Comparative diagnostic test evaluation of serum procalcitonin and C-reactive protein in suspected bloodstream infections in children with cancer

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Abstract

Purpose. To compute diagnostic test properties of C-reactive protein (CRP) and serum procalcitonin (PCT) levels in bloodstream infections in children with cancer and suspected sepsis, in comparison with blood culture as the gold standard.

Methodology. Consecutive paediatric cancer patients, aged ≤14 years, with clinically suspected bloodstream infections were evaluated with blood culture and assay of PCT and CRP levels. Blood culture was taken as the gold standard for comparison. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio (LR) and receiver operating characteristic (ROC) with area under ROC curve (AUC) were calculated to assess the diagnostic test performance for PCT and CRP.

Results/Key findings. The ROC curve for PCT was better than that for CRP, with an AUC of 0.751 for PCT at a cut-off of 2.25 ng ml−1. The AUC for CRP was 0.638 at a cut-off of 8.0 mg dl−1. Among the three cut-off values of PCT selected from the ROC curve applicable to the patients under study, the cut-off value of ≥0.49 ng ml−1 had the maximum sensitivity of 81.4% and an NPV of 94.67%; ≥2.25 ng ml−1 had a sensitivity and specificity of 65.12 and 71.6%, respectively, and ≥6.47 ng ml−1 had a maximum specificity of 82.10%. For CRP, the cut-off value of ≥5.3 mg dl−1 had the maximum sensitivity of 72.09%; ≥8.0 mg dl−1 had a sensitivity and specificity of 58.14 and 68.09%, respectively, and ≥8.4 mg dl−1 had the maximum specificity of 70.04%.

Conclusion. PCT is a better serological marker for excluding bloodstream infections than CRP. The cut-off value of 0.49 ng ml−1 with a negative predictive value of 94.67% will be ideal in a clinical setting of immune-compromised children with suspected sepsis.

INTRODUCTION

Opportunistic infections, especially bloodstream infections, constitute a major cause of morbidity and mortality in paediatric cancer patients. Although fever is the most common manifestation of such infections, life-threatening infections can also occur without fever, especially in neutropenic patients. The similarity of certain non-infectious states (such as transfusion reaction, graft-versus-host disease and fever associated with tumour lysis) to sepsis and the protein manifestations of bloodstream infections in cancer patients present the physician with diagnostic challenges [1].

A reliable marker for timely diagnosis of such life-threatening infections with high specificity and sensitivity is necessary. Microbial cultures and serological tests are the current options available that aid the clinician in diagnosing bloodstream infections. Blood cultures currently represent the ‘gold standard’ for diagnosis of septicaemia. Nonetheless, they have limitations. Positive results require hours to days of incubation. No single culture medium or system in use has been shown to be best for detecting all potential bloodstream pathogens. Some micro-organisms grow poorly, or not at all, in conventional blood culture media and systems. Delay in diagnosis is a very important factor affecting prognosis, so clinicians often resort to empirical
antibiotic therapy, which may be counterproductive when given to patients without infection [2].

A great deal of effort has been made to determine laboratory parameters that could help in the quick diagnosis of sepsis. These include serological markers like C-reactive protein (CRP) and procalcitonin (PCT).

CRP is the most widely used inflammatory marker for diagnosing infections [3]. It is an acute-phase protein which increases in infections, connective tissue disorders and neoplastic disease. High levels of CRP are found during bacterial infections compared to viral infections [4]. However, CRP lacks specificity for identification of systemic bacterial infections [2].

PCT has been proposed as a reliable marker for improved diagnosis of bacterial infections and to guide antibiotic therapy. It has several advantages over other inflammatory markers. PCT is produced in response to endotoxins or mediators released in response to bacterial infections like IL-1β, TNF-α, IL-6 etc. and its level strongly correlates with the extent and severity of bacterial infections [5]. PCT rises after the elevation of TNF-α and IL-6, but before the rise in CRP. An increase in levels of PCT in the early stage of infection, with a rapid decrease once the infection is controlled, makes PCT ideal for the evaluation of sepsis in oncology settings [2, 6].

Many studies are available from different parts of the world assessing the test properties of PCT and CRP [7–11]. But there is a dearth of information on the use of PCT in cancer patients. The majority of these patients are neutropenic and have an impaired immune response. The effects of these parameters on production and kinetics of PCT and CRP are not well studied. Available data suggests that PCT is superior to CRP [12]. Some studies have also shown that PCT can be elevated in non-infectious states such as acute graft-versus-host disease and during T-cell-directed immuno-modulatory treatment [13]. Extrapolation of the results from such studies to paediatric cancer patients has to be done with caution. Hence we planned to study the usefulness of biomarkers like PCT and CRP in bloodstream infections in paediatric cancer patients using diagnostic test evaluation methods.

Diagnostic accuracy studies address the agreement between a proposed (index) test and a reference standard (gold standard) for the ability to identify a target condition. The performance characteristics of the test are evaluated in comparison with the gold standard. Characteristics assessed in diagnostic test evaluations include sensitivity, specificity, predictive values and likelihood ratios. The starting point is the construction of a 2×2 table with the index test results on one side and those of the reference standard on the other: four possibilities may arise for each cut-off value of the index test: true positive (TP); false positive (FP); false negative (FN); and true negative (TN). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio (LR) are calculated as per the formulae given in Fig. 1.

Plotting the receiver operator characteristic (ROC) curve and determining the area under the ROC curve (AUC) is another measure of test performance. The ROC curve graphically displays the trade-off between sensitivity and specificity, and is useful for assigning the best cut-offs for clinical use. An ROC curve is constructed by plotting the TP rate (sensitivity) against the FP rate (1-specificity) over a range of cut off values. The best cut-off point is at or near the shoulder of the ROC curve, unless there are clinical reasons for minimizing either FNs or FPs. Tests that perform well will crowd towards the upper left corner of the ROC curve as the sensitivity is progressively increased; there is little or no loss in specificity until very high levels of sensitivity are achieved. Tests that perform less well have curves that fall closer to the diagonal running from lower left to upper right. Overall accuracy is sometimes expressed as the AUC and provides a useful parameter for comparing test performance. The greater the AUC, the better the test [14].

**METHODS**

**Objective**

To compute the standard test properties of serum levels of CRP and PCT, in comparison with blood culture as the gold standard, in bloodstream infections among paediatric cancer patients with suspected sepsis.
Study design
Diagnostic test evaluation – cross-sectional method of data collection.

Setting
Division of Pediatric Oncology and Division of Microbiology, Regional Cancer Centre, Thiruvananthapuram, Kerala, India.

Inclusion criteria
Consecutive paediatric cancer patients aged ≤14 years who presented with clinically suspected bloodstream infections during the study period (July 2014 to February 2015) were included in the study.

Cancer patients undergoing chemotherapy with or without neutropenia, presenting with fever, tachycardia and tachypnoea were suspected to have bloodstream infections. Patients with other localizing signs of infection and who satisfied the criteria for systemic inflammatory response syndrome (SIRS) were also included in the study.

Exclusion criteria
(1) Children who received treatment with antibiotics within the previous 48 h.
(2) Children whose parents did not give consent for inclusion in the study.

The sample size was calculated to be approximately 300 patients.

Data collection
The study was approved by the institutional ethics committee. Informed consent was obtained from the parents of children before collecting blood samples for the study.

Laboratory procedures
On clinical suspicion of bloodstream infection, two samples of 3–5 ml of blood were collected in two automated blood culture bottles (BACT/Alert FAN aerobic bottles; bioMérieux) for culture from each child before initiation of antimicrobial therapy. The samples were collected under aseptic precautions from two different sites. One central line sample and one peripheral vein sample were collected from patients with an indwelling central line catheter, and two peripheral samples were collected from patients without a central line. Bottles flagged as positive by the Bact/Alert system were sub-cultured and interpreted according to the standard protocols.

Blood (5 ml) was collected in vacutainers for PCT and CRP estimation. Serum was separated and PCT levels were assayed quantitatively using a VIDAS BRAHMS enzyme-linked fluorescent assay (bioMérieux) as per the manufacturer’s instructions. The cut-off point for an elevated serum PCT level was taken as ≥0.1 ng ml⁻¹.

CRP levels were estimated quantitatively using VITROS Chemistry Products CRP slides in a VITROS 5600 Integrated System (Ortho-Clinical Diagnostics, Johnson & Johnson). The cut-off point for an elevated serum CRP level was taken as ≥1 mg dl⁻¹.

Analysis
The data collected from all patients were entered into a Microsoft Excel spreadsheet and analysed using SPSS software version 16 and functions available in Microsoft Excel 2010. A patient was diagnosed to have bloodstream infection when both blood culture samples drawn from two different sites flagged positive and the same organism was isolated from both blood culture samples. Diagnostic performance of PCT and CRP was assessed in comparison with blood culture as the gold standard by calculating the sensitivity, specificity, positive predictive value, NPV and likelihood ratios (both positive and negative LR). ROC curves were also drawn using SPSS software. The output consisting of sensitivity and specificity against each cut-off value of PCT and CRP was obtained from the SPSS software while constructing the ROC curves. NPV, PPV and LR for the selected cut-offs of PCT and CRP were calculated using functions available in Microsoft Excel 2010. The AUCs of PCT and CRP were calculated and compared using MedCalc 15 statistical software.

RESULTS
Three hundred paediatric oncology patients with a presumed clinical diagnosis of bloodstream infection were included in the study.

Demographic data
Among 300 patients with suspected bloodstream infections, 173 (57.7 %) were male and 127 (42.3 %) were female. Mean age was 6.5±4.1 years. 32.6 % patients had indwelling central lines.

Laboratory diagnosis
Reference test (gold standard) – blood culture
Forty-three patients (14.3 %) had positive blood cultures. 25.6 % of the blood-culture-positive cases had infection with Gram-positive organisms, 65.1 % had infections with Gram-negative organisms and 9.3 % had infections with Candida species.

Tests under evaluation – PCT and CRP
The median total PCT level was 0.49 ng ml⁻¹. The median PCT level in the culture-positive group was 7.03 ng ml⁻¹, while in the culture-negative group it was 0.38 ng ml⁻¹. Among the culture positives, median PCT level was highest in bloodstream infections due to Gram-negative pathogens (19.12 ng ml⁻¹) followed by Gram-positive pathogens (0.54 ng ml⁻¹) and Candida species (0.32 ng ml⁻¹).

The median total CRP level was 5.6 mg dl⁻¹. The median CRP level in the culture-positive group was 8.5 mg dl⁻¹, while in the culture-negative group it was 5 mg dl⁻¹. Median CRP levels were also higher in Gram-negative sepsicaemia (9.3 mg dl⁻¹) compared to Gram-positive sepsicaemia (7 mg dl⁻¹).
Diagnostic test properties of PCT

An ROC curve was generated for PCT and sensitivity and specificity were analyzed simultaneously for different cut-off points of PCT from the ROC curve. As the PCT levels increased, the sensitivity decreased and the specificity increased. When the PCT levels decreased, the sensitivity increased, but specificity decreased. Hence, from the ROC curve, a PCT value of 2.25 ng ml\(^{-1}\) that had 65.12 % sensitivity and 71.6 % specificity was chosen as the optimum cut-off level. ROC curve analysis also showed an AUC of 0.751 for PCT (Fig. 2).

Three cut-off values for PCT were selected from the ROC curve applicable to the patients under study. The selected cut-off based on the maximum sensitivity with moderate specificity was \(\geq 0.49\) ng ml\(^{-1}\) with a sensitivity and specificity of 81.40 and 55.30 %, respectively. The cut-off selected with moderate sensitivity and specificity was \(\geq 2.25\) ng ml\(^{-1}\), which had a sensitivity and specificity of 65.12 and 71.6 %, respectively; the third cut-off value \((\geq 6.47\) ng ml\(^{-1}\)) had moderate sensitivity and excellent specificity (53.49 and 82.10 %, respectively). Across the three chosen cut-off values of PCT, the sensitivity was found to drop from 81.4 to 53.49 % and the specificity was found to increase from 55.30 to 82.10 % (Table 1). However, there was no corresponding significant change in NPV across various cut-offs (i.e. 94.67, 92.46 and 91.34 %, respectively). The PPV was lower when compared to NPV across different cut-offs and ranged from 23.33 to 33.33 %.

**Table 1. Summary of test properties of PCT versus blood culture at selected cut-offs of PCT**

<table>
<thead>
<tr>
<th>PCT (ng ml(^{-1}))</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>LR+</th>
<th>LR−</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\geq 0.49)</td>
<td>81.4</td>
<td>55.3</td>
<td>23.33</td>
<td>94.67</td>
<td>1.82</td>
<td>0.34</td>
</tr>
<tr>
<td>(\geq 2.25)</td>
<td>65.12</td>
<td>71.6</td>
<td>27.72</td>
<td>92.46</td>
<td>2.29</td>
<td>0.49</td>
</tr>
<tr>
<td>(\geq 6.47)</td>
<td>53.49</td>
<td>82.1</td>
<td>33.33</td>
<td>91.34</td>
<td>2.99</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Diagnostic test properties of CRP

ROC curve analysis of CRP revealed an AUC of 0.638. Sensitivity and specificity were analyzed simultaneously at different cut-off levels of CRP from the ROC curve. As in the case of PCT, the cut-off value chosen for CRP from the ROC curve was 8.0 mg dl\(^{-1}\) with a sensitivity and specificity of 58.14 and 68.09 %, respectively (Fig. 3).

Three cut-off values of CRP were selected from the ROC curve: two values with reasonable sensitivity and specificity (\(\geq 8.0\) and \(\geq 8.4\) mg dl\(^{-1}\)) and one value (\(\geq 5.3\) mg dl\(^{-1}\)) with maximum sensitivity and moderate specificity, i.e.72.09 and 51.36 %, respectively (Table 2). Pair-wise comparison of the area under ROC curves of PCT and CRP using MedCalc statistical software showed a significant difference \((P=0.0130)\) in the AUC.

**DISCUSSION**

Sepsis is the major cause of morbidity and mortality in pediatric patients with cancer. Prompt antibiotic treatment improves outcome and survival. Fever in oncology patients may be due to infections or due to the inherent inflammatory state of cancer itself. Empirical antibiotic treatment in non-infectious states can lead to the emergence of antibiotic resistance and an increase in the cost of treatment. Specific biomarkers with high sensitivity, specificity and NPV that can diagnose or exclude suspected sepsis are essential for timely initiation or withholding of antibiotic therapy [15].

In terms of sensitivity and specificity, NPV and AUC, PCT was found to be a better marker than CRP in excluding bloodstream infections in this cohort. The ROC curve for PCT was better than that for CRP, having an AUC of 0.751 for PCT at the cut-off level of 2.25 ng ml\(^{-1}\) when compared to CRP, which had an AUC of 0.638 at the cut-off level of 8.0 mg dl\(^{-1}\). For the PCT value of 2.25 ng ml\(^{-1}\) selected from the ROC curve, the sensitivity (65.12 %) and specificity (71.6 %) were reasonably good and the NPV (92.46 %) was very high. The PPV was lower (27.72 %). However, considering the clinical setting of immune-compromised children with suspected sepsis, where false negativity is hazardous due to the life-threatening situation, a cut-off value of 0.49 ng ml\(^{-1}\) can also be considered as a good cut-off with a sensitivity of 81.4 %, accepting the relatively low specificity of 55.30 %. The NPV of 94.67 % shows that PCT has a good NPV for excluding bloodstream infections even though the PPV was low (23.33 %). Our data suggests that PCT is better suited for excluding bloodstream infections in
 paediatric cancer patients as the NPV was very high (ranging between 91.34 and 94.67 % across the three selected cut-offs of ≥6.47 ng ml\(^{-1}\), ≥2.25 ng ml\(^{-1}\) and ≥0.49 ng ml\(^{-1}\)) than for the diagnosis of bloodstream infections (low PPV, i.e. 33.33 %, 27.72 and 23.33 %, respectively). The optimal cut-off value for PCT from various studies ranged from 0.6–5 ng ml\(^{-1}\) [12, 16, 17].

In the case of CRP, the cut-off value selected as per the ROC curve analysis was 8.0 mg dl\(^{-1}\), which had a low sensitivity of 58.14 % and specificity of 68.09 %. The current accepted cut-off level of CRP (≥1 mg dl\(^{-1}\)) has a high sensitivity of 97.67 %, but specificity is very low (4.67 %), which is unacceptable. Similarly, in the case of PCT, at the usual considered cut-off value of ≥0.1 ng ml\(^{-1}\), sensitivity is 95.35 %, but specificity is only 17.51 %.

Miedema \textit{et al.} evaluated CRP and PCT as predictors for bacterial infection in paediatric oncology patients with febrile neutropenia and found that PCT levels were significantly increased in patients with a bacterial infection, in contrast to CRP [18]. An updated systematic review and meta-analysis of the predictive value of serum biomarkers in the assessment of febrile neutropenia in children with cancer by Haeusler \textit{et al.} also showed that PCT had better discriminatory ability than CRP in predictions of serious infection in children with febrile neutropenia [19].

Although the study carried out by Veeresh \textit{et al.} showed that PCT was a better marker than CRP for identifying bacterial sepsis, the study was not confined to paediatric cancer patients [16]. Their cut-off value of 2.42 ng ml\(^{-1}\) for PCT chosen from the ROC curve showed a reasonable sensitivity and specificity of 84 and 64.9 %, respectively. The AUC for PCT was 0.78 and that of CRP was 0.64.

A retrospective study carried out by Hattori \textit{et al.} in an unselected population above 18 years of age with suspected bloodstream infections showed that a cut-off value of 0.9 ng ml\(^{-1}\) for PCT can be used to predict bacteraemia [17].

In a study conducted by Mina Hur \textit{et al.} in patients with suspected sepsis, but not confined to paediatric cancer patients, it was seen that both PCT and CRP showed significant differences between patients with positive and negative blood cultures (PCT, 8.47 versus 2.44 ng ml\(^{-1}\); CRP, 11.05 versus 5.98 mg l\(^{-1}\)). The AUCs for PCT and CRP were 0.720 and 0.558, respectively; the significant difference suggesting the superiority of PCT over CRP [7]. Results from these studies are comparable with those obtained from our study.

It was also observed that PCT levels were high in Gram-negative sepsis when compared to Gram-positive and candida sepsis. The sample size was found to be inadequate for making meaningful comparisons when the patients were categorized into groups based on the organisms causing sepsicaemia. Hence further studies are warranted to determine whether there is a significant difference in the levels of PCT in Gram-negative and Gram-positive bacteraemia in order to use PCT for differentiating Gram-positive and Gram-negative sepsicaemia.

**Conclusion**

Sepsis in paediatric cancer patients is a matter of life and death. Blood culture, the current gold standard for diagnosing sepsis, is time consuming. In this scenario, it becomes all the more important to choose a biomarker that is reliable in identifying bloodstream infections. When the biomarkers CRP and PCT were compared, the test performance of CRP was poor. A cut-off value of 0.49 ng ml\(^{-1}\) for PCT with a negative predictive value of 94.67 % is useful for excluding bloodstream infections in a clinical setting of immunocompromised children with suspected sepsis.

**Table 2. Summary of test properties of CRP versus blood culture at selected cut-offs of CRP**

<table>
<thead>
<tr>
<th>CRP (mg dl(^{-1}))</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>LR+</th>
<th>LR−</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5.3</td>
<td>72.09</td>
<td>51.36</td>
<td>19.87</td>
<td>91.67</td>
<td>1.48</td>
<td>0.54</td>
</tr>
<tr>
<td>≥8.0</td>
<td>58.14</td>
<td>68.09</td>
<td>23.36</td>
<td>90.67</td>
<td>1.82</td>
<td>0.61</td>
</tr>
<tr>
<td>≥8.4</td>
<td>53.49</td>
<td>70.04</td>
<td>23</td>
<td>90</td>
<td>1.79</td>
<td>0.66</td>
</tr>
</tbody>
</table>

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study was approved by the Institutional Ethics Committee (HEC No: 08/2014 dt. fifteenth February 2014). Informed consent was obtained from the parents of paediatric oncology patients before collecting blood samples for the study.

References

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