 5.3*</td>
</tr>
<tr>
<td>GII</td>
<td>74.5 ± 4.3</td>
<td>68.4 ± 5.0</td>
<td>72.3 ± 5.6</td>
</tr>
<tr>
<td><strong>Creatinine, mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gl</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>GII</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
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<tr>
<td><strong>Urea, mg/dL</strong></td>
<td></td>
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<tr>
<td>Gl</td>
<td>40.0 ± 4.5</td>
<td>39.1 ± 2.8</td>
<td>44.5 ± 4.7</td>
</tr>
<tr>
<td>GII</td>
<td>32.3 ± 2.4</td>
<td>37.2 ± 2.3</td>
<td>39.5 ± 3.0</td>
</tr>
<tr>
<td><strong>EF₁₅₀, %</strong></td>
<td></td>
<td></td>
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<tr>
<td>Gl</td>
<td>91.6 ± 9.7</td>
<td>74.8 ± 9.9</td>
<td>83.0 ± 11.8</td>
</tr>
<tr>
<td>GII</td>
<td>95.2 ± 10.7</td>
<td>77.6 ± 10.0</td>
<td>95.6 ± 10.0</td>
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<tr>
<td><strong>Fluid balance, mL</strong></td>
<td></td>
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<tr>
<td>Gl</td>
<td>–</td>
<td>100.0 ± 136.7</td>
<td>637.3 ± 161.1</td>
</tr>
<tr>
<td>GII</td>
<td>–</td>
<td>-342.9 ± 237.0</td>
<td>136.1 ± 289.0</td>
</tr>
</tbody>
</table>

*P < .01 compared to preoperative time point. EF₁₅₀ indicates fractional sodium excretion.

Table 4. Renal Biomarker Measurements

<table>
<thead>
<tr>
<th></th>
<th>Preoperation</th>
<th>Postoperative 6th hour</th>
<th>Postoperative 24th hour</th>
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</thead>
<tbody>
<tr>
<td><strong>NGAL, ng/mL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gl</td>
<td>9.3 ± 4.0</td>
<td>26.2 ± 6.4*</td>
<td>–</td>
</tr>
<tr>
<td>GII</td>
<td>9.0 ± 3.2</td>
<td>22.4 ± 8.8</td>
<td>–</td>
</tr>
<tr>
<td><strong>T-SH, µmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gl</td>
<td>44.0 ± 6.0**</td>
<td>69.7 ± 7.3</td>
<td>34.8 ± 5.6**</td>
</tr>
<tr>
<td>GII</td>
<td>50.4 ± 6.8</td>
<td>74.4 ± 7.7</td>
<td>48.5 ± 8.9</td>
</tr>
<tr>
<td><strong>AOPP, µmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gl</td>
<td>1421.0 ± 122.9</td>
<td>1143.9 ± 98.8</td>
<td>1354.6 ± 145</td>
</tr>
<tr>
<td>GII</td>
<td>1425.3 ± 129.3</td>
<td>1243.3 ± 105.6</td>
<td>1307.3 ± 173.6</td>
</tr>
</tbody>
</table>

NGAL indicates neutrophil gelatinase-associated lipocalin; AOPP, advanced oxidative protein products; T-SH, total thiol.

*P < .05 compared to preoperative time point; ** P < .001 compared to postoperative 6th hour

with ECC, such as those with pre-existing kidney dysfunction or anemia [Karkouti 2012].

Erythrocytes undergo progressive, interrelated biochemical and morphological changes during storage [Redfors 2010; Comporti 2002; Almac 2007; Timmou 2006] and moreover up to 30% of the transfused erythrocytes are hemolysed in intravascular space after transfusion [Luten 2008; Lasocki 2011]. Thus, transfusion of packed RBC can in particular lead to high concentrations of free Hb and iron in the circulation, which is highly toxic to kidneys and other organs [Baek 2012; Hod 2011; Vermeulen Windsant 2012]. Moreover cardiac surgery with ECC is itself an important aggravating factor, as ECC progressively damages native erythrocytes, further increasing free Hb and iron levels [Rosner 2006; Stafford-Smith 2008; Vercaemst 2008].

Two recent studies included only non-transfused patients to rule out the confounding effect of transfusion, and found anemia to be independently related to AKI [Ranucci 2010; Loor 2012]. In another recent study, it was illustrated that the AKI risks of transfusion are more pronounced in anemic than non-anemic patients [Karkouti 2011]. Thus, it seems that the independent effects of perioperative transfusion and perioperative anemia on AKI may be synergistic [Karkouti 2012].

Moreover, several studies attempted to assess the risks of transfusion by randomizing patients to restrictive or liberal transfusion arms [Karkouti 2011; Carson 2012]. The main finding of these studies was that the restrictive transfusion strategy significantly reduced the number of transfusions at the cost of a significant increase in the severity of anemia [Carson 2012]. Only three of the studies reported renal outcomes and they did not find any differences between groups in the rate of AKI [Carson 2012], suggesting that transfusions do not cause AKI. However, assessing the effects of transfusion on AKI was not the primary endpoint of these studies,
and they were also underpowered for this outcome (in total, there were only 38 reported cases of AKI in these studies) [Carson 2012]. Even if these studies were adequately powered, they can’t be used to ascertain the risks of transfusion on AKI [Deans 2012].

In an animal study, studying the effects of acute dilutional anemia on several tissues to quantify organ-specific tolerance of different levels of acute anemia, the heart, brain, kidneys, liver, small intestine, and skeletal muscle experienced tissue hypoxia at different degrees of acute anemia. They have stated that the heart, brain, and liver showed no signs of tissue hypoxia at Hb of 4%. It was concluded that hemodynamics, metabolic parameters, or oxygen consumption did not indicate that tissue oxygenation was restricted before reaching critical Hb and utilization of anemia tolerance reduces the need for any risks of perioperative transfusion [Lauscher 2013].

In our study the statistically significantly lower Hb and Htc in Group II was only recorded at T5. The similar results of both groups at T6 and T7 (after the end of ECC) strongly suggest that liberal transfusion strategy does not improve results regarding Htc and Hb. Moreover, intubation, intensive care unit stay, and hospital stay durations of the patients in both groups did not have any statistically significant differences, suggesting that transfusion for Htc between 21-24% has a positive impact on outcome. But in Group II, unlike in Group I, a statistically significant increase of total thiol and NGAL at postoperative hour 6 compared to preoperative measurement; a decrease of total thiol at postoperative hour 24 compared to postoperative hour 6; and a decrease of GFR at postoperative hour 24 compared to preoperative measurements suggests that RBC transfusion results in some kidney injury (may be not progressing to AKI) in these elective patients with normal renal functions. But regarding this statistical knowledge, it is probable to claim that patients with borderline renal functions may experience AKI induced by transfusion.

The most commonly used traditional clinical biomarkers for the detection of AKI are creatinine and urea. All have serious limitations as early detectors of AKI. The release of creatinine varies with age, gender, diet, muscle mass, drugs, and vigorous exercise. Moreover, secretion accounts for 10-40% of creatinine clearance, which could mask a decrease in GFR. Creatinine becomes abnormal only when more than 50% of GFR is lost and may require up to 24 hours before sufficient increases in blood concentration are detectable. Level of urea is also inversely related to GFR, but several factors affect its production and clearance, limiting its value in estimating GFR. Urea also requires time to accumulate, does not reflect real-time changes in GFR, and delays diagnosis. In response to these problems, innovative technologies such as functional genomics and proteomics have facilitated the detection of several potential earlier biomarkers of AKI. One of the most promising biomarkers is NGAL, which has now been evaluated in several populations. The expression of NGAL in early, acute tubular injury was identified using functional genomics. NGAL as a measure of tubular stress concentration increases dramatically in response to tubular injury and precedes rises in serum creatinine by >24 hours [Moore 2010; Schmidt-Ott 2006].

An acute rise in urinary NGAL has been reported to accurately identify evolving AKI in both pediatric and adult populations within 2 to 8 hours of cardiac surgery [McCloy 2010; Bennett 2008; Dent 2007; Mishra 2005; Xin 2008; Tuladhar 2009].

This would allow timely implementation and evaluation of potential protective therapies, either intraoperatively or on ICU admission, targeted to those at high risk of AKI. Indeed, the consideration of pre- to postoperative changes in NGAL concentration could also aid medical decision making, which might lead to improved outcomes. Furthermore, if NGAL reflected the likelihood for timely discharge, it could be useful in surgical cases and ICU bed planning [Moore 2010].

The spectral characteristics of AOPP correspond to several chromophores, which include dityrosine, carbonyls, and pentosidine [Breusing 2010]. Oxidative modifications of cellular proteins, as in AOPP formation, result in a loss of protein function. When transfused patients were compared to non-transfused anemic group, the impaired redox homeostasis was not determined by means of AOPP concentration changes. However, impaired protein redox homeostasis, which appears to occur in the transfused group, may be an enhancing factor in the propagation of protein oxidation, as indicated by the thiol concentration changings. It is known...

Table 5. Durations of Intubation, Intensive Care Unit Stay, and Hospital Stay

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intubation duration, hours</td>
<td>9.4 ± 1.2</td>
<td>8 ± 0.8</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Stay in intensive care unit, hours</td>
<td>26.6 ± 3.4</td>
<td>21.3 ± 0.5</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Hospital stay, days</td>
<td>6.7 ± 0.2</td>
<td>6.8 ± 0.4</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

Overall fluid balance through time points. P > .05 between groups for all time points.
that thiol groups in proteins have been working as endogenous antioxidants [Cremers 2013].

In our study, statistically significant changes in NGAL and total thiol are poorly correlated with traditional renal injury markers. Only GFR decreased at postoperative hour 24, where urea and creatinine had no statistically different changes during the operative timeline. So it is obvious that specific renal biomarkers like NGAL give the clinician the chance to predict renal injury several hours before clinical onset.

There are some limitations in our study. Long-term and clinical follow-up with more patients would give us better results. Also, leukocyte filtration and the age of packed RBCs that would have impact on renal functions would be issues to consider in future studies.

Conclusion

Transfusion is a possible cause for renal injury in OHS patients. Transfusion to increase Htc of 21-24% in OHS patients experiencing postoperative anemia may result in renal injury, which may progress to AKI. Following up the renal functions with traditional diagnostic markers—urea, creatinine, GFR—may delay the diagnosis. Early diagnosis and therapy for prevention of AKI is possible by monitoring specific renal biomarkers.

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