Evaluation of Cytokine Levels and Pulmonary Function in Patients Undergoing Coronary Artery Bypass Graft

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Background and objectives: Systemic inflammatory response syndrome is commonly observed in coronary artery bypass grafts (CABG) with cardiopulmonary bypass (CB). The objective of this study was to evaluate the systemic and pulmonary levels of cytokines and their correlation with lung function in patients undergoing myocardial revascularization (MR) with CB.

Methods: This study was approved by the Institutional Ethics Committee, and 13 patients undergoing MR with CB were evaluated. After anesthetic induction and at the end of CB, plasma and bronchoalveolar lavage levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α were determined. The duration of CB and surgery, PaO2/FiO2 ratio, alveolar-arterial oxygen gradient (A-a gradient), shunt, and lung compliance were evaluated. Results were submitted to analysis of variance for repeated measurements (*p < 0.05) and Spearman’s correlation coefficient.

Results: We observed increased levels of cytokines in plasma and bronchoalveolar lavage after CB and a direct relationship between the increase in IL-1β and decrease in lung compliance (p = 0.0439), as well as the inverse relationship between the increase in IL-10 and a decrease in compliance (p = 0.0326). The increase in IL-6 was directly related to the duration of CB (p = 0.012), while the increase in IL-8 was directly related to the duration of surgery (p < 0.0001). Levels of interleukin-1β, IL-8, and TNF-α in bronchoalveolar lavage were higher than in plasma.

Conclusions: There is an increase in cytokine levels in plasma and bronchoalveolar lavage after CB, as well as a correlation between increased cytokine levels and CB duration and surgery and changes in lung compliance.

Keywords: Cytokines; Myocardial Revascularization; Extracorporeal Circulation; Systemic Inflammatory Response Syndrome; Respiratory Function Tests.

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INTRODUCTION

Patients undergoing surgical treatment for coronary disease by myocardial revascularization (MR) with cardiopulmonary bypass (CB) invariably develop an inflammatory process of varying severity that may compromise postoperative evolution 1. According to Kollef et al. 2, the incidence of multiple organ dysfunction syndrome (MODS) in MR with CB may reach 11%, and this group presents a mortality rate of about 41%.

Inflammation may be understood as a protective response to eliminate the initial cause of cellular injury (bacteria, toxins, trauma, etc.), as well as the main consequence of such injury: cellular and tissue necrosis 3. The inflammatory response consists of a systemic process that develops even in the absence of infection, and is best known as systemic inflammatory response syndrome (SIRS) 4. Due to its multifactorial character, some prefer to use the terminology PIRO (predisposition, insult or infection, response, organ dysfunction) instead of SIRS 5. In the absence of an inflammatory process, one would expect the spread of infection, lack of wound healing, and the damaged organ would lose its function permanently; however, depending on the intensity of the inflammatory process, it is potentially harmful 3.

To clinically identify the patient with SIRS, the presence of at least two of the following criteria has been used: tachycardia with HR > 90 bpm, tachypnea with RR > 20 ipm or volume > 10 L.min⁻¹, or PaCO₂ < 32 mmHg, hypothermia or hyperventilation.
hyperthermia (Temp < 35.5°C or > 38°C), and leukocytosis or leucopenia (WBC > 12,000 or < 4,000.dL⁻¹) 4,6,7.

Systemic inflammatory response syndrome may progress to organ dysfunction, especially with changes in pulmonary function, shock, renal failure, and MODS 4.

Although CB is among the main risk factors for SIRS in coronary artery bypass grafts (CABG), its etiology and clinical importance after CABG are still poorly understood, and the development of a clinical and laboratorial method to quantify the intensity (diagnosis), to predict which organs will be more affected (clinical correlation), and the establishment of the correct treatment are still a challenge 6. The relationship between the severity of SIRS and target organ injury is yet to be established 6. In his study, Brix-Christensen found no relationship between the plasma levels of cytokines and the expression of messenger RNA corresponding to this cytokine in the lungs, kidneys, and heart 9.

In the lungs during CB tissue perfusion is done only by the non-pulsatile flow from bronchial arteries, and after CB the process of ischemia-reperfusion is observed. Those changes in pulmonary physiology trigger the local production of inflammatory mediators characterizing the lung as the main organ responsible for perpetuating this process 10-12, indicating the possibility of correlation between postoperative pulmonary function and MODS in CABG.

The objectives of the present study was to evaluate the changes in blood and bronchoalveolar lavage (BAL) levels of cytokines in patients undergoing myocardial revascularization with CB and the correlation with the duration of CB and changes in pulmonary function observed in the postoperative period.

METHOD

After approval of the study by the Institutional Ethics Committee and signing of the informed consent, 13 patients who were scheduled for elective myocardial revascularization were enrolled. Patients with a recent history of smoking (abstinence period lower than six weeks), chronic obstructive pulmonary disease (COPD), pulmonary infection or neutropenia (WBC > 12,000 or < 4,000.dL⁻¹), liver failure, presence of radiologic pulmonary changes, and obesity (BMI ≥ 35), were excluded. Patients who took steroidal anti-inflammatory drugs in the last 30 days prior to surgery, patients classified as ASA ≥ P4, or with moderate or higher risk for surgery, according to Higgins et al. 13, were also excluded. Surgery without CB was another exclusion criterion.

Patients were fasted for at least 8 hours. Oral midazolam, 0.1 to 0.3 mg.kg⁻¹ (maximum of 15 mg), was given 30 minutes before surgery. Upon admission to the operating room patients were monitored with pulse oximeter and continuous 5-lead electrocardiogram evaluating the derivations D₃ and V₅. After local anesthesia of the vascular puncture sites, peripheral venipuncture was performed with a 16G or 14G catheter, and percutaneous radial artery puncture was performed with a 20G catheter for monitoring of invasive blood pressure. Patients received 1 g of intravenous methylprednisolone. All patients underwent the same anesthetic technique and after pre-oxygenation for 3 minutes general anesthesia was induced with midazolam 0.1 to 0.3 mg.kg⁻¹, sufentanil 0.1 to 0.5 µg.kg⁻¹, and etomidate 0.15 to 0.30 mg.kg⁻¹. Atracurium 0.5 mg.kg⁻¹ was used for muscle relaxation. Patients were then ventilated with a face mask with 100% O₂ and after complete effect of the neuromuscular blocker, tracheal intubation was performed with an ET tube of adequate caliber. After lung auscultation and P₆CO₂ monitoring by the sidestream method, controlled mechanical ventilation cycled by volume was instituted (Cicero, Drager, Germany) with a volume of 6 to 8 mL.kg⁻¹, respiratory rate 12 bpm (later guided by P₆CO₂), limited to a pressure of 25 cmH₂O, flow of 2 L.min⁻¹, fE = 1.2, FiO₂ of 50% (oxygen and compressed air), and PEEP of 5 cmH₂O.

After tracheal intubation, the right internal jugular vein was punctured and the central venous catheter was introduced. After fixing the central venous catheter, the monitoring process also included diuresis and nasopharyngeal temperature. Anesthesia was maintained with fractioned doses of sufentanil, 10 µg every 30 minutes, associated with isoflurane 0.5 to 1.0 MAC (expired fraction monitored by the respirator Cicero, Drager, Germany). During CB, patients were maintained unconscious with target-controlled infusion of propofol in order to maintain a target-concentration of 1.0 to 2.5 µg.ml⁻¹. Warmed Ringer’s lactate was used for hydration.

After full anti-coagulation with heparin, patients were placed in CB with membrane oxygenator (Braile, São José do Rio Preto, Brazil) with non-pulsatile flow. The initial CB flow was obtained by calculating 2.2 times the body surface and, afterwards, titrated to maintain a blood pressure of at least 60 mmHg, Ringer’s lactate 1,500 mL, mannitol 250 mL, and heparin 10,000 units were used as perfusate. The duration of CB was evaluated and, at the end of the surgery, variable doses of vasodilators and/or inotropics were introduced according to clinical indication.

Two samples of BAL were collected from each patient, all performed by the same anesthesiologist. The first one was collected immediately after tracheal intubation (Pre-CB) and the second at the end of the procedure immediately after reversion of anticoagulation with protamine (Post-CB). The device was introduced in the middle lobe or lingula of the left lung due to the higher percentage recovery of lavage in these regions 14. Through the orotracheal tube, after applying 10% spray of lidocaine three times, the fiberoptic bronchoscope (Pentax- FB-15bs) with 4.8 mm diameter and 2 mm canal was introduced. During the procedure, patients were ventilated with 100% oxygen. Sixty to 100 mL of 0.9% saline warmed to 37°C and divided in 20 mL aliquots were used. After infusion of 60 mL saline solution through the canal of fiberoptic bronchoscope, it was aspirated manually using a syringe after respiratory incursions from the ventilator. If the recovered volume was enough, the remaining aliquots (40 mL) were not infused. Samples were stored in polyethylene tubes, to avoid macrophage adherence to the glass, at 5°C until the second sample collection (mean time 120 minutes). After collecting
samples, the tubes were sent for laboratorial processing. At the time of BAL collection, blood samples were also collected through the arterial catheter to determine the plasma levels of cytokines. Samples were stored at 5°C until the end of surgery and subsequently sent to the clinical laboratory. The material was centrifuged at 3,000 rpm for 10 minutes at a temperature of 10°C. The supernatant (BAL) was pipetted. Aliquots were stored at -25°C to be analyzed later. After all materials were collected, they were thawed in room temperature. To determine the levels of cytokines, a semi-automated and immunometric system using specific antibodies and chemiluminescent enzyme (IMMULITE; DPC-Medlab, Las Angeles, CA) was used.

Blood samples for levels of hemoglobin, hematocrit, arterial and venous blood gases were collected after anesthetic induction, at the end of surgery, one hour after the end of surgery, three and six hours after surgery, and in the first postoperative day. The results were used to calculate the following parameters:

- The relationship between the partial pressure of oxygen and inspired fraction of oxygen; obtained directly by the relationship PaO2/FiO2, considering normal values above 200.
- Alveolar-arterial gradient of oxygen (A-aO2): calculated by the difference between the alveolar oxygen pressure and arterial oxygen pressure. The formula PAO2 = [(BP - PH2O) x FiO2] – PCO2, in which PAO2 = alveolar oxygen pressure, PaO2 = arterial oxygen pressure, BP = barometric pressure, PH2O = water vapor pressure, FiO2 = inspired oxygen pressure, and PaCO2 = arterial CO2 pressure was used to calculate the alveolar oxygen pressure (PAO2). As normal levels for A-aO2, we considered the values of 10 to 15 mmHg for a FiO2 of 21% and 10 to 65 mmHg for a FiO2 of 100%.
- Pulmonary shunt: Shunt was calculated using the formula (CcO2 - CaO2)/CcO2, in which CcO2 represents capillary oxygen content, CaO2 arterial oxygen content, and CvO2 venous oxygen content. The capillary oxygen content was calculated by the following formula [(Hb x 1.34) + (PaO2 x 0.0031)], in which Hb is the hemoglobin level (g.dL-1) and PaO2 is the alveolar oxygen pressure. The arterial oxygen content (CaO2) was calculated with the following formula: [(1.34 x Hb x SaO2/100) + (PaO2 x 0.0031)], in which SaO2 represents the arterial oxygen saturation and PaO2 the arterial oxygen pressure. The venous oxygen content (CvO2) was calculated with the following formula: [(1.34 x Hb x SvO2/100) + (PvO2 x 0.0031)], in which SvO2 represents the venous oxygen saturation and PvO2 the venous oxygen pressure. Pulmonary shunts of 3% to 5% were considered normal.

Dynamic lung compliance (tidal volume/peak pressure) was also investigated. This parameter was evaluated in the beginning and at the end of surgery, and 1 hour and 3 hours after surgery. Variations in cytokine levels (V%/IL) were calculated as [100 x (post-CB IL - pre-CB IL)/pre-CB IL], in which positive values indicate an increase in cytokine levels and negative values indicate a decrease in cytokine levels both from the initial to the final moment. To analyze the relationship between cytokine levels and pulmonary function parameters, only the plasma levels and variations between the beginning and end of surgery were considered, and the values of those variables were analyzed in modules. To evaluate the results normally, we used the Shapiro-Wilk test, with logarithmic transformation of the variable whenever necessary. Spearman’s correlation coefficient was used to measure the association between cytokine levels and the study parameters. To compare the levels of cytokines in plasma and BAL samples and moments of collection (before and after CB), we used analysis of variance for repeated measurements. A level of p < 0.05 was considered alpha error.

RESULTS

Among the patients enrolled in this study, five were female and eight male. Regarding the functional classification of patients, according to the New York Heart Association, 11 were classified as class 2, while the remaining were classified as class 3; and according to the Higgins surgical risk classification, eight were classified as minimal risk, and five as low surgical risk. Descriptive data regarding age, body mass index (BMI), and duration of CB and surgery are shown in Table I.

Regarding the analysis of bronchoalveolar lavage, the first sample was collected 35.00 ± 13.84 minutes after intubation (mean ± SD), and the mean volume infused was 67.69 ± 17.39 mL with 29.77% recovery of total volume. The second sample was collected 43.23 ± 22.58 minutes after the end of CB, and 69.23 ± 19.35 mL were infused and 25.1% were recovered. Plasma and BAL levels of cytokines are presented in Table II. Data regarding blood oxygenation and lung compliance are presented in Table III; non-measured levels of compliance correspond to the moments patients were extubated.

Table IV shows the Spearman’s correlation coefficient for variations in plasma cytokine levels and pulmonary function parameters at the beginning and end of surgery. We observed positive correlations between the variations in IL-1β and IL-10 and the variation in lung compliance. The increase

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.46 ± 5.36</td>
<td>55.00</td>
<td>46.00</td>
<td>66.00</td>
</tr>
<tr>
<td>BMI (kg.m-2)</td>
<td>27.33 ± 2.81</td>
<td>28.20</td>
<td>20.90</td>
<td>30.70</td>
</tr>
<tr>
<td>Duration of CB (min)</td>
<td>90.46 ± 41.84</td>
<td>80</td>
<td>45</td>
<td>201</td>
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</tbody>
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SD: standard deviation.
Table II – Levels (mean ± SD) of Cytokines in Plasma and Bronchoalveolar Lavage (pg.mL⁻¹)

<table>
<thead>
<tr>
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<th>Pre-CB</th>
<th>Post-CB</th>
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<tbody>
<tr>
<td>Log IL-1β</td>
<td>-0.73 ± 0.84</td>
<td>-0.35 ± 0.87</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.32 ± 1.29</td>
<td>1.01 ± 1.48</td>
</tr>
<tr>
<td>BAL</td>
<td>0.36 ± 1.30</td>
<td>54.71 ± 67.38</td>
</tr>
<tr>
<td>BAL</td>
<td>0.68 ± 1.43</td>
<td>3.22 ± 9.00</td>
</tr>
<tr>
<td>Log IL-8</td>
<td>1.88 ± 0.35</td>
<td>3.08 ± 0.98</td>
</tr>
<tr>
<td>Plasma</td>
<td>2.37 ± 2.22</td>
<td>3.85 ± 1.60</td>
</tr>
<tr>
<td>BAL</td>
<td>2.64 ± 1.99</td>
<td>1491.25 ± 963.60</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.58 ± 0.54</td>
<td>2.40 ± 0.62</td>
</tr>
<tr>
<td>Bal</td>
<td>5.76 ± 2.56</td>
<td>14.65 ± 13.75</td>
</tr>
<tr>
<td>BAL</td>
<td>17.12 ± 1.40</td>
<td>17.10 ± 1.49</td>
</tr>
</tbody>
</table>

in IL-1β showed correlation with a decrease in lung compliance (p = 0.044 and rho = 0.589), and the higher the increase in IL-10, the lower is the decrease in lung compliance (p = 0.032 and rho = -0.593).

Table V shows the Spearman’s correlation coefficient between variations in plasma levels of cytokines and duration of CB and surgery. The correlation between the duration of CB and the absolute variation in IL-6 levels, i.e., the greater the duration of CB the greater the absolute variation in IL-6 levels (p = 0.012 and rho = 0.671), was identified as being important. It was observed an important percentage variation of IL-8, which showed a significant correlation with the duration of surgery, and the longer the surgery, the higher the variation in IL-8 (p < 0.0001 and rho = 0.895).

Table IV – Spearman’s Correlation Coefficient between the Variations in Plasma Levels of Cytokines and Parameters of Lung Function at the Beginning and End of Surgery (rho/p)

<table>
<thead>
<tr>
<th></th>
<th>PaO2 / FiO2</th>
<th>A-a O2 (mmHg)</th>
<th>Shunt (%)</th>
<th>Compliance (mL / cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning of surgery</td>
<td>334.62 ± 85.05</td>
<td>265.92 ± 82.66</td>
<td>16.69 ± 6.08</td>
<td>38.65 ± 10.04</td>
</tr>
<tr>
<td>End of surgery</td>
<td>193.77 ± 64.96</td>
<td>398.15 ± 70.43</td>
<td>32.53 ± 7.19</td>
<td>31.85 ± 6.94</td>
</tr>
<tr>
<td>1 hour after surgery</td>
<td>212.15 ± 67.81</td>
<td>379.77 ± 72.66</td>
<td>21.55 ± 6.28</td>
<td>31.38 ± 5.12</td>
</tr>
<tr>
<td>3 hours after surgery</td>
<td>240.23 ± 84.40</td>
<td>181.15 ± 177.27</td>
<td>18.34 ± 8.74</td>
<td>30.75 ± 5.83</td>
</tr>
<tr>
<td>6 hours after surgery</td>
<td>251.31 ± 79.68</td>
<td>122.46 ± 45.50</td>
<td>15.37 ± 7.64</td>
<td></td>
</tr>
<tr>
<td>24 hours after surgery</td>
<td>212.85 ± 53.73</td>
<td>136.38 ± 37.70</td>
<td>19.67 ± 6.69</td>
<td></td>
</tr>
</tbody>
</table>

The value of p refers to the changes in means along the evaluations.
The direct causal relationship between this inflammatory response to CABG and the clinical postoperative outcome is not well defined, and therapeutic interventions will not be completely justified in the absence of a clear cause-effect relationship. On the other hand, the increase in airway resistance after CB, similar to what was observed in this study, has been well demonstrated. The increased cellularity observed in the bronchoalveolar lavage of patients undergoing CB may be related to the inflammatory response.

Some studies have demonstrated that the increase in cytokine levels may occur in 5 minutes to 2 hours after CB. In the present study, the second sample collection of post-CB plasma and BAL, usually less than 2 hours after the first one, could not have detected the peak in the increase in cytokine levels in some cases.

The use of corticosteroids in patients undergoing CABG can alter expected concentrations of proinflammatory and anti-inflammatory cytokines. Although the indication of corticosteroids has not been established, its use can minimize postoperative changes in lung function. It has been demonstrated that methylprednisolone can reduce the production of IL-6 and increase the production of IL-10, although it does not affect the duration of mechanical ventilation or length of hospitalization after CABG. In the present study, patients received steroid after anesthetic induction and we observed a significant increase in levels of IL-10 at the end of surgery, besides the negative correlation between the levels of this interleukin and reduction of lung compliance. Corticotherapy may have contributed to the absence of significant increase in post-CB IL-1 and TNF levels. Those results might suggest a beneficial effect of corticotherapy on pulmonary function.

Besides corticosteroids, other immunomodulatory drugs such as endotoxins, anticytokine antibodies, and cytokine receptor agonists have been proposed to inhibit the inflammatory response. The use of specific monoclonal antibodies to block the effects of proinflammatory cytokines, such as TNF, can also minimize the myocardial depressive action of these substances.

The presence of genetic polymorphism that determines different levels of cytokine production after a triggering event represents a limitation of the present study. The polymorphism of IL-10 gene can lead to a lower release of this interleukin after CB. In other cases, an increase in systemic inflammatory response may be observed. The size of the study population did not take into account the presence of this polymorphism, and this factor may partially explain the high variability observed in cytokine levels. In the present study, patients who required transfusion of packed-red blood cells were not excluded, and it was demonstrated that allogeneic transfusion of non-deleukocyted blood leads to an increase in cytokine levels.

Considering the objectives of this study, we can conclude that myocardial revascularization with CB causes increased levels of cytokine in plasma and bronchoalveolar lavage and that there is a correlation between the increased cytokine levels and a decrease in lung compliance, and between the increase in cytokine levels and the duration of extracorporeal circulation and surgery.
REFERÊNCIAS / REFERENCES


