Complement and neutrophil activation during cardiopulmonary bypass
A randomized comparison of hypothermic and normothermic circulation

Massimo Chelloa,*, Pasquale Mastrorobertoa, Rossana Romanoa, Raimondo ASCIONEb,
Donato Pantaleob, Vincenzo De Amiciss

aDepartment of Cardiac Surgery, Medical School of Catanzaro, Catanzaro, Italy
bDepartment of Cardiac Surgery, Medical School of Naples, Naples, Italy
cDepartment of Clinical Chemistry, Hospital Nuovo Pellegrini, Naples, Italy

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Abstract

Objective: Activation of both complement and neutrophils has been demonstrated to be involved in many pathological reactions following cardiopulmonary bypass (CPB). The aim of the present study is to evaluate the effect of normothermic and hypothermic CPB on both complement and neutrophil activation. Methods: Two groups of patients (n = 20 each) scheduled for elective coronary artery bypass grafting, underwent CPB with intermittent warm or cold blood cardioplegia. Plasma concentration of C3a, C5a and C5b-9, as well as nitro-blu tetrazolium (NBT) scores of circulating neutrophils were measured before anesthesia, 10 and 30 min after the beginning of CPB, and 8, 16 and 24 h, postoperatively. Results: In both groups, CPB determined a significant complement activation, evidenced as a significant increase in plasma concentration of C3a, C5a and C5b-9. This in turn triggered the neutrophil activation, documented as a significant increase of NBT scores in circulating neutrophils at the end of CPB and in the early postoperative period. Interestingly, in the warm group the extent of both complement and neutrophil activation was significantly higher compared with the cold group during the whole sampling period. Conclusion: In conclusion, our study clearly demonstrates that warm CPB is associated with an increased ability to activate complement and neutrophils in patients undergoing coronary surgery. Copyright © 1997 Elsevier Science B.V.

Keywords: Temperature; Cardiopulmonary bypass; Complement; Neutrophils

1. Introduction

Cardiopulmonary bypass (CPB) has been demonstrated to promote significant activation of the human complement cascade that results in the production of both C3a and C5a anaphylatoxins [2,5,14]. Furthermore, CPB associated complement activation, acts as a potent stimulator of polymorphonuclear leukocytes (PMN) suggesting that a complement-derived mediator is responsible for the transient pulmonary vascular PMN sequestration observed in patients who have undergone CPB. [10,21] This sequence of events can be explained by the production of the chemotactic peptide, C5a, which is a cleavage product generated during complement activation. Activated neutrophils in turn, release a number of factors which are potentially toxic and include toxic products of oxygen metabolism as well as proteinases such as neutrophil elastase and metalloproteinase [16], which may contribute to adverse clinical and biochemical changes [11,15].

In addition to initiating neutrophil-endothelial cell interactions, C5a may also contribute to inflammatory
reactions by stimulating the release of other mediators, such as interleukin 1 [7], cationic proteins and leukotrienes [28]. Thus, inflammatory responses begun by complement activation may be prolonged and potentiated by the action of mediators released by C5a.

Activation products from this reaction may be responsible for postoperative organ dysfunction frequently observed after CPB procedures [14]. Because the extent of complement activation during CPB can be correlated to the risk of postoperative morbidity [14], efforts to reduce activation are of potential clinical importance.

This study was designed to compare the effects of normothermic and hypothermic CPB on both complement and neutrophil activation in 40 patients undergoing elective coronary surgery.

2. Materials

Forty surgical patients scheduled to have non-urgent aortocoronary bypass grafting at the Department of Cardiac Surgery of the Medical School of Naples, were randomly assigned by hospital number to one of two groups. All patients had angina on entry and were receiving some combination of nitrate vasodilators, /-blockers and calcium-channel blocking agents. The two groups were similar in mean age, male dominance, preoperative hemodynamic data and number of coronary arteries revascularized (Table 1). Patients had good left ventricular function as judged by preoperative cardiac angiogram (left ventricular ejection fraction > 0.50) and had no evidence of pulmonary disease as judged by chest roentgenogram and lung volume.

The same standard anesthesia was used in all patients. After pre-medication, a Swan-Ganz catheter was positioned into the central pulmonary artery and a radial artery cannula was inserted. Anesthesia was induced with thiopental sodium and muscle relaxation was achieved with pancuronium; analgesia was produced with thiopental sodium and muscle relaxation was sustained by the action of C5a. After cardiac arrest, additional doses of low potassium (26 mEq/l) blood cardioplegia were infused into the aortic root at 200–300 ml/min until diastolic arrest was achieved. In the warm group this initial dose was given at 37°C, whereas in the cold group the initial dose was administered at 5°C.

3. Temperature groups

Blood cardioplegia was prepared by mixing four parts of oxygenated blood with each part of hyperkaliemic crystalloid solution (SIENA 1) and was delivered via a separate circuit consisting of a roller pump (Sarns), a reservoir (Terumo), a heat exchanger/bubble trap (Medtronics), and an in-line ultrasonic flow probe. In all patients, cardiac arrest was achieved by an infusion of a high potassium (26 mEq/l) blood cardioplegia infused into the aortic root at 200–300 ml/min until diastolic arrest was achieved. In the warm group this initial dose was given at 37°C, whereas in the cold group the initial dose was administered at 5°C.

3.1. Warm group

After cardiac arrest, additional doses of low potassium cardioplegia (12 mEq/l) at 37°C were repeated after construction of each distal anastomosis or after 15 min. All distal anastomoses were performed first and proximal anastomoses were performed under partial occlusion. The patient’s body temperature was actively warmed to 37°C during bypass.

3.2. Cold group

Cardioplegia was given as in the warm group, but at a temperature of 5°C. Myocardial septal temperature ranged from 10 to 15°C with this technique. The esophageal temperature was maintained between 25 and 28°C during the aortic cross-clamping period and rewarming to 37°C was begun during construction of the last distal anastomosis. Warm cardioplegic reperfusion before aortic unclamping was never used.

Arterial blood samples were taken before induction of anesthesia, 10 and 30 min after the beginning of CPB, at the end of CPB and 8, 16 and 24 h, postoperatively. The samples were collected in heparinized cooled syringes that were immediately capped and stored in ice until separation and analysis. Blood samples were assayed for oxygen and carbon dioxide tension, pH and oxygen saturation. Neutrophil count was determined on fresh blood by an automated cell counter. The study protocol was approved by the Ethical Committee of the Medical School of Naples. Informed consent was obtained from each patient.

3.3. Assay of complement activation

C3a was measured by means of a radioimmunoassay kit. C5a was measured using a monoclonal anti-C5/C5a antibody reaction as described by Mollnes and co-workers [20] and C5b-9 was measured in serum by an enzyme-linked immunosorbed assay.
3.4. Nitro-blu tetrazolium

One specific form of neutrophil activation is the capacity to reduce nitro-blu tetrazolium (NBT) dye, which is associated with an enhanced generation of superoxide anion radicals. This is the pivotal phenomenon of the respiratory burst after neutrophil activation. The NBT test was performed on fresh blood as described by Neumann et al. [22]. In brief, 0.2 ml heparinized blood were incubated at 37°C for 10 min. Then, 0.2 ml of a 0.1% solution of NBT in phosphate-buffered saline was added. The mixture was incubated at 37°C for 15 min and then kept at room temperature (21–25°C) for another 15 min. Smears were prepared by allowing a drop of the NBT and blood mixture to rapidly run down a pre-cleaned slide. After air drying, the smears were stained by Wright’s stain. On each slide, 100 neutrophils were counted. The percentage of neutrophils containing formazan deposits of at least the size of a lobe of the nucleus was designed as positive NBT score.

3.5. Statistical methods

Data are expressed as mean ± S.E.M. A repeated measures analysis of variance followed by Scheffé’s multiple comparison analysis was used to test for significant changes over the time course within groups. Unpaired Student’s t-test was used to compare intergroups means. Linear regression analysis was used to test relations between independent variables. A P value of less than 0.05 was considered significant.

4. Results

4.1. Operative data

There was no operative death. No patient sustained a Q wave myocardial infarction or subendocardial myocardial infarction. No patient required intra-aortic balloon counterpulsation during the postoperative period. There were no significant hemodynamic differences between the two groups (Table 2). During the first postoperative hours, systemic vascular resistance was significantly lower in the warm group (warm, 954 ± 53.25 vs. cold, 1186 ± 64.24 dyne s/cm², P < 0.01) with similar values on the morning of the first postoperative day. Mean infusion rate of vasodilators during the same period were equal. Two patients in the cold group and three in the warm group required dopamine infusion for low cardiac output syndrome (defined as requirement for inotropic support because of a CI of less than 2.1 l/min per m² and a systolic blood pressure <90 mmHg despite optimization of preload and afterload and correction of any electrolyte disorder). CI was significantly higher in patients after normothermic CPB as compared with patients after hypothermic CPB (3.6 ± 0.06 vs. 3.1 ± 0.07 l/min per m², P < 0.01), without significant difference on the first postoperative day. During the stay in ICU, heart rate and main arterial blood pressure were similar in both groups. There were no differences in regard to total volume administered, mean urinary output, need for diuretics or total fluid balance during the postoperative period. Also, there were no differences with regard to time to extubation (cold group, 8.4 ± 0.4 h; warm group, 7.9 ± 0.5 h; P = NS) and postoperative stay in ICU.

4.2. C3a

The C3a level at different sampling points is shown in Fig. 1. It is seen that soon after the beginning of CPB the C3a level rapidly rose in both groups, such that by

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cold group</th>
<th>Warm group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI (l/min per m²)</td>
<td>3.1 ± 0.07</td>
<td>3.6 ± 0.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systolic PAP (mmHg)</td>
<td>42 ± 5</td>
<td>39 ± 4.4</td>
<td>NS</td>
</tr>
<tr>
<td>MLAP (mmHg)</td>
<td>14.1 ± 0.7</td>
<td>15.4 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>SVR (dyne s/cm²)</td>
<td>1186 ± 64.2</td>
<td>954 ± 53.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVSWI (gm/m²)</td>
<td>4 h, 31.5 ± 3</td>
<td>32.5 ± 3.7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12 h, 28 ± 3.7</td>
<td>31.2 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>24 h, 30.5 ± 3.1</td>
<td>33.1 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>86 ± 6.2</td>
<td>82 ± 8.3</td>
<td>NS</td>
</tr>
<tr>
<td>LOS (No.)</td>
<td>2</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Dopamine (µg/kg per min)</td>
<td>4 ± 0.5</td>
<td>3.7 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

CI, cardiac index; PAP, pulmonary artery pressure; MLAP, mean left atrial pressure; SVR, systemic vascular resistance; LVSWI, left ventricular stroke work index; HR, heart rate; LOS, low output state.
10 min, the plasma concentration of C3a was $415 \pm 35.8$ ng/ml ($P < 0.05$ vs. baseline) and $582 \pm 38.6$ ng/ml ($P < 0.01$ vs. baseline) respectively in the cold and warm groups. Thereafter, C3a concentration further increased in a linear fashion, reaching a peak level at the end of CPB (cold group, $1036.8 \pm 71.2$ ng/ml; $P < 0.01$ vs baseline; warm group, $1527 \pm 104.7$ ng/ml; $P < 0.01$ vs. baseline). In the cold group, a linear reduction of C3a plasma concentration was observed during the postoperative period, with return toward the baseline values 24 h postoperatively. In the warm group, plasma levels of C3a showed a similar postoperative pattern but smaller in magnitude, and at 24 h postoperatively the C3a level was still significantly higher than the baseline value ($538.5 \pm 40$ ng/ml, $P < 0.01$ vs. baseline). Of interest is that, after the beginning of CPB and through the whole sampling period, the plasma concentration of C3a was significantly greater in the warm group compared with the cold group.

4.3. C5a

The C5a level at different sampling points is shown in Fig. 2. A significant increase of plasma C5a value was noted in both groups 30 min after the beginning of CPB. Thereafter, C5a plasma concentrations increased in a linear fashion until 24 h postoperatively, the point at which peak values were observed in both cardioplegic groups (cold, $9.5 \pm 0.5$ ng/ml; $P < 0.01$ vs. baseline; warm, $14.5 \pm 1$ ng/ml; $P < 0.01$ vs. baseline). Interestingly, from the end of CPB through 24 h postoperatively, plasma values of C5a were significantly higher ($P < 0.01$) in the warm group compared with the cold group.

4.4. C5b-9

The C5b-9 levels at different sampling point is shown in Fig. 3. In both groups, the pattern of C5b-9 during the whole sampling time strictly resembled that of C3a. Serum concentrations of C5b-9 increased significantly 10 min after the beginning of CPB, therefore increasing in a linear fashion to reach the peak at the end of CPB (cold, $811.3 \pm 103$ ng/ml; $P < 0.01$ vs. baseline; warm, $1098 \pm 113.4$ ng/ml; $P < 0.01$ vs. baseline). In the cold group, a linear reduction of C5b-9 serum concentrations was observed during the postoperative period, with return to the baseline values 24 h postoperatively. Serum levels of C5b-9 showed a similar postoperative pattern in the warm group but lesser in magnitude and at 24 h postoperatively the C5b-9 level was still higher than the baseline value, though the difference was not significant ($378 \pm 46.2$ vs. $200 \pm 19.6$ ng/ml). Of interest is that, after the beginning of CPB and through the whole sampling period, the serum concentration of C5b-9 was significantly greater in the warm group compared with the cold group.
Fig. 4. Neutrophil count in arterial samples (during and after CPB) for a patient in the two cardioplegia groups. Each symbol represents mean ± S.E.M. Abbreviations as in Fig. 1.

4.5. Neutrophils

The neutrophil count at different sampling point is shown in Fig. 4. In both groups, initiation of CPB resulted in a rapid but not significant decline of neutrophils compared with the basal value (cold, $3.8 ± 0.16 \times 10^3$ cells/mm$^3$; warm, $3.7 ± 0.2 \times 10^3$ cells/mm$^3$) such that by 10 min, the neutrophil count in the circulation was 86 and 90% of its basal value in the warm and cold groups, respectively. Thereafter, the neutrophil count increased and 30 min after initiation of CPB it returned to approximately baseline level, a linear increase was then observed in both cardioplegic groups after the discontinuation of CPB through 24 h postoperatively, with peak values at 24 h postoperatively (cold group, $10.6 ± 0.46 \times 10^3$ cells/mm$^3$; warm group, $11.6 ± 0.5 \times 10^3$ cells/mm$^3$).

4.6. NBT

The values of NBT scores are shown in Fig. 5. The maximum NBT score was recorded in both groups at the end of CPB (cold, $13.1 ± 1.3\%$; $P < 0.01$ vs. baseline; warm, $18.1 ± 1.5\%$; $P < 0.01$ vs. baseline). However, in the cold group, values of NBT scores decreased in a linear fashion during the postoperative period, returning toward baseline values 24 h postoperatively. In the warm group, NBT scores were still high 8 h postoperatively, and although tending downward 24 h, postoperatively they were still significantly higher compared with baseline values ($10.7 ± 0.7\%$, $P < 0.01$ vs. baseline). Interestingly, during the whole sampling period, the values of NBT score were significantly higher ($P < 0.01$) in the warm group compared with the cold group.

When examined by linear regression analysis, no significant correlation was found between NBT score and C3a plasma concentration, whereas significant positive correlation were found between both C5b-9 and NTB scores ($r = 0.5$, $P < 0.01$) and C5a levels and NTB scores ($r = 0.68$, $P < 0.01$).

4.7. Discussion

Over the last decade, there has been a significant increase in the number of risk factors in patients with coronary artery disease referred for coronary artery bypass grafting, including advancing patient age, worsening ventricular condition, the increasing frequency of comorbid diseases. This change in patient demographics parallels to the increasingly aggressive attempt to provide maximal myocardial protection to resuscitate the ischemic myocardium. The systemic inflammatory response that occurs to some extent in all subjects after CPB remains a major cause of morbidity and mortality, especially in infants and children and although technical progress has improved the safety of cardiac operations, postoperative dysfunction of lung and other organs still occurs frequently [25]. A large body of evidence suggests that the extent of complement activation is correlated with the postoperative development of cardiac, renal and pulmonary dysfunction, as well as abnormal bleeding and pulmonary insufficiency [14,24].

Our present study confirmed the presence of both complement and PMN activation during CPB. This was evident as the generation and accumulation of activated complement components in plasma and the detection of NBT positive granules in circulating neutrophils. Moreover, our current investigation clearly demonstrates that normothermic CPB is associated with an increased activation of complement compared with hypothermic CPB; this in turn is capable of inducing a more accentuated neutrophil activation response.

To the best of our knowledge, this is the first in vivo demonstration of a temperature dependent complement activation during CPB and it is in agreement with the results of previous in vitro studies. Moore and colleagues [21] demonstrated that exposure of normal neutrophils to C5a in vitro caused an increase in C3b receptors in circulating neutrophils which was depen-
dent on temperature, is evident from a nine fold increase of specific neutrophil fluorescence from 25 to 37°C. Generation of C3a and C5a in normal serum by zymosan was also temperature dependent, C5a increased more than 1000 folds from 25 to 37°C and C3a production also showed a 120 fold increase from 25 to 37°C.

Furthermore, the results of this study confirm the important relationship between complement and neutrophil activation during CPB. Although no correlation was found between the formation of C3a and the NTB scores, a significant correlation was found between either the formation of C5b-9 or C5a and NTB scores. This is in agreement with the results of several studies demonstrating the important role that both C5b-9 and C5a play in the activation of circulating neutrophils. Nonlethal amounts of C5b-9 that are deposited on cell membranes at sites of complement activation have been shown to stimulate reactive oxygen metabolite production by neutrophils [9]. On the other hand, C5a is the most potent granulocyte activator in the complement cascade; nevertheless, in many studies, the pattern of C5a production during CPB appears still controversial and many authors failed to detect significant increase of C5a either during CPB or early after CPB discontinuation [8-13,27]. This difficulty may in part be due to the rapid and irreversible binding of C5adesArg to specific receptors on neutrophils and monocytes within minutes after formation [6] and its short half-life of less than 2 min in the circulation [29], that would preclude its detection at times other than the maximum complement activation and associated maximum neutropenia. In the current study we used a more sensitive assay, based on the method described by Mollnes and co-workers [20] to detect plasma levels of C5a. Our results are congruent with those of these authors, who reported significant elevation of C5a plasma levels 30 min after the start of CPB and during the whole observation period of 48 h.

Although a correlation between complement and PMN activations has been demonstrated during CPB, several other inflammatory mediators, in addition to C5a and C5b-9, have been shown to contribute to neutrophil activation. For example CPB, either directly or through activated complement products [1,4,23], stimulates the release of a variety of biologically active cytokines such as IL-1 and IL-8 [12] as well as the tumor necrosis factor [4] which has been proposed to mediate many of the responses associated with CPB [2]. According to this consideration, our data are consistent with the results of several recent articles that documented an increased release of cytokines in patients undergoing normothermic CPB and the significance of cytokines in the pathogenesis of multisystem organ failure [3].

Menasché and co-workers [17] demonstrated that the release of IL-1ra and elastase was consistently higher in patients undergoing warm CPB than in those who received conventional hypothermic bypass. Moreover, the same authors [19] also reported, in two groups of patients undergoing normothermic (33.5–37°C) or hypothermic (28°C) CPB, a greater production in the warm group of interleukin-1 receptor antagonist, soluble intercellular adhesion molecule 1 and elastase, which are markers of cytokine production, cytokine upregulated endothelial ligands for neutrophil adhesion molecules and degranulation secondary to adhesion of neutrophils to endothelial cells, respectively. Heffner-Cavaillon and co-workers [8] also demonstrated that IL-1 production by activated monocytes drastically decreased between 37 and 26°C.

Finally, our study failed to demonstrate, in both cardioplegic groups, the neutropenia probably due to pulmonary sequestration, previously demonstrated in other studies immediately after CPB [10]. In our study, an immediate fall in leukocyte number occurred at the onset of CPB, probably due to hemodilution or adhesion of neutrophils to the absorbed protein layer in the extracorporeal circuit [18,26]. A later leukocytosis was observed 12 and 24 h postoperatively, due to mobilisation of margined cells and release of immature neutrophils from bone marrow. These results are in agreement with those of Butler and co-workers [1], who proposed as a possible explanation for these different results the considerable shorter duration of CPB of his study compared with those in previous reports. Significant correlation between the extent of pulmonary vascular leukosequestration, the duration of aortic cross-clamping and the duration of CPB has been demonstrated [5] and pulmonary histological changes increased with extended CPB, particularly when in excess of 150 min [24].

In conclusion, our experimental results clearly demonstrate that different CPB temperatures show significant differences in their ability to activate both complement and neutrophils. Although a significant correlation between activation of either complement or neutrophils and hemodynamic variables or pulmonary function was not demonstrated in this study, it is important to consider that this was a relatively low risk population and none of the 40 patients had any adverse effects during or after their myocardial revascularization procedures. It is therefore important to further analyse complement and neutrophil activation in higher risk patients, particularly in those in whom hemodynamic instability and multiorgan failure are likely to develop after CPB [13].
References


