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Diagnostic and Prognostic Value of Serum Calprotectin in Septic Shock Patients

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Abstract: Calprotectin is a potent acute phase reactant with increases of more than 100 fold during inflamed conditions. We measured the diagnostic and prognostic value of serum calprotectin (SC) in septic shock. We enrolled 50 adult shocked patients admitted to intensive care unit. Then, classified into 2 groups; septic group (25) with well-defined septic shock with positive cultures. Non-septic group (25) with negative cultures or no source of sepsis. Blood samples for SC), C-reactive protein (CRP) and white blood cell count (WBCC) in the first 6 hours of ICU admission and re-obtained again on day 3. We observed the weaning of vasopressor and 7-days in ICU mortality. SC measured on day 1 was significantly higher in the septic group than the non-septic group ($p<0.001$). SC showed a good correlation with weaning of vasopressor (AUC was 0.764; $p<0.028$), while it showed relative correlation with 7-days in ICU mortality (AUC was 0.752; $p<0.057$) compared with other markers in the study. SC may aid in rapid identification of septic shock from non-septic shock at a cutoff of 2 µg/dl (sensitivity 92% and specificity 84%). Also the change in SC level may aid in prognostication of septic shock.

Keywords: Critical, Septic Shock, Vasopressor, Mortality, Calprotectin, CRP

1. Introduction

Sepsis is a major public health concern. [1] It is among the most common reason for admission to intensive care units (ICUs) throughout the world. [2] Severe sepsis and septic shock are associated with 30-60% mortality rate which is very high compared to other common diseases, such as myocardial infarction or breast cancer. [3] Sepsis is now defined as a "life-threatening organ dysfunction due to a dysregulated host response to infection". In this new definition the concept of the non-homeostatic host response to infection is strongly stressed while the systemic inflammatory response syndrome (SIRS) criteria have been removed. The SIRS criteria are considered overly non-specific and of poor clinical utility. [4]

Septic shock is now defined as a "subset of sepsis where underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality". Clinical criteria identifying such condition include the need for vasopressors to obtain a mean arterial pressure (MAP) ≥65 and an increase in lactate concentration more than two mmol/L, despite adequate fluid resuscitation. [4] 65The Sepsis-related Organ Failure Assessment (SOFA) score is widely used in the critical care setting and is a reliable tool to characterize septic patients clinically, but it requires some laboratory investigations. [4]

An important factor in optimizing survival rates is the speed of diagnosis, allowing rapid, effective intervention.[5-7] However, diagnosing sepsis is not always straightforward, especially in critically ill patients who often have complex ongoing disease processes. Many of these patients will also recently have received antimicrobial therapy that can render microbial cultures negative, even when cultures are positive, results are time consuming so it may delay the diagnosis. [8-

10] The traditional approach to sepsis diagnosis was based on clinical signs and symptoms (or markers) of sepsis, such as fever, tachycardia and tachypnea, supported by relevant microbiological data. More recently, biological laboratory markers (biomarkers) have been used, ranging from the relatively simple white blood cell count (WBCs) and C-reactive protein (CRP) to more complex biomarkers, such as procalcitonin (PCT) or cytokine levels. [11]

Importantly, all of these markers are more helpful at ruling out than at ruling in an infection. Virtually, all patients in ICU have some inflammatory response associated with fever at one time or another, but these responses do not all require antibiotic administration. Hence, sepsis biomarkers could be helpful to decrease the use of antibiotics or unnecessary diagnostic tests, such as computerized tomography (CT) scans, to identify a source of sepsis. [11] In addition to aid diagnosis, biomarkers of sepsis can potentially be used for prognostication to predict the development of organ dysfunction, to guide antibiotic therapy and to evaluate the response to therapy. [12] The need for such biomarkers in sepsis was also recognized, and the literature began to see increasing numbers of papers related to potential biomarkers. Indeed, as the pathophysiology of sepsis began to be unraveled, multiple potential candidate biomarkers were proposed and tested. More than 170 different compounds have been suggested as potential biomarkers of sepsis [12] and one of them is serum calprotectin.

Calprotectin is a calcium and zinc binding protein found predominantly in the cytosol of neutrophils where it accounts for 30 –40% of the protein content [13, 14]. Calprotectin is a complex consisting of one S-100: A8 and one S-100: A9 molecule or multimeres of these molecules[15]. The protein is released when the neutrophils are activated and the granule content released. [16] Calprotectin has several functions intracellularly and extracellularly. The intracellular functions involve the activation of the neutrophilic NADPH-oxidase and modulation of the cell cytoskeleton during migration of phagocytes [17-19] The extracellular effects include pro-inflammatory, antimicrobial, oxidant scavenging and apoptosis-inducing activities. [20, 21] When neutrophils and monocytes are stimulated, they respond by secreting calprotectin into the extracellular fluid. [22] Calprotectin is a potent acute phase reactant with increases of more than 100 fold during inflamed conditions.[23] In vitro studies have showed that calprotectin has both bacteriostatic and fungistatic properties and is resistant to enzymatic degradation. Calprotectin enters into pus and abscess fluid during neutrophil cell death, along with other antimicrobial proteins. [24]

2. Methods

After the approval by the Medical Ethics Committee of Alexandria Faculty of Medicine, we conducted this prospective observational study from January 2016 to January 2017. Informed consents was taken from patients or their next of kin. We enrolled all adult patients admitted to the ICU with diagnosis of shock, patients were subdivided into two groups: septic and non-septic group. We excluded all pregnant females, patients above 65 years, hemodynamically stable patients, immunocompromised patients, patients with inflammatory bowel diseases and patients with Rheumatoid arthritis. Any patient was with shock of mixed origin was excluded from the study.

All enrolled patients (n=50) were subjected to microbiological culturing (sputum, blood and urine cultures, cultures of secretions and tissue, Gram stain and urine analysis) within 6 hrs of admission and prior to any antibiotic administration. Then we classified patients into two groups. Septic group was formed of 25 patients with well-defined septic shock with positive cultures. Non-septic group was formed of 25 patients with negative cultures, active bleeding, poor systolic function, or the shock state was proved to be non-septic in origin. Cardiogenic shock patients were presented with acute myocardial infarction or cardiotoxic substances ingestion with documented echocardiography of poor systolic function and positive cardiac enzymes. Obstructive shock patients were presented with tension pneumothorax due to direct chest trauma with documented chest radiographs and clinical signs and symptoms. Hypovolemic shock patients were presented with active evident gastrointestinal bleeding or traumatic bleeding either internal documented by abdominal ultrasonography and CT or evident external bleeding. All non-septic group patients had no clinical evidence of any infections.

We collected all data about patient's demographics, principal diagnosis and all clinical, laboratory and radiological parameters at time of enrollment. Initial severity of illness was determined using Simplified Acute Physiology II Score (SAPS II) [25], Acute Physiology and Chronic Health Evaluation II score (APACHE II) [26] and Sequential organ failure assessment score (SOFA) [27] and Quick SOFA (qSOFA). [4] Blood samples for Serum Calprotectin (SC), C-reactive protein (CRP) and white blood cell count (WBCC) in the first 6 hours of ICU admission and re-obtained again on day 3. SC was obtained in serum evacuated separator tubes and centrifuged for the separation of serum and processed on the same day. It was measured using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA).

All patients were followed-up from time of enrollment till the day of discharge or demise and evaluated by, complete physical examination, laboratory investigations (complete blood count (CBC) with differential, red cell distribution width (RDW) and mean platelet volume (MPV), coagulation studies (e.g., prothrombin time [PT], activated partial thromboplastin time [aPTT]), blood chemistry (e.g., sodium, chloride, magnesium, phosphate, glucose), renal and hepatic function tests (e.g., creatinine, blood urea nitrogen, bilirubin, alanine aminotransferase, aspartate aminotransferase, albumin, lipase), CPK: Creatine phosphokinase, ABG and Pao_2/ Fio_2), radiological investigations (chest, abdominal, extremity radiography, abdominal ultrasonography and CT of the abdomen).

Data were collected onto an electronic spreadsheet and Statistical Package (Version 24, SPSS) was used for statistical analyses. The Kolmogorov- Smirnov test was used to verify the normality of distribution of variables. Descriptive statistics were reported as raw percentages or means and standard deviations. A Student's t-test or Mann-Whitney test was used when appropriate to compare means for parametric or non-parametric data respectively. A chi-square test or Fisher's exact test was performed for comparison of categorical variables. $P < 0.05$ was considered statistically significant. Receiver operating characteristic curve (ROC) was used to determine the diagnostic performance of the markers. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. Agreement between markers was done using Sensitivity, Specificity, PPV and NPV. Significance of the obtained results was judged at the 5% level.

3. Results

Regarding the main patient's characteristics, there was no statistically significant difference in sex of the two studied groups (*p* value=0.777). Mean age was significantly lower in non-septic group patients than septic patients (39.00 Vs 53.88 years, *p*<0.001). There were no any statistically significant differences between the two groups regarding SAPS II, APACHE II and SOFA score (p values = 0.768, 0.367, 0.06 respectively). (Table 1).

Table 1. The baseline characteristics of the two studied groups.

	Septic Group (n=25)	Non-Septic group (n=25)	*Test of sig.*	*p value*
Sex				
Male	14	13	χ^2= 0.081	0.777
Female	11	12		
Age (years)				
Mean (Range)	53.88 (23.0 – 65.0)	39.0 (20.0 – 65.0)	t= 4.164	<0.001*
SAPS II				
Mean (range)	62.52 (33.0 – 89.0)	61.28 (35.0 – 82.0)	t=0.297	0.768
APACHE II				
Mean(range)	22.04 (9.0 – 35.0)	20.36 (9.0 – 33.0)	t=0.912	0.367
SOFA Score				
Mean(range)	11.48 (7.0 – 16.0)	10.12 (3.0 – 16.0)	t=2.009	0.06

Data are presented as mean (range), n (percentage) as appropriate. Significance testing was performed by:
χ^2, p: χ^2 and p values for Chi square test for comparing between the two groups
t, p: t and p values for Student t-test for comparing between the two groups
*: Statistically significant at p ≤ 0.05

Regarding main outcomes, there was no statistically significant difference in the failure of weaning from vasopressor between septic and non-septic groups (12 patients (48%) Vs 14 (56%), *p*=0.571). There was no statistically significant difference in 7-days in ICU mortality between septic and non-septic groups (17 patients (68%) Vs 15 (60%), *p*=0.556). Regarding the source of infection in the septic group, Pneumonia was the leading cause (84%), followed by urinary tract infections (48%), then diabetic foot infection (16%), then catheter related infection (8%) and at last Intra-abdominal sepsis (4%). Regarding the etiology of shock in the non-septic group, cardiogenic shock due to toxicological substance was the leading cause (44%), followed by Post-MI cardiogenic shock (24%) and hypovolemic shock due to bleeding (24%) then obstructive shock due to tension pneumothorax (8%).

Regarding main study marker, serum calprotectin (SC) measured on day 1 was significantly higher in the septic group than the non-septic group (*p*<0.001), the median SC level in the septic group was 4.5 µg/dl while in the non-septic group was 1.5 µg/dl. CRP was significantly higher in the septic group than the non-septic group (*p*<0.001), the median serum CRP in the septic group was 140 mg/dl while in the non-septic group was 5 mg/dl. WBCC was not statistically different between the two groups (*p*=0.67).

Receiver operating characteristic (ROC) curve was constructed to evaluate the ability of SC, CRP and WBCC to identify septic shock. The area under the curve (AUC) for SC, CRP and WBCC were 0.973 (CI 0.937-1.0; *p*<0.001), 0.976 (CI 0.939-1.0; *p*<0.001) and 0.542 (CI 0.379-0.704; *p*=0.614) respectively. (figure 1) For SC, the best cut-off value of 2 µg/dl showed sensitivity 92% and specificity 84% with positive predictive value 85.2 and negative predictive value 91.3, while for CRP, best cut-off value of 30 mg/dl showed sensitivity 92% and specificity 80% with positive predictive value 82.14 and negative predictive value 90.91. (Table 2)

Table 2. *Agreement (sensitivity, specificity and accuracy) for study markers with identification of septic shock.*

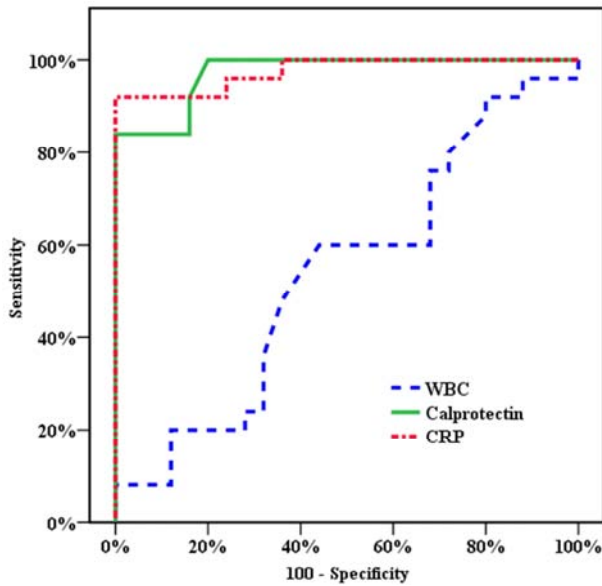| 1st day	Cut off	Sensitivity	Specificity	PPV	NPV
WBCC	>11	76.0	32.0	52.8	57.1
SC	>2	92.0	84.0	85.2	91.3
CRP	>30	92.0	80.0	82.14	90.91

Figure 1. ROC curve analysis for study markers in the diagnosis of sepsis.

Regarding the results of SC, CRP and WBCC On day 3 with SOFA score, Delta (Δ) of all these values were calculated and correlated with weaning form vasopressor and 7 days ICU mortality. ROC curve was constructed to evaluate the ability of Δ SC, Δ CRP, Δ WBCC and Δ SOFA score to predict the prognosis in the septic group of patients. As regards weaning from vasopressor, the AUC for Δ SC, Δ CRP and Δ WBCC were 0.764 (CI 0.560-0.968; *p*<0.028), 0.719 (CI 0.505-0.933; *p*<0.069) and 0.587 (CI 0.349-0.825; *p*=0.587) respectively. Δ SOFA score was 0.927 (CI 0.813-1; *p*<0.001). (figure 3) Δ SC best cut-off value of >-0.4 showed sensitivity and specificity of 83.33% and 61.54% respectively, while Δ SOFA score, best cut-off value of >-1 showed sensitivity and specificity of 91.67% and 92.31% respectively (table 3)
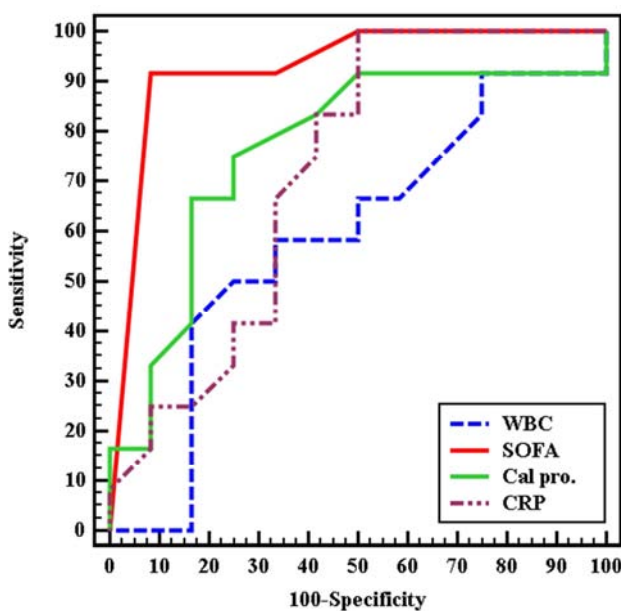

Figure 2. ROC curve analysis for study markers in predicting failure of weaning of vasopressor in septic group patients.

Table 3. *Agreement (sensitivity, specificity and accuracy) for changes of SOFA and SC with failure of weaning of vasopressor.*

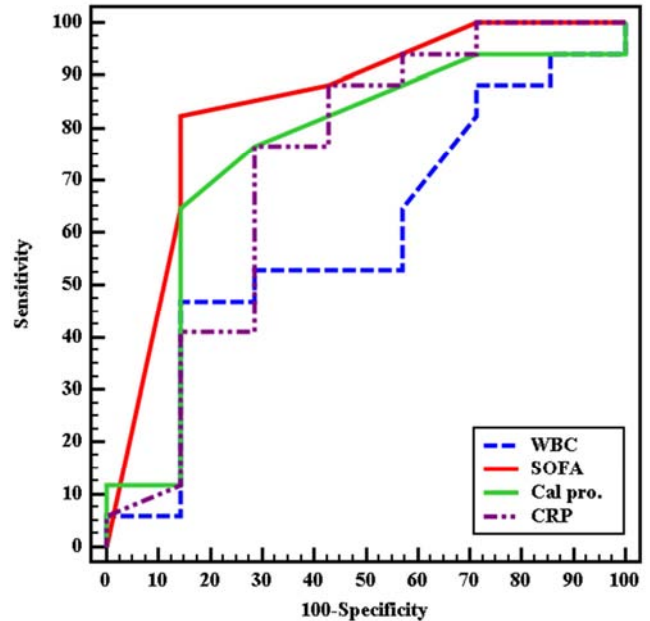| Delta of	Cut off	Sensitivity	Specificity	PPV	NPV
SOFA Score	>-1	91.67	92.31	91.7	92.3
SC	>-0.4	83.33	61.54	66.7	80.0

Figure 3. ROC curve analysis for study markers in predicting mortality in septic group patient.

As regards mortality, the AUC for Δ SC, Δ CRP and Δ WBCC were 0.752 (CI 0.516-0.988; *p*<0.057), 0.727 (CI 0.470-0.984; *p*<0.086) and 0.592 (CI 0.338-0.847; *p*=0.485) respectively. Δ SOFA score was 0.845 (CI 0.651-1; *p*=0.009). (figure 3) Δ SC best cut-off value of >-0.4 showed sensitivity and specificity of 76.47% and 75% respectively while Δ SOFA best cut-off value of >-1 showed sensitivity and specificity of 82.35% and 87.5% respectively (table 4)

Table 4. *Agreement (sensitivity, specificity and accuracy) for changes of SOFA score and SC with mortality.*

Delta of	Cut off	Sensitivity	Specificity	PPV	NPV
SOFA	>-1	82.35	87.50	93.3	70.0
SC	>-0.4	76.47	75.0	86.7	60.0

4. Discussion

Up to the present day, little is known about the course of serum calprotectin (SC) levels over time and its relationship with development of sepsis, although it was suggested by several studies as a potential marker for diagnosis in neonatal sepsis [28-30]. Only one study up till now studied the value of SC in identifying sepsis in adult in post-operative patients. [31] In our study we tried to highlight the diagnostic and prognostic value of SC in septic shock and compare it with traditional sepsis markers as WBCC and CRP. SC and CRP were significantly higher in the septic group than the non-septic, while WBCC was not. as regards SC and CRP, the AUC were nearly equal 0.973 (p<0.001) and 0.976 (p<0.001)

respectively.

The only study to evaluate the value of serum calprotectin in identifying sepsis was carried among 162 patients admitted to 10-bed Surgical ICU after major operations between January 2015 and January 2016. According to whether the patients developed sepsis within postoperative 7 days, the patients in the study group were further divided into the sepsis subgroup and the non-sepsis subgroup. Blood samples were collected from patients on postoperative days (POD) 1, 2, 3, 5, and 7. SC was measured. The results showed that The SC levels were significantly higher in septic patients compared with those without sepsis on POD 1, 2, 3, 5, and 7, there was a significantly higher level of SC on POD 3 compared with those at other time points. The ROC curves showed that ΔSC1–3 was significantly lower in the non-sepsis subgroup compared with that of the sepsis subgroup. The area under the curve on ΔSC1–3 was 0.86. More specifically, using an optimal cutoff point of 2.5 μg/mL ΔSC1–3 value was able to differentiate patients with sepsis from those without sepsis. In addition, the diagnostic accuracies of ΔSC1–3 (sensitivity 87%, specificity 89%) for new-onset sepsis were greater than other ΔSCs. [31]

CRP was widely studied as a marker for sepsis with variable results, several studies suggested a diagnostic value in identifying sepsis. However, different cut-off values were suggested, Póvoa P et al suggested cut-off value of 50 mg/l or more was highly suggestive of sepsis with sensitivity 98.5% and specificity 75% [32], in another study conducted included 112 ICU patients, it was reported that a serum CRP concentration of >8.7 mg/dl had a sensitivity of 93.4% and a specificity of 86.1% for identifying sepsis. [33] while another one conducted on 255 patients, cut-off value of 128 mg/l was suggested for identifying sepsis with sensitivity, specificity, 61% and 87%, respectively. However, it was not useful in distinguishing sepsis from severe sepsis or septic shock. [34] Other studies contradicted with these results, Barati M. et al showed that CRP did not have significant difference between septic and non-septic burn patients [35], Also In another study conducted on 75 patients over a 12-month period to test diagnostic and prognostic value of procalcitonin and CRP in septic shock, CRP level was the same between both groups. i.e it was not helpful in identifying septic shock [36].

In this study total WBCC failed to show significant difference between the two groups, the AUC was 0.542 (p 0.614), the cut-off value 11 c/mm3 which was previously assumed as a part of SIRS to help in diagnosis of sepsis[37] showed sensitivity and specificity of 72% and 32% respectively. SIRS was criticized of being a tool for identifying sepsis by 2 studies, either for being too nonspecific and having poor discriminant validity [38], or being not that sensitive for identification of septic patients and having poor concurrent validity. [39]

This study also tried to evaluate the prognostic value of these markers, as regards weaning from vasopressor, Δ SC was the only marker to show significant correlation (p=0.028), with sensitivity its optimum cut-off value of >-0.4 μg/dl was 83.33%, with NPV 80.0%, along with of Δ SOFA

having sensitivity at its optimum cut-off value of >-1 μg/dl was 91.67%, with NPV 92.3%, while as regards mortality in 7 days, Δ SC was the only marker to show relatively significant correlation (p=0.057), with sensitivity its optimum cut-off value of >-0.4 μg/dl was 76.47%, with NPV 60.0%, along with of Δ SOFA having sensitivity at its optimum cut-off value of >-1 μg/dl was 82.35%, with NPV 70%. This agreed with a study for evaluation the diagnostic and prognostic evaluation of the different biological markers (Procalcitonin PCT, CRP and WBCC) conducted on 88 patients with septic shock [40], Plasma PCT and CRP were determined, together with the leukocyte count upon admission to the ICU and after 72 h and calculated the clearance of each marker based on the following formula: [(initial value − final value/initial value) × 100]. CRP and WBCC did not correlate with mortality unlike PCT. In the ROC curve analysis, the best area under the curve corresponded to PCT clearance (0.79), which exceeded that corresponding to CRP (0.64) and leukocyte count (0.60) [41], also in another study where 75 patients were included in the study, of whom 62 were classified as having septic shock [37] and 13 non-septic shock. Among patients with septic shock, those who died in the ICU had significantly higher PCT concentrations than those who were alive at ICU discharge, at all the assay time points whereas CRP was not helpful in predicting mortality [36].

SOFA score was widely studied as a prognostic marker in sepsis. [42]. In a study was carried on 248 subjects aged admitted to the ICU showed that a positive relationship was found between the Δ SOFA (change in SOFA from day 1 to day 3) and in-hospital mortality (p< 0.001). Subjects with a Δ SOFA of ≥ 2 points had a two-fold higher mortality rate (42%) than did the entire cohort (21%). Any increase in Δ SOFA (score worsened over 72 hours) was associated with a 35% in-hospital mortality rate. [43] at last but not least, The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) suggested that organ dysfunction can be represented by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more, which is associated with an in-hospital mortality greater than 10%. [4]

Regarding prognosis, SC showed a good correlation with weaning of vasopressor (AUC was 0.764; p<0.028), while it showed relative correlation with 7-days in ICU mortality (AUC was 0.752; p<0.057) compared with other markers in the study. The main limitation of this study is that it is a single-center study. A multicenter study may be required to extrapolate these findings to other clinical settings, the sample size was small (50 patients), further evaluation of SC and comparison with established sepsis markers as PCT should be studied.

5. Conclusion

Serum calprotectin (SC) may aid in rapid identification of septic shock from non-septic shock at a cutoff of 2 μg/dl (sensitivity 92% and specificity 84%, positive predictive

value 85.2 and negative predictive value 91.3), so helping initiate timely antibiotic therapy, whereas reducing inappropriate use of antibiotics and unnecessary radiological studies. Also the change in SC level may aid in prognostication of septic shock and may give a clue about the failure of the ongoing therapy. However, because the number of patients in this study was only 50, further studies would be required to substantiate these findings.

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