Acute Lung Injury During Cardiopulmonary Bypass
Are the Neutrophils Responsible?

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To test the hypothesis that acute lung injury during cardiopulmonary bypass (CPB) is related to the activation of neutrophils and the body temperature during bypass, we determined the differential WBC count, plasma elastase concentrations, and lung function before, during, and after CPB in 38 patients undergoing elective coronary artery bypass surgery. The patients were randomly assigned to receive either normothermic (n=19, rectal temperature: 35.9±0.1°C, mean±SE) or hypothermic (n=19, 29.2±0.5°C) CPB. The cellular response to the extracorporeal circulation was significantly delayed in the hypothermic group with a later onset of neutrophilia and a later increase in plasma elastase levels during bypass. Lung function deteriorated significantly after CPB as assessed by respiratory index, alveolar-arterial oxygen gradient, and intrapulmonary shunt, independent of bypass temperature. There was a positive correlation between peak elastase concentrations and postoperative respiratory index as well as intrapulmonary shunt (R²=0.5, p=0.002 and R²=0.45, p=0.003, respectively). Besides peak plasma elastase levels, multiple regression revealed no significant influence of other independent factors on postoperative lung dysfunction in our patients.

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**Key words:** cardiopulmonary bypass; leukocyte activation; lung injury

Cardiopulmonary bypass (CPB), essential to most cardiac operations, is suspected to cause a systemic inflammatory response partly induced by the contact of blood with nonphysiologic surfaces. This response includes endothelial injury and increased microvascular permeability that may result in pulmonary dysfunction with a variable degree of clinical expression.1-3 CPB is a recognized cause of ARDS.4 As in ARDS,5,6 there is increasing evidence that supports the hypothesis that lung injury during CPB follows an accumulation of activated neutrophils in the pulmonary circulation with subsequent release of toxic substances, leading to tissue injury.7,8 Neutrophil elastase, stored in the azurophilic granules of polymorphonuclear leukocytes and released after cellular activation,9 is a potent protease, able of attacking even intact cells.10,11 Elevated levels of elastase are found in plasma of patients after CPB12-15 and in plasma and BAL fluid of patients with ARDS.16,17 The aim of this study was to evaluate a relationship between neutrophil activation and pulmonary dysfunction after CPB and to assess the potential influence of CPB temperature on cellular activation.

**METHODS**

The investigation was approved by the institutional ethical committee. Informed consent was obtained from each patient.

**Patients**

Thirty-eight patients undergoing elective coronary artery bypass grafting were enrolled in the study. Only patients who were scheduled for first operations of the day were included. Patients with an ejection fraction below 35%, age older than 70 years, prior cardiac procedures, cardiac valvular disease, or impaired lung function (FVC [percent of normal]+FEV₁ [percent of FEV₁]<150) were excluded from the study. Nineteen patients were randomly assigned for normothermic CPB (warm group), 19 for hypothermic CPB (cold group). The preoperative clinical profiles of the patients are summarized in Table 1. After premedication with flunitrazepam (1 to 2 mg), anesthesia was induced and maintained with flunitrazepam and fentanyl as required. Muscle relaxation was achieved with pancuronium bromide.

**Operative Technique**

After median sternotomy and heparinization (300 IU/kg of body weight), standard cannulation techniques through the ascending aorta and right atrium were used to complete CPB circuit. CPB was performed with disposable membrane oxygenators and primed with Ringer's lactate, 5,000 IU/L of heparin, and 2 million IU of aprotinin (Bayer; Leverkusen, Germany). After institution of CPB, pa
Table 1—Patient Characteristics and Perioperative Data*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Warm Group (n=19)</th>
<th>Cold Group (n=19)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59±2</td>
<td>62±2</td>
<td>0.50</td>
</tr>
<tr>
<td>Men/women</td>
<td>18/1</td>
<td>17/2</td>
<td>0.54</td>
</tr>
<tr>
<td>History of smoking, No. (%)</td>
<td>15 (79)</td>
<td>16 (84)</td>
<td>0.70</td>
</tr>
<tr>
<td>Hypertension (patient history), No. (%)</td>
<td>11 (58)</td>
<td>10 (53)</td>
<td>0.75</td>
</tr>
<tr>
<td>Diabetes (not insulin dependent), No. (%)</td>
<td>7 (37)</td>
<td>1 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Obesity (&gt;10% normal body weight), No. (%)</td>
<td>12 (63)</td>
<td>8 (42)</td>
<td>0.32</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>64±3</td>
<td>62±2</td>
<td>0.70</td>
</tr>
<tr>
<td>No. of bypass grafts</td>
<td>3.9±0.2</td>
<td>4.1±0.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Use of internal thoracic artery, No. (%)</td>
<td>18 (95)</td>
<td>17 (89)</td>
<td>0.61</td>
</tr>
<tr>
<td>CPB time, min</td>
<td>84±7</td>
<td>94±8</td>
<td>0.29</td>
</tr>
<tr>
<td>Rectal temperature during CPB, °C</td>
<td>35.9±0.1</td>
<td>39.2±0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total volume administered during CPB, L</td>
<td>3.8±0.2</td>
<td>4.0±0.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Total volume administered after CPB, L</td>
<td>2.9±0.3</td>
<td>2.6±0.2</td>
<td>0.35</td>
</tr>
<tr>
<td>Transfused packed RBC volume, mL</td>
<td>566±119</td>
<td>1,050±158</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean postoperative rectal temperature, °C</td>
<td>37.5±0.1</td>
<td>37.0±0.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values are mean±SEM.

Plasma concentrations of elastase (as α1-antiprotease-elastase complex) were determined by enzyme-linked immunosorbent assays (PMN Elastase IMAC; Merck Diagnostica; Darmstadt, Germany).

In all patients, a pulmonary thermoligation catheter was introduced after induction of anesthesia. Serial blood samples for arterial and mixed venous blood gas analysis were obtained from the radial artery and pulmonary artery catheter after induction of anesthesia, after sternal closure, and in 3-h intervals until the first postoperative day.

Alveolar-arterial Po2 gradient, respiratory index (alveolar-arterial Po2 gradient/PaO2), and intrapulmonary shunt (Qsp/Qt) were used as a measure of pulmonary dysfunction. Alveolar-arterial Po2 gradient was calculated using the alveolar gas equation, assuming a respiratory quotient of 0.8 and an alveolar PaO2 equal to arterial Po2. Intrapulmonary shunt was derived from standard shunt equation. Capillary O2 contents were computed by the Kelman routine, assuming full equilibrium between alveolar and capillary Po2.

**Statistics**

All values are reported as mean±SEM and were analyzed by means of a statistical system (StatView 4.0). Differences among groups for continuous data were assessed with unpaired Student's t test or Mann-Whitney test according to distribution. χ2 analysis and Fisher's Exact Test were used for categorical data. The Bonferroni method was used for adjustments of p values obtained from comparison of cell counts and elastase levels at multiple time points. The significance of differences for comparing changes within groups on two occasions only was assessed by Student's t test for paired data. Summary measures (peak values, area under the curve) were used for comparison of elastase values and hemodynamic measurements between groups. Simple and multiple regression analyses were used to define the correlations of variables measured. Differences were considered significant by a probability level of p less than 0.05.

**RESULTS**

Patient demographics and perioperative data are presented in Table 1. There were no significant differences between groups in risk factors for coronary artery disease and risk factors for perioperative morbidity and mortality, with the exception of a lower prevalence of diabetic patients in the cold group. No differences were seen between the two groups in CPB time, number of performed bypass grafts, use of internal thoracic artery, and administration of IV fluid during and after CPB. Because of increased postoperative bleeding in patients after hypothermic CPB, volume of transfused packed RBCs was higher in this group (p=0.02).

Rectal temperature was significantly lower in the cold group during CPB (cold, 29.2±0.5°C; warm, 35.9±0.1°C; p<0.0001). At arrival to the ICU and during the first postoperative hours, rectal temperatures were slightly higher in patients after normothermic CPB with an average difference of 0.5°C.

**Cell Counts**

Leukocyte numbers dropped after the start of CPB in both the cold and warm group to similar levels (Fig 1). During the first 30 min of CPB, there was a signif-
significant increase in leukocyte counts in the warm group (p<0.0001), whereas in cold patients, leukocyte count did not change (p=0.97) (30 min CPB: cold, 3.5±0.4×10^9/L, vs warm, 5.1±0.4×10^9/L; p=0.039). After CPB until the first day, leukocyte numbers were equal in both groups.

The course of changes in neutrophil counts during and after CPB were identical with those of total leukocyte counts (Fig 1). There was an initial drop at the beginning of CPB with a significant increase during CPB in the warm group only (p<0.0001) (30 min CPB: cold, 2.0±0.3×10^9/L, vs warm, 3.4±0.3×10^9/L; p=0.006). After rewarming and weaning from CPB, neutrophil values were equal in both groups.

After induction of CPB, there was an immediate increase in percentage of band forms in warm patients (p=0.0001) whereas in cold patients, the values remained unchanged during hypothermic CPB (p=0.29) (15 min CPB: cold, 11±1%, vs warm, 16±1%; p=0.098; 30 min CPB: cold, 13±2%, vs warm, 20±2%; p=0.062). During rewarming and weaning from CPB, the percentage of band forms increased steeply in cold patients to reach similar levels as in warm patients (post-CPB: cold, 28±2%; and warm, 26±2%; p=0.34).

**Elastase**

Logarithmic transformation of elastase values was used to yield normal distribution of data. In warm patients, plasma elastase levels rose significantly (p<0.0001) in the first 30 min of CPB (Fig 2), whereas in cold patients, elastase values remained almost unchanged during the same period (p=0.12) (log elastase: 30 min CPB: cold, 1.61±0.7, vs warm, 1.87±0.7; p=0.08). Maximum elastase levels were observed at the end of CPB with a constant decrease in elastase concentrations during the first postoperative hours in both groups. Calculation of area under the curve and peak elastase levels revealed no significant differences between groups (log area under the curve: cold, 2.02±0.05, vs warm, 1.99±0.06; p=0.76; log peak levels: cold, 2.27±0.08, vs warm, 2.22±0.07; p=0.69).

**Pulmonary Function**

There was a deterioration of all pulmonary parameters after CPB. Mean alveolar-arterial P02 gradients were significantly increased after CPB (cold, p=0.002; warm, p=0.002, Fig 3) without differences between the cold and warm group. The same held true for mean intrapulmonary shunt (cold, p=0.01; warm, p=0.002). Mean respiratory index increased in both groups after CPB, without reaching significance in the warm group (cold, p=0.008; warm, p=0.07). During the first postoperative hours, alveolar-arterial P02 gradients, intrapulmonary shunt, and respiratory index decreased equally in cold and warm patients. All values reached baseline levels on the first postoperative day, independent of CPB temperature.

The area under the curve for postoperative respiratory index correlated significantly with the logarithm of
peak elastase levels and area under the curve of plasma elastase concentration with $R^2$ values of 0.5 and 0.43, respectively ($p=0.002$ and $p=0.005$, respectively) (Fig 4). Besides peak plasma elastase levels, multiple regression revealed no significant influence of several independent factors such as CPB time, CPB temperature, age, smoking, total amount of infused packed RBCs, and postoperative administered fluids on postoperative respiratory index in our patients (Table 2). In addition, there was a positive correlation between elastase levels and intrapulmonary shunt, too (area under the curve of intrapulmonary shunt vs logarithm of peak elastase levels: $R^2=0.45$, $p=0.003$). Correlations and regressions were accomplished with pooled data from patients who remained intubated until the first postoperative day only ($n=18$: cold, $n=9$: warm, $n=9$).

Patients after normothermic CPB remained intubated for an average of 13.3±1.7 h, patients after hypothermic CPB for 16.4±1.5 h ($p=0.47$).

**DISCUSSION**

CPB results in activation of neutrophils and postoperative pulmonary dysfunction with impaired respiratory index, alveolar-arterial oxygen gradient, and increased intrapulmonary shunt. There was a significant correlation between neutrophil activation and degree of lung dysfunction. Hypothermia during CPB caused a delay in cellular activation but had no influence on total activation of neutrophils and on postoperative pulmonary dysfunction.

Pulmonary dysfunction after surgery involving CPB remains an important clinical problem despite refinements in techniques of extracorporeal circulation and postoperative intensive care. Although changes in lung function after cardiac surgery are well known, their pathogenesis is complex and not completely understood. Known factors that contribute to changes in lung function include the mechanical effects of median sternotomy, hydrostatic pulmonary edema, retention of tracheobronchial secretions, and phrenic nerve dysfunction. There is considerable evidence that cellular factors, such as the activation of leukocytes, may play an important role in the pathogenesis of postbypass lung injury. Several studies have demonstrated activation of leukocytes during CPB with sequestration of neutrophils in the pulmonary circulation causing neutrophil adhesion-related injury of endothelial cells. Leukocyte depletion and inhibition of neutrophil adhesion during CPB resulted in

**Table 2—Multiple Linear Regression Between Postoperative Respiratory Index and Seven Independent Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard Coefficient</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak elastase</td>
<td>0.73</td>
<td>0.03</td>
</tr>
<tr>
<td>Total volume administered after CPB</td>
<td>0.21</td>
<td>0.40</td>
</tr>
<tr>
<td>History of smoking</td>
<td>0.24</td>
<td>0.29</td>
</tr>
<tr>
<td>Rectal temperature during CPB</td>
<td>-0.24</td>
<td>0.36</td>
</tr>
<tr>
<td>CPB time</td>
<td>-0.13</td>
<td>0.63</td>
</tr>
<tr>
<td>Age</td>
<td>0.05</td>
<td>0.85</td>
</tr>
<tr>
<td>Transfused packed RBC volume</td>
<td>0.11</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Pooled data of patients who remained intubated until the first postoperative day, $n=18$. 

**Figure 3.** Respiratory index, alveolar-arterial oxygen gradient (A-a P02), and intrapulmonary shunt before, during, and after normothermic (warm) and hypothermic (cold) CPB. In both groups, lung function deteriorated significantly after CPB, independent of CPB temperature. Values are mean±SEM.

**Figure 4.** Correlation between area under the curve for postoperative respiratory index and logarithm of peak elastase levels.
improved postoperative pulmonary function in experimental animal models. However, none of the previous studies demonstrated a direct relationship between postoperative pulmonary dysfunction and the degree of leukocyte activation during CPB. In addition, to our knowledge, there exist no data about the influence of body temperature during the bypass procedure on the activation of neutrophils. A first clinical study showed some evidence of improved lung function after normothermic CPB.24

Our results demonstrate a clear relationship between CPB temperature and neutrophil kinetics and activation during extracorporeal circulation. After an initial fall in neutrophil count mainly due to hemodilution in both groups, neutrophil values increased continually during normothermic bypass, whereas in hypothermic patients the number of neutrophils remained unchanged until the patients were rewarmed. These results are consistent with an earlier study demonstrating delayed neutrophilia during hypothermic as compared with normothermic CPB.25 Activation of neutrophils, assessed by plasma elastase concentrations, followed a similar pattern as neutrophil counts during CPB. During hypothermic bypass, the decrease of plasma elastase concentrations remained delayed until the patients were rewarmed, whereas in normothermic CPB, elastase levels increased gradually to reach similar values at the end of bypass. Since plasma elastase levels dropped already during the first postoperative hours with neutrophil numbers still increasing during that period, the rise in elastase reflects neutrophil injury or stimulation during bypass and is not a mere expression of neutrophilia.

These results suggest that hypothermia has an inhibitory effect on neutrophil kinetics and activation. This is in concordance with in vitro studies that showed a substantially reduced response of neutrophils to complement activation products and to complement activation itself during hypothermia.26 Active rewarming, however, seems to provoke a burst of cellular activation at the end of bypass. Therefore, hypothermic bypass does not have a protective effect against cellular activation during extracorporeal circulation per se but merely delays activation of neutrophils without influence on total activation.

Similar to ARDS, lung injury after CPB is characterized by an increase in extravascular lung water due to endothelial damage and atelectasis.3,5,20,25,27,28 These alterations result in an impaired gas exchange and increased intrapulmonary shunting after CPB.13,20 To assess postoperative pulmonary dysfunction, we measured respiratory index, alveolararterial oxygen gradient, and intrapulmonary shunt in all patients before and after CPB until the first operative day. Our results demonstrate a marked deterioration of all parameters at the end of operation, independent of bypass temperature. During the postoperative hours, lung function improved gradually and had returned to baseline levels by the first postoperative day. There was a significant correlation between degree of lung injury and activation of neutrophils. Peak elastase concentration and area under the curve of elastase concentration correlated significantly with the postoperative respiratory index and intrapulmonary shunt. The square of the correlation coefficient (R²) resulted between 0.43 and 0.50 indicating that 43 to 50% of the variability of postoperative lung injury can be explained by changes in elastase concentration. This is in accordance with studies showing a relation of elastase levels and lung injury in other pulmonary disease states such as ARDS.16,17 It seems, therefore, that beside poor lung reexpansion and inadequate clearance of secretions, activation of neutrophils might be an important factor leading to postoperative pulmonary dysfunction. Hence, manipulation of neutrophils by inhibition of neutrophil adhesion,23 the removal of leukocytes during CPB,8 or improved biocompatibility of bypass equipment to reduce cellular activation29 may be promising approaches to reduce postoperative lung injury.

REFERENCES
Clinical Investigations
