Presepsin a Diagnostic Marker for Sepsis in Intensive Care Unit Patients

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Presepsin a Diagnostic Marker for Sepsis in Intensive Care Unit Patients

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The aim of the study was to evaluate the usefulness of presepsin as a diagnostic marker of sepsis in intensive care unit (ICU) patients. Presepsin was measured by a rapid method based on a chemiluminescent enzyme immunoassay (PATHFAST). The clinical usefulness of presepsin to diagnose sepsis and septic shock was studied and compared with procalcitonin, C-reactive protein and total leucocytic count. This study was conducted on 53 individuals divided into 3 groups. Group I included 28 adult ICU patients with at least two diagnostic criteria for systemic inflammatory response syndrome (SIRS) as patient group, Group IIa 15 patients admitted to ICU for any medical cause but with no evidence of infection were enrolled as patient control group and further 10 apparently healthy subjects as healthy control group. Patients were further subdivided retrospectively according to the final diagnosis into: patients with sepsis 16 (57.1%) and septic shock 12 (42.9%), from which 17 (59.3%) improved while 11 (39.3%) did not survive. The presepsin values were significantly higher in patients with sepsis than the control groups. The area under ROC curve (AUC) for discriminating sepsis from non septic conditions for presepsin was greater than the AUC of PCT, CRP or TLC. This suggests that presepsin has high specificity and sensitivity for sepsis diagnosis. In conclusion, presepsin can be used as a useful biomarker for the diagnosis of sepsis. It is readily available, cost-effective and able to distinguish septic patients in a complex population.

Systemic inflammatory response syndrome (SIRS) is a systemic inflammatory response to a variety of insults (ischemia, trauma or inflammation). The response is manifested by two or more of the following criteria “The American College of Chest Physicians/Society of Critical Care Medicine and the International Surviving Sepsis Campaign Guidelines Committee” ACCP/SCCM criteria: temperature > 38°C or <36°C, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or PaCO2 <32 mmHg and white blood cell count > 14,000 cells/cmm or < 4,000 cells/cmm (Levy et al., 2003). Sepsis is a SIRS in the presence of a confirmed or suspected infection. Severe sepsis is sepsis associated with organ dysfunction, hypoperfusion or hypotension. Septic shock is sepsis induced hypotension despite adequate fluid resuscitation (Russel, 2011). The inflammatory cascade is a complex process that involves innate, humoral and cellular responses, and complement and cytokine cascades. Important mediators include platelet-activating factor, tumor necrosis factor alpha and the interleukins 1, 6, 8 and 10. SIRS may be seen in emergency admissions but is also often seen in ward patients who have developed a complication (Griffiths & Anderson, 2009).

The activation of innate immunity is central to the manifestation of sepsis, and toll-like receptor (TLR4) plays an important role in this activation process. CD14 is a glycoprotein expressed on the membrane surface of monocytes/macrophages and serves as a receptor for complexes of lipopolysaccharides (LPS) and LPS binding protein (LPB), activating the TLR4...
Simultaneously, CD14 is shed from the cell membrane into the circulation forming soluble CD14 (sCD14). However, plasma protease activity generates also another sCD14 molecule called sCD14 subtype (sCD14-ST) or presepsin. The levels of presepsin were significantly higher in septic patients than apparently healthy individuals (Yaegashi et al., 2005).

The traditional approach to sepsis diagnosis was based on clinical signs and symptoms of sepsis, such as fever, tachycardia and tachypnea, supported by relevant microbiological data includes culturing blood, urine and cerebrospinal fluid. More recently, biological laboratory markers (biomarkers) have been used, ranging from the relatively simple white blood cell count and CRP to more complex biomarkers, such as PCT or cytokine levels such as TNF-α, IL-1β, or IL-6 (Vincent & Beumier, 2013).

Presepsin is thought to be a more specific and sensitive marker for the diagnosis and prognosis of sepsis compared with interleukin-6 and PCT. Presepsin concentrations in blood were increased faster than PCT and CRP in sepsis patients (Agilli et al., 2012).

The aim of the study to evaluate the usefulness of presepsin as a diagnostic marker of sepsis in intensive care unit patients, alone and compared with serum PCT and CRP.

Subject and Method

A total of (53) subjects were included in the study. (28) Of them were enrolled as patient group (19 males & 9 females). Their ages ranged between (20 & 84) years admitted to ICU at Ain Shams University hospitals, Egypt; who met at least two criteria for SIRS (temperature > 38°C or <36°C, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or PaCO2 <32 mmHg and white blood cell count > 14.000cells/cmm or < 4.000 cells/cmm). Sepsis is a SIRS in the presence of a confirmed or suspected infection. Severe sepsis is sepsis associated with organ dysfunction, hypoperfusion or hypotension. Septic shock is sepsis induced hypotension despite adequate fluid resuscitation. (15) Non septic ICU patients were enrolled as patient control group (11 males & 4 females), their ages ranged between (25 &74) years. Other (10) healthy subjects were included as normal control group (7 males and 3 females). Their ages ranged between (27 & 83) years. Patients ≤ 18 yrs, with severe or prolonged cardiogenic shock, after major trauma or major surgical intervention or severe burns were excluded. After informed written consent from patients or their relatives, all ICU patients included in this study were subjected to the following:

- Full history taking and complete clinical examination.
- Complete blood picture Performed on 5 part differential automated cell counter coulter® LH 750 cell counter (Coulter Corporation, Florida, USA).
- Quantitative measurement of the level of C - reactive protein extended range (RCRP): performed on dimension® clinical chemistry system (Siemens health care diagnostic products GmbH, Malburg/ Germany) using Flex® reagent cartilage, based on particle enhanced turbidimetric immunoassay technique. The cut off value for CRP is 3.0 mg/L.
- Measurement of the level of Procalcitonin (PCT): Quantitative analysis of PCT was performed with an automated electrochemiluminescence immunoanalyzer; Cobas e411 ® platforms (Roche Diagnostics, Mannheim, Germany). It is based on Sandwich immunoassay principle.

Measurement method:

1st incubation: Antigen in the sample (30µL), a biotinylated monoclonal PCT-specific antibody, and a monoclonal PCT-specific antibody labelled with a ruthenium complex are act to form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. This action mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed...
with Pro Cell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

The cut off value for PCT is 0.1 ng/mL.

Measurement of presepsin:
It is done on PATHFAST based on non-competitive chemiluminescent enzyme immunoassay. The presepsin binds to (ALP) labelled polyclonal Abs and monoclonal Abs coated magnetic particles. The luminescence intensity is related to the presepsin concentration of the sample which is calculated by means of a standard curve. The assay time doesn’t exceed 17 minutes.

Volume Method:
1) 100 μl of whole blood were dispensed into the wells of the reagent cartridge.
2) To 25 μl of the specimen, 25 μl dilution solution, 50 μl magnetic latex reagent & 50 μl labeled Ab reagent were added and allowed to react at 37°C for 5 min. Then washing was done three times with the wash solution provided.
3) 100 μl luminescent substrates were added. The luminescence intensity generated was related to presepsin concentration in the sample.
4) The presepsin concentration was measured by comparison with the amount of luminescence of a calibration agent (CAL-1 & CAL-2) that has been subjected to the same procedure as the sample.
5) The steps from (2) to (4) were automatically processed with an immunoassay analyzer (PATHFAST: Mitsubishi Chemical Medicine Corporation, Japan).

Quality Control Assay (QC Assay):
A QC assay is performed after every calibration to check the calibration curves and to obtain data from QC samples for quality control. A QC assay is indispensable for assuring validity of sample results. After each calibration, with each new shipment of previously calibrated test kit, or whenever the institution wishes to verify the performance of the system, analyze two levels of quality control material with known concentrations of presepsin (sCD14-ST).

-Validation of presepsin assay results:
Assay range of the presepsin kit is: 20 - 20,000 pg/ml.
Normal reference range: 60 - 365 pg/ml.

Statistical Analysis
Data were analyzed using SPSS (version 20) statistical software package under Windows 7 operating system for IBM compatible PC. The statistical tests used were: Arithmetic mean, standard deviation, for categorized parametric data and median in case of non parametric data. Student's t-test: was used for comparison of quantitative data, while Chi-Square test (X2) was used for comparison of qualitative data. For multi-group comparisons, Kruskal–Wallis one-way analysis of variance was applied, and two-group comparison was performed non parametrically using the Mann–Whitney U test and parametrically using ANOVA- test. ROC curve was done to determine the best cut off value of the marker to determine the highest value of sensitivity and specificity on this point. The level of significance was ≤ 0.05.

Results
A total of 53 subjects were included in the study. 28 of them were enrolled as patient group. They were 19 males (67.9%) & 9 females (32.1%). Their ages ranged between 20 & 84 (mean 53.46 ± 16.54) years. 15 non septic ICU patients were enrolled as patient control group. They were 11 males (73.3%) & 4 females (26.7%). Their ages ranged between 25 & 74 (mean 48.73 ± 13.93) years. Other 10 healthy subjects were included as normal control group 7 of them males (70%) and 3 females (30%). Their ages ranged between 27& 83 (mean 50.3 ± 17.46) years. The patients are further subdivided retrospectively according to the final diagnosis into: patients with sepsis 16 (57.1%) and patients with septic shock 12 (42.9%). From which 17 (59.3%) improved while 11(39.3%) did not survive. The primary site of infection in the group I were 43% due to intestinal and pulmonary causes, 11% urinary tract infection and 3% wound
infection. The culture results obtained from the patient’s medical records revealed the predominance of gram negative infection (81.8%) over the gram positive (4.5%). While (13.6%) of cultures were reported as negative as showing no growth.

In this study we notice that median values for presepsin is higher in patient group than control group as follows: 331.5 pg/ml (Range: 157 to 422) in healthy controls, 555 pg/ml (Range: 489 to 809) in patient controls, 1273.5 pg/ml (Range: 755 to 1786.5) in sepsis patients and 4417.5 pg/ml (Range: 2585 to 6469.5) in septic shock patients (Figure 1). The patient group had a significantly higher level of presepsin than the healthy and patient control groups ($P<0.0001$). Although we recorded a small elevation of presepsin in the patient control group, nevertheless, the levels were significantly higher than those in the healthy group ($P<0.0001$).

![Figure 1. Median presepsin values among the groups included in the study.](image)

Presepsin and PCT values did not differ significantly according to the primary sites of infection (intestinal, pulmonary, urinary tract or others) among patients with a confirmed diagnosis of sepsis or septic shock.

We also compared the various studied parameters namely presepsin, PCT, CRP and TLC levels in septic patients (including sepsis and septic shock patients) with their levels in patient control group as shown in (table 1). The comparisons revealed that; There is a highly statistically significant difference among the groups ($P<0.001$) in presepsin, PCT and CRP, and ($P=0.003$) in TLC.

Compared with the patient control group, presepsin, PCT and CRP levels were significantly higher in patients with sepsis and septic shock, but there was no difference for CRP levels in sepsis than in septic shock, as shown in (table 2).
Table 1. Comparison between the various studied parameters in the groups included in the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient Control</th>
<th>Sepsis Patients</th>
<th>Septic Shock Patients</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presepsin (pg/ml)</td>
<td>555.0</td>
<td>1273.5</td>
<td>4417.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range):</td>
<td>(489.0-809.00)</td>
<td>(755.0-1786.50)</td>
<td>(2585.0-6469.50)</td>
<td></td>
</tr>
<tr>
<td>Procalcitonin (ng/ml)</td>
<td>0.2</td>
<td>1.15</td>
<td>7.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range):</td>
<td>(0.1-0.4)</td>
<td>(0.65-2.31)</td>
<td>(3.25-39.95)</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.8</td>
<td>12.95</td>
<td>16.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range):</td>
<td>(1.0-4.2)</td>
<td>(7.8-17.45)</td>
<td>(12.0-29.7)</td>
<td></td>
</tr>
<tr>
<td>TLC (10^9/L)</td>
<td>8.76±5.46</td>
<td>16.43±7.19</td>
<td>18.96±10.19</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean ± 2SD:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 is significant

Table 2. Comparative statistics of presepsin, PCT and CRP between the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Pt Control</th>
<th>Sepsis</th>
<th>*P-value</th>
<th>Sepsis</th>
<th>*P-value</th>
<th>Sepsis</th>
<th>*P-value</th>
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<td>(755.0-1786.5)</td>
<td>(2585.0-6469.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procalcitonin (ng/ml)</td>
<td>0.2</td>
<td>1.15</td>
<td>0.001</td>
<td>1.15</td>
<td>0.002</td>
<td>7.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range):</td>
<td>(0.10-0.40)</td>
<td>(0.65-2.315)</td>
<td></td>
<td>(0.65-2.315)</td>
<td>(3.25-39.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.8</td>
<td>12.95</td>
<td>0.001</td>
<td>12.95</td>
<td>NS</td>
<td>2.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Median (Range):</td>
<td>(1.0-4.2)</td>
<td>(7.8-17.45)</td>
<td></td>
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<td>(12.0-29.7)</td>
<td>(1.0-4.2)</td>
<td></td>
</tr>
</tbody>
</table>

*P>0.05 is not significant (NS).

Value of presepsin for diagnosing sepsis

To discriminate between presepsin values in healthy control group and the patient control group, ROC curve was constructed and revealed that the area under the curve (AUC) of presepsin was 0.847 (P=0.004). When the cut-off value for presepsin was set at 455.5pg/mL, clinical sensitivity was 80.0% and specificity was 80.0% (Figure 2).
To better prove the usefulness of presepsin as a marker in the diagnosis of sepsis, we used the ROC analysis to discriminate the non-septic ICU patients (patient control group) from the sepsis group. ROC analysis revealed that the AUC of presepsin was 0.905 which was higher than the AUC of PCT (0.892), CRP (0.846), or TLC (0.809), respectively (Figure 3). This suggests that presepsin has high specificity and sensitivity for sepsis diagnosis.

<table>
<thead>
<tr>
<th>AUC</th>
<th>cut off point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presepsin</td>
<td>0.847</td>
<td>455.5</td>
<td>80.0%</td>
<td>80.0%</td>
</tr>
</tbody>
</table>

**Figure 2.** ROC curve of presepsin value in healthy control group compared to patient control group show that presepsin level at 455.5 pg/mL can differentiate both groups.

**Figure 3.** ROC curves of the four biomarkers including presepsin for diagnosis of sepsis. At the cut-off value 863pg/mL, presepsin may be able to discriminate between patients with and without sepsis with sensitivity and specificity of 85.2% and 86.7%, respectively. Using a PCT cutoff value of 0.5ng/ml for diagnosing sepsis, the sensitivity was 88.9% and the specificity was 86.7%.
Value of presepsin for predicting septic shock

The ROC curves of presepsin, PCT, CRP and TLC for predicting Septic shock in septic patients are displayed in (Figure 4); The AUC of presepsin was (0.883), which is higher than that of PCT (0.828), while area under the curves of both TLC and CRP were insignificant. These results shows that presepsin has superior prognostic accuracy.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presepsin</td>
<td>0.883</td>
<td>0.001</td>
</tr>
<tr>
<td>PCT</td>
<td>0.828</td>
<td>0.004</td>
</tr>
<tr>
<td>CRP</td>
<td>0.683</td>
<td>NS</td>
</tr>
<tr>
<td>TLC</td>
<td>0.556</td>
<td>NS</td>
</tr>
</tbody>
</table>

Figure 4. ROC curves of the four biomarkers including presepsin for predicting septic shock. At the cutoff value 1781.0 pg/ml presepsin predicting septic shock, the sensitivity was 83.3% and the specificity was 73.3%. And by using cutoff value of 2.37 ng/ml for procalcitonin, the sensitivity was 83.3% and the specificity was 80.0%.

Discussion

Sepsis is a systemic deleterious host response to infection, possibly leading to severe sepsis or septic shock. According to the most recent guidelines, published by the Surviving Sepsis Campaign, early recognition of these conditions, speed and appropriateness of therapy in the initial hours after presentation considerably influence the outcomes of septic patients (Dellinger et al., 2013).

Blood culture is the gold standard method for detecting the presence of microorganism in the blood stream. However, it has limited usefulness for early detection of infection because it usually requires several days for results to be known. Blood culture may also be plagued by some false negative cases, especially in patients undergoing antibiotic therapy. This can lead to a delay in antibiotic administration and consequently to increased mortality (Romualdo et al., 2014).

Plasma CRP and PCT values more than two standard deviations (SD) above the normal levels, if associated with infection documented or only suspected, are now part of the definition of sepsis (Dellinger et al., 2013).

Presepsin is a soluble CD14 subtype normally present in very low concentrations in the serum of healthy individuals and has been shown to increase in response to bacterial infections, according to the severity of the disease (Shozushima et al., 2011). In addition, sCD14-ST levels increased in the first 6h after the onset of sepsis. These changes in concentration occurred on a
much faster time scale than those observed for PCT or CRP (Yaegashi et al., 2005).

Furthermore, a rapid assay method for presepsin called PATHFAST® Presepsin assay, is now available and could be used on a point-of-care testing basis, thus allowing the emergency physician to get presepsin values in a short time from whole blood samples (Okamura & Yokoi, 2011).

We designed our study to validate the usefulness of presepsin as a diagnostic marker of sepsis in intensive care unit patients, alone and compared with serum procalcitonin and C-reactive protein. ICU patients presented with at least two clinical characteristics of SIRS and documented or suspected infection were enrolled, definitive diagnoses were made and according to the analysis of the clinical records, the population were divided into two main groups: (15) patient control group (patients admitted to ICU for any medical cause but with no evidence of infection) and patient group included (28) patients classified into patients with sepsis (16) and patients with septic shock (12) according to ACCP/SCCM criteria (Levy et al., 2003). Further (10) apparently healthy subjects were enrolled as healthy control group. All groups in the study were homogeneous in terms of size and demographic characteristics.

In our study we measured presepsin levels in all the groups included. Generally we noticed that the levels of presepsin among the groups were higher than the levels obtained by Liu et al. (2013) and Endo et al. (2012), who conducted their studies in Japan. While our results were closer to those obtained by Vodnik et al. (2013) and Ulla et al. (2013), whom study were conducted in Serbia and Italy respectively. We suggest this difference is due to different geographical distribution of the population involved in the study as Egypt is an endemic area for many infectious diseases. And also it may be due to the limited number of subjects in the present study.

In this study we compared presepsin levels in all the groups included. It revealed that the presepsin levels were significantly higher in septic patient group than the non septic control groups. This comes in accordance with Yaegashi et al. (2005), Shozushima et al. (2011) and Liu et al. (2013).

Although we found a small elevation of presepsin in the patient control group, the levels of presepsin in patients (sepsis and septic shock) were significantly higher. Our results were in agreement with that of Vodnik et al. (2013).

ROC curve was used to evaluate the value of the four markers (presepsin, PCT, CRP and TLC) in the diagnosis of sepsis; the results showed that presepsin was the best, followed by PCT, CRP and TLC. Our results agreed with those of Endo et al. (2012), Vodnik et al. (2013) and Liu et al. (2013).

Ulla et al. (2013), suggested a cut-off value 600 pg/ml of presepsin for diagnosis of sepsis which gives sensitivity 78.95% and specificity 61.9%. Liu et al. (2013), chose a point 550pg/ml improving sensitivity to 85 % and reported specificity was 63.6%. By applying the recommended cut-off 550pg/ml proposed by Liu et al. (2013), in our present study, improving the sensitivity to 92.6% but worsen the specificity to 46.7%. While by applying the point 600pg/ml as a cut-off as recommended by Ulla et al. (2013), Vodnik et al. (2013), and Endo et al. (2012), the sensitivity was 92.6% but still low specificity 53.3%.

In our study Presepsin level at point 713.5pg/ml the sensitivity was 85.2% but the specificity was 60% while cut-off point of 863pg/ml has the same sensitivity 85.2% but
even better specificity 86.7%. So our suggestion is to use this cut-off point (863 pg/ml) for sepsis diagnosis. The higher cut-off points in our study could be explained as a correlation to higher levels of presepsin in the population we studied as mentioned previously.

In our study, the cut-off value of 0.5ng/mL for PCT provides a sensitivity of 88.9% and specificity of 86.7% for this parameter. This value for the PCT is in accordance with the already established interval for the PCT (low risk of sepsis < 0.5 ng/mL) and with which recommended by Endo et al. (2012), and Vodnik et al. (2013).

Presepsin as a biomarker is not only suitable for the early diagnosis of sepsis, but also for the assessment of its severity and prognosis. The presepsin and PCT levels were significantly higher in septic shock than in sepsis patients. The present study demonstrated that plasma presepsin levels were a good parameter for reflecting the severity of sepsis which is agreed with Spanuth et al. (2011), Vodnik et al. (2013), and Liu et al. (2013).

On the contrary, Ulla et al. (2013), observed that no difference in presepsin levels was found between the sepsis and septic shock groups. They suggest that the concentration of the biomarker is not related to the severity of the disease in the very first hours as their patients were recruited from the emergency department not from the ICU as our study.

In this study, ROC analysis to discriminate sepsis from septic shock showed that the AUC of presepsin was higher than that of PCT and displayed higher sensitivity in predicting septic shock which further indicated that presepsin was superior to PCT in the assessment of prognosis which is agreed with Liu et al. (2013).

The median levels of presepsin were significantly higher in nonsurvivors than in survivors although the AUC of presepsin was slightly lower than that of PCT. This comes in accordance with Liu et al. (2013) and Ulla et al. (2013). While Masson and his colleagues (2014), showed that, compared with PCT, presepsin measured on day-1 predicted 90-day mortality in these selected patients. Also Charles et al. (2009), showed that the magnitude of PCT elevation on day 1 does not reliably predict the outcome in patients with sepsis.

In conclusion, the presepsin can be used as a biomarker for early diagnosis, assessment of severity and prognosis of sepsis. It is readily available, cost-effective and able to distinguish septic patients in a complex population as ICU patients. The PATHFAST Presepsin assay reveals its results within 17 min, so it can be used on a point-of-care testing basis, thus allowing the emergency physician to get presepsin values in a short time from whole blood samples.

References


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7. Liu B, Chen YX, Yin Q, Zhao YZ, Li CS (2013). Diagnostic value and prognostic evaluation of Presepsin for sepsis in an emergency department. Critical Care: 17: R244.


