Procalcitonin does discriminate between sepsis and systemic inflammatory response syndrome

Ronaldo Arkader¹, Eduardo Juan Troster¹, Marcel Rezende Lopes¹, Roberto Raiz Júnior¹, Joseph A Carcillo², Claudio Leone³, Thelma Suely Okay¹

¹University of São Paulo, Brazil
²University of Pittsburgh School of Medicine
³Hospital das Clínicas

Corresponding author:
Thelma Suely Okay
Laboratório de Investigação Médica – LIM/36 - Departamento de Pediatria, Faculdade de Medicina, Universidade de São Paulo
Av. Dr. Enéas de Carvalho Aguiar, 647
05403-900 São Paulo - SP Brazil

Phone number: 55 (11) 3069-8606
Fax number: 55 (11) 3069-8506
Email: tsokay@icr.hcnet.usp.br

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Abstract

Objective: To evaluate whether procalcitonin (PCT) and C reactive protein (CRP) are able to discriminate between sepsis and systemic inflammatory response syndrome (SIRS) in critically ill pediatric patients.

Design and Setting: Prospective, observational study in a pediatric intensive care unit. The study was divided in two parts: I) kinetics of PCT and CRP in patients undergoing open heart surgery with cardiopulmonary bypass (CPB), representing the SIRS model (group I) [1]. II) Kinetics of PCT and CRP in patients with confirmed bacterial sepsis (group II).

Patients: Fourteen patients with confirmed bacterial sepsis (group II).

Measurements and main results: In group I PCT and CRP concentrations were determined in five different times: before CPB; immediately after CPB; 24h; 48h and 72h after the procedure. Although all 14 patients of group I had negative cultures, the sampling time “before CPB” was obtained in order to ensure that baseline laboratory markers were within reference values, but these results were not used in statistical analyses between groups. PCT median concentration was 0.24 ng/mL thus confirming that patients were not infected (reference value < 2.0 ng/mL). There was an increment of PCT concentrations which peaked immediately after CPB (median 0.58 ng/mL), then decreased to 0.47 ng/mL at 24 h; 0.33 ng/mL at 48h and 0.22 ng/mL at 72 h. CRP median concentrations remained high on POD1 (36.6 mg/L) and POD2 (13.0 mg/L). In septic children (group II), PCT and CRP were measured four times: at admission; 24 h, 48 h and 72 h after hospitalization. PCT concentrations were high at admission (median 9.15 ng/mL) and unlikely CRP, decreased afterwards in 11 of 14 patients who evolved favorably (median 0.31 ng/mL). Conversely, CRP levels were high in only 11 out of 14 patients at admission. CRP persisted high in 13 of 14 patients at 24 h; in 12 of 14 at 48 h; and finally in 10 of 14 patients at 72 h. Median values were: 95.0 mg/L; 50.9 mg/L 86.0 mg/L and 20.3 mg/L, respectively. The area under the receiver operating characteristic curve (ROC) was 0.99 for PCT and 0.54 for CRP. Cut off concentrations to differentiate SIRS from sepsis were > 2 ng/mL for PCT and > 79 mg/L for CRP.

Conclusion: PCT is able to differentiate between SIRS and sepsis. CRP lacked sensitivity as it did not detect 3 septic patients at admission. Moreover, CRP did not modulate according to patients outcome as did PCT. The latter returned to reference values in 11 of 14 patients who evolved favorably, persisting high in the 3 patients who died.
Introduction

Bacterial sepsis is a major cause of morbidity and mortality in neonates and children.[2] Rapid detection of bacterial sepsis is difficult because the first signs of disease are usually nonspecific.[3] Early diagnosis of severe infections and the prompt initiation of adequate antimicrobial therapy are essential for successful treatment. [4]

Cardiac surgery and the use of cardiopulmonary bypass (CPB) have significantly improved prognosis of pediatric patients with congenital heart disease. However, extended blood contact with foreign surfaces, the use of hypothermia, myocardial ischemia, reperfusion and surgical trauma trigger activation of the immune system, the complement pathway and cytokines release leading to systemic inflammatory response immediately after CPB. During the first hours or days after surgery, it is cumbersome to differentiate between normal inflammatory response and infectious complications.[5][6]

Laboratory parameters such as CRP and leukocyte count are often abnormally elevated after cardiac surgery albeit the absence of infection.[5][7] PCT has been recently proposed as a more specific marker of infection, being able to discriminate between systemic inflammatory responses and sepsis.[8]

We present herewith the second part of a study conducted in critically ill patients presenting with bacterial sepsis. We aimed at comparing this septic group of children with the cases of SIRS from the first part of the study.[1]

Patients

This study has received the approval of the Ethics Committee of the School of Medicine, University of São Paulo, Brazil. After informed consent of parents, 14 children were enrolled in this second part of the study. Group II consisted of children presenting with bacterial sepsis confirmed by either hemoculture (n=10), cerebrospinal fluid (CSF) culture (n=1) or urine culture (n=3). Seven boys and 7 girls were included with age varying from 3 days to 192 months. Bacterial sepsis was defined according to the American College of Chest Physicians and Society of Critical Care Medicine, slightly modified to fit both neonatal and pediatric population.[9] All pediatric patients with bacterial sepsis received antibiotics according to the decision of the attendant physician. Patients who had received antibiotics, anti-inflammatory drugs or corticosteroids prior to hospitalization were excluded. Children presenting with endocrine, liver or renal dysfunction were also excluded because these conditions might have decreased production and clearance of acute phase proteins such as CRP.

Material and Methods
Three milliliters of arterial blood were drawn in sterile vacuum tubes with no additives (Becton Dickinson-BD), centrifuged, and serum aliquots have been stored at −20°C until the time of analysis. Patients of group II were tested at admission (before administration of antibiotics); on the first (24h), second (48h) and third day (72h) of treatment.

CRP concentrations have been determined in serum samples by immunonephelometry (nephelometer -2, Dade-Behring, La Défense, France; reference values were < 5.0 mg/L).[7] Briefly, polystyrene particles coated with monoclonal antibodies specific to human CRP are aggregated when mixed with serum samples of patients containing CRP. These aggregates scatter a beam of light passed through the sample. The intensity of scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

Procalcitonin has been evaluated by an immunoluminometric assay (LUMItest’ PCT, BRAHMS Diagnostica, GmbH, Germany, recommended reference interval for SIRS is 0.5-2.0 ng/mL). Briefly, 20 µl of serum or plasma samples are added to a tube coated with an anti-katacalcin antibody. Samples are incubated at room temperature for 1 hour, and a second antibody anti-calcitonin labelled with a luminescent acridine derivative is added to the reaction. Then, samples are placed in a luminometer and hydrogen peroxide and sodium hydroxide solutions are automatically injected. These substances react with the acridine derivative bound to the anti-calcitonin antibody leading to emission of light as the acridine turns into acridone. The intensity of emitted light is directly proportional to the PCT concentration.[10]

**Statistical Analysis**

PCT and CRP concentrations were presented as median (min.-max.). Results comparing sampling times within group II were made by means of the Wilcoxon test. Values of p<0.01 were considered statistically significant. The Friedman test was used to evaluate statistical significance of differences between group I (SIRS) and II (sepsis). Sampling times were compared two by two: after CPB versus admission; POD1 versus 24 h; POD2 versus 48 h; POD3 versus 72 h. Values of p<0.01 were considered statistically significant.

Sensitivity, specificity and predictive values were calculated for different concentrations of PCT and CRP by means of the Receiver Operator Curve (ROC), for patients of group II (sepsis).

**Results**

Characteristics of group II patients (sepsis) are shown in Table 1. The median of age in group I was 1.5 months (0.1-192.0), and the median of hospital staying was 20.0 days (4.0-72.0).
Table 1 – Characteristics of the 14 patients with sepsis (group II).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age Month</th>
<th>Gender</th>
<th>ICU stay days</th>
<th>Culture</th>
<th>outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>F</td>
<td>4</td>
<td><em>Klebsiella pneumoniae</em> BC</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>F</td>
<td>6</td>
<td><em>Pseudomonas aeruginosa</em> BC</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>F</td>
<td>24</td>
<td><em>Pseudomonas aeruginosa</em> BC</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>192</td>
<td>M</td>
<td>12</td>
<td><em>Staphylococcus aureus</em> BC</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>M</td>
<td>8</td>
<td><em>Streptococcus sp</em> BC</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>F</td>
<td>17</td>
<td><em>Pseudomonas aeruginosa</em> BC</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>0,5</td>
<td>M</td>
<td>41</td>
<td><em>Staphylococcus aureus</em> BC</td>
<td>NS</td>
</tr>
<tr>
<td>8</td>
<td>0,8</td>
<td>M</td>
<td>48</td>
<td><em>Proteus mirabilis</em> UC</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>0,6</td>
<td>M</td>
<td>9</td>
<td><em>Escherichia coli</em> UC</td>
<td>S</td>
</tr>
<tr>
<td>10</td>
<td>0,1</td>
<td>M</td>
<td>27</td>
<td><em>Staphylococcus epidermidis</em> BC</td>
<td>S</td>
</tr>
<tr>
<td>11</td>
<td>0,5</td>
<td>F</td>
<td>5</td>
<td><em>Staphylococcus aureus</em> BC</td>
<td>S</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>F</td>
<td>25</td>
<td><em>Escherichia coli</em> UC</td>
<td>S</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>M</td>
<td>33</td>
<td><em>Neisseria meningitidis</em> CSF</td>
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</tr>
<tr>
<td>14</td>
<td>0,1</td>
<td>F</td>
<td>72</td>
<td><em>Enterobacter sp</em> BC</td>
<td>S</td>
</tr>
</tbody>
</table>

ICU = Intensive Care Unit  
M = male  
F = female  
BC = blood culture  
UC = urine culture  
CSF = cerebrospinal fluid  
NS = non survivor  
S = survivor
Results of group I patients have already been reported.[1] All 14 patients evolved favorably, with no signs or symptoms of infection.

Figure 1 shows PCT concentrations in the 14 patients of group I (SIRS) and 14 patients of group II (Sepsis). There was an increment of PCT concentrations in all 14 patients at admission. Median values (min.-max.) were: 9.15 ng/mL (2.1-607.7) at admission (adm); 6.25 ng/mL (1.5-619.9) at 24 h (D1); 3.22 ng/mL (0.1-149.1) at 48 h (D2), and 0.31 ng/mL (0.1-153.5) at 72 h (D3).

Figure 2 shows CRP concentrations of group I (SIRS) and group II (sepsis). CRP levels were above the reference interval (> 3.5 mg/L) in only 11 out of 14 patients at admission; in 13 of 14 patients at 24 h (D1); in 12 of 14 at 48 h (D2), and finally in 10 of 14 patients at 72 h (D3). Median values (min.-max.) were: 95.0 mg/L (3.1-322.0) at admission (adm); 50.9 mg/L (3.1-393.1) at 24 h (D1); 86.0 mg/L (3.1-148.7) at 48 h (D2); 20.3 mg/L (3.5-200.0) at 72 h (D3).

Sensitivity (SE), specificity (SP), positive predictive values (PPV) and negative predictive values (NPV) have been calculated by means of the Receiver Operator Curve (ROC). Results are shown in Table 2. The area under ROC curve was 0.99 for PCT (95% of confidence interval; 0.97 to 1) and 0.54 for CRP (95% CI 0.38 to 0.72).
Table 2 – Diagnostic value of procalcitonin (PCT) and C-reactive protein (CRP) at various thresholds considering the 14 patients of group II (sepsis).

<table>
<thead>
<tr>
<th></th>
<th>SE</th>
<th>SP</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCT (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>73</td>
<td>67</td>
<td>64</td>
<td>75</td>
</tr>
<tr>
<td>1.0</td>
<td>71</td>
<td>92</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>1.5</td>
<td>68</td>
<td>98</td>
<td>97</td>
<td>79</td>
</tr>
<tr>
<td><strong>2.0</strong></td>
<td><strong>88</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>86</strong></td>
</tr>
<tr>
<td>5.0</td>
<td>41</td>
<td>100</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>76</td>
<td>40</td>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td>10.0</td>
<td>70</td>
<td>44</td>
<td>50</td>
<td>64</td>
</tr>
<tr>
<td>30.0</td>
<td>52</td>
<td>70</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>50.0</td>
<td>45</td>
<td>80</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>100.0</td>
<td>30</td>
<td>97</td>
<td>89</td>
<td>63</td>
</tr>
</tbody>
</table>

SE = sensitivity
SP= specificity
PPV = positive predictive value
NPV= negative predictive value
Discussion

The present study aimed at analyzing the kinetics of PCT and CRP in two different situations: SIRS and sepsis, and verifying whether these two laboratory markers might be used to discriminate between the two entities. In this second part of the study (group II), 14 pediatric patients presenting with confirmed sepsis (group II) were enrolled and compared with 14 patients of group I (SIRS).[1]

Following cytotoxic chemotherapy, fever might be a signal of invasive bacterial disease or a drug reaction associated with SIRS. Stryjewski et al. found that PCT and interleukin-8 could be used to detect bacterial sepsis in febrile, neutropenic children.[11] Kuse et al. showed that PCT allows differentiation between rejection and infection in patients presenting with fever of unknown origin (FUO) and receiving prednisolone following liver transplantation.[12] Other study from Sauer et al. concluded that serum PCT correlates with the severity of sepsis among deeply immunosuppressed pediatric bone marrow transplant recipients, and that it may reliably identify children at risk to develop graft versus-host disease, who received prophylaxis with prednisone.[13] Our study showed that corticosteroids did not affect either PCT or CRP kinetics in group I (SIRS), as we could observe an increment of PCT and CRP after CPB, and of CRP in the other sampling times. However, it is noteworthy that PCT increments found after CPB did not exceed the reference interval for SIRS (< 2 ng/mL).

There are several studies in the literature evaluating the ability of PCT to diagnose infection in patients with different underlying pathologies.[14][15][16] In septic children, those reports tend to indicate that PCT could be used as a laboratory marker to discriminate between SIRS and sepsis.[15][17][18][19] In our study, PCT concentrations of group II patients have increased in all 14 patients already at admission (median 9.15 ng/ml), thus confirming its ability to diagnose infection. These data corroborate the study of Gendrel et al. who have compared the concentrations of PCT, CRP, interleukin-6 and IFN in pediatric patients, aiming at discriminating between viral and bacterial infections.[20] They concluded that the best laboratory parameter was PCT (83% of sensitivity and 93% of specificity) when the cut off was 1.0 ng/mL. Moreover, 47% of children presenting with viral infections showed CRP concentrations above 10.0 mg/L; and 26.9% above 20.0 mg/L, corroborating the lack of specificity of CRP. In our study, PCT concentrations in septic patients were high at admission and at 24 h. However, when concentrations found at admission (median 22.12 ng/mL) were compared with those obtained at 48h (median 3.22 ng/mL) or at 72h (median 0.42 ng/mL), a statistically significant difference was found (p<0.001). These data have confirm that PCT concentrations modulate more quickly than CRP, thus indicating that it could be used to test response to antibiotic therapy.[21] Moreover, in the 11 survivors of group II (sepsis), PCT concentrations returned to the SIRS interval (0.5 - 2.0 ng/mL) until the last time of sampling (72h). On the contrary, PCT concentrations remained above reference interval until 72h, exactly in the three patients that evolved to lethal exit.[22][23]

Taking CRP concentrations into consideration with respect to group II, CRP did not detect 3 of 14 septic patients at admission, what might postpone the introduction of antibiotics.
Moreover, CRP remained high until 72 h showing that, unlikely PCT, it could not be used to test response to antibiotics.[17][24]

Early identification of patients with insidious sepsis would allow early therapeutic intervention, what might influence patients outcome.[25] In our study, PCT was also able to discriminate between post-CBP time (representing SIRS) and the first sampling time of septic patients (admission). Comparisons were made by means of the Friedman test (p< 0.001). Besides, when post-CBP concentrations were confronted to the 24 h sampling time, there was a statistically significant difference (p < 0.001). Contrastingly, CRP did not succeed in discriminating these situations tested two by two, confirming its inability to differentiate SIRS and sepsis (p<0.01).[26]

In the present study, PCT proved to be more specific than CRP, and also to have a higher positive predictive value to diagnose sepsis in comparison with CRP (Table 2). Similar specificity was observed by Lopez and Enguix (94% and 100%, respectively).[15][18] However, in the first study the best cut-off for PCT was 0.59 ng/mL, while it was 8 ng/mL in the second report. In a systematic review, Simon et al. reported that the diagnostic accuracy of PCT was higher than that of CRP to distinguish between bacterial infection and SIRS among hospitalized patients.[27]

Conclusions

We conclude that SIRS (group I) did influence serum CRP concentrations immediately after surgery, and at 24h, 48 h and 72h, while PCT concentrations remained within predicted SIRS range (0.5-2.0 ng/mL) at all sampling times. Therefore, PCT was able to discriminate between SIRS and sepsis, while CRP was not. In the present study, unlikely CRP, PCT proved to modulate according to the evolution of sepsis. These data might indicate that PCT, but not CRP, could be used to test response to antibiotics.

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References


**Figure 1** - Plot of procalcitonin (PCT) versus time in group I patients (SIRS) and group II (Sepsis).
Figure 2 – Plot of C reactive protein (CRP) versus time in group I (SIRS) and II (sepsis) patients.