

# Diagnostic Value of Presepsin in Neonatal Sepsis

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Presepsin has recently been described as biomarker of sepsis. The aim of this study was to investigate the diagnostic accuracy of presepsin in diagnosing neonatal sepsis and discriminating sepsis from non-infectious systemic inflammatory response syndrome (SIRS). The study included 70 full term neonates divided into three groups: 1) Sepsis group (22 patients with clinically suspected sepsis and positive blood cultures) 2) Non-infectious SIRS group (28 patients with clinically suspected sepsis and persistently negative blood cultures) 3) Control group (20 healthy neonates without any clinical signs of infection). Plasma presepsin level was measured by chemiluminescent enzyme immunoassay (CLEIA) and results were compared with that of C-reactive protein (CRP) assay. The results revealed that presepsin levels were significantly higher in sepsis group than in non-infectious SIRS group and controls ( $P < 0.001$ ). The area under the receiver operating characteristics (ROC) curve (AUC) for discriminating sepsis from non-infectious SIRS patients was 0.990 for presepsin and it was significantly higher than that of CRP (0.804). The best cut-off value for presepsin was 812 pg/ml, which was associated with sensitivity, specificity and negative predictive value of 95.5%, 91.7% and 97.8% respectively. In conclusion, presepsin is a sensitive and accurate biomarker and is useful for the diagnosis of sepsis and discrimination from non-infectious SIRS in neonates.

Neonatal sepsis is a systemic inflammatory response syndrome (SIRS) caused by infection, and it is a significant contributor to morbidity & mortality (Okamura, 2015). Beyond being a life-threatening condition, sepsis can cause sequelae in survivors and significantly impairs neurodevelopmental outcome. Because the disease can progress rapidly to septic shock and multiple organ dysfunction syndrome, early diagnosis and treatment are crucial to improve survival (Pugni *et al.*, 2015). Similarly, ruling out sepsis in SIRS patients has great benefits, including reduction of hospitalization and limit the unnecessary use of antimicrobial therapy (Romualdo *et al.*, 2014).

The accurate and timely diagnosis of septicemia in the neonatal population is challenging and problematic. Although blood culture has been considered as the gold standard, this analysis is still too slow and limited by false negative results (Mishra *et al.*, 2006). Also, the use of C-reactive protein (CRP), the most widely used sepsis

biomarker, is hampered by a physiological 3-day increase, resulting in a low sensitivity to detect sepsis at an early stage, therefore, there is a gap between the rapid clinical evolution of a systemic inflammation to severe sepsis/septic shock and changes in CRP serum level. CRP also lacks the specificity to consistently discriminate between infections and non-infectious inflammatory conditions (Hofer *et al.*, 2012).

As such, there is much interest in developing rapid, sensitive and reliable biomarkers to differentiate between infected and non-infected newborns. An approach that has gained particular attention is detection of soluble CD14 subtype (sCD14-ST: named as presepsin) which is a soluble form of CD14 (Mussap *et al.*, 2012).

Cluster of differentiation 14 (CD14) is a glycoprotein expressed on the membrane surface of various cells, such as monocytes, macrophages and granulocytes. It serves as a pattern recognition receptor for bacterial molecules, namely lipopolysaccharides (LPS) from Gram-negative bacteria and

peptidoglycans together with lipoteichoic acid from Gram-positive bacteria, activating the toll-like receptor 4 (TLR4)-specific pro-inflammatory signaling cascade against infectious agents. After TLR4 activation, the complex LPS-CD14 is internalized into a phagolysosome. Soluble forms of CD14 (sCD14) are produced and released into circulation either by secretion following phagocytosis or through proteolytic cleavage on activated monocytes (Bas *et al.*, 2004). Presepsin is a 13-kDa truncated form of sCD14 consisting of 64 amino acid residues. This truncated form, also directly synthesized and secreted by the liver (Zou *et al.*, 2014).

Since one of the production mechanisms of presepsin is related to the phagocytosis of bacteria, the biological characteristics of presepsin are different from those of other inflammatory markers. Presepsin has three features in comparison with procalcitonin (PCT), CRP, and interleukin-6 (IL-6): 1) Presepsin can be detected earlier after the onset of infection; 2) Presepsin is not affected by severe trauma, severe burn, or invasive surgical procedures, which lead to SIRS, therefore, it is specifically expressed in sepsis; 3) Presepsin levels reflect the clinical condition of septic patients (Okamura, 2015).

In recent years, several authors evaluated the role of presepsin as a biomarker in adults. Based on the results of these studies, presepsin appears to be a promising biomarker in the diagnosis of sepsis, as well as for assessing the severity and predicting the outcome in septic patients (Endo *et al.*, 2012, Vodnik *et al.*, 2013, Romualdo *et al.*, 2014, Masson *et al.*, 2015). Nevertheless, very few studies were conducted in pediatric population (Mussap *et al.*, 2012, Poggi *et al.*, 2015, Topcuoglu *et al.*, 2015, Mussap *et al.*, 2015).

The aim of our study was to investigate the diagnostic accuracy of presepsin in diagnosing neonatal sepsis and in

discriminating sepsis from non-infectious SIRS.

## Subjects and Methods

### Study Design and Population

This is a prospective study, conducted during the period from June 2015 to January 2016. The study included 50 full term neonates with suspected blood stream infections admitted in Neonatal Intensive Care Unit (NICU) at Benha University Hospital and 20 healthy neonates age and sex matched collected from neonatal outpatient clinic coming for follow up served as a control group.

Exclusion criteria were congenital malformations, previous antibiotic treatment, and refusal of parental consent. Permission from the Research Ethics Committee in Benha Faculty of Medicine and an administrative permission were obtained before conducting this work. A written informed consent (in Arabic language) was obtained from the neonate's guardian before participation.

Suspicion of sepsis by the caring neonatologists was based on the criteria and definitions of the International Pediatric Sepsis Consensus Conference guidelines (Goldstein *et al.*, 2005).

Blood was sampled at the first time of suspicion of sepsis for blood culture, complete blood count (CBC) (for calculation of hematological sepsis scoring which used as indicator of sepsis) and for measuring presepsin and CRP prior to the initiation of empirical antibiotic therapy or any medical treatment.

According to clinical data, hematological sepsis scoring (HSS) and results of blood culture, patients with suspected sepsis were divided into:

- 1) Sepsis group: 22 patients with clinically suspected sepsis,  $HSS \geq 3$  (range:3-7) (mean  $\pm$  SD:  $4.36 \pm 1.17$ ) and positive blood cultures.
- 2) Non-infectious SIRS (28 patients with clinically suspected sepsis,  $HSS < 3$  (range:0-2) (mean  $\pm$  SD:  $0.857 \pm 0.803$ ), with persistently negative blood cultures and without any evidence of local infections. Local infections were excluded by complete systemic clinical and microbiological examination and radiological investigations.

### Measurement Methods

- CRP Assay

CRP was immediately measured in serum by nephelometry (Turbox CRP Orion Diagnostica Cat. No.

67559). It is considered positive when the titer is  $>6$  mg/L.

- **Presepsin Assay**

Plasma samples for presepsin were immediately aliquoted, frozen and kept at  $-80^{\circ}\text{C}$  until tested. Presepsin levels were measured with a commercially available chemiluminescent immunoassay on a PATHFAST immunoanalyzer (PATHFAST®, Mitsubishi Chemical Medience Corp., Tokyo, Japan).

The test principle is based on non-competitive chemiluminescent enzyme immunoassay (CLEIA). During incubation of the sample with alkaline phosphatase labeled anti-presepsin polyclonal antibody and anti-presepsin monoclonal antibody coated magnetic particles, the presepsin of the sample binds to the anti-presepsin antibodies forming an immune-complex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound substances, a chemiluminescent substrate is added. After a short incubation, the luminescence intensity generated by the enzyme reaction is measured. The luminescence intensity is related to the presepsin concentration of the sample which is calculated by means of a standard curve.

- **Blood Culture**

Using BD BACTEC Ped Plus/ F Culture vials, Soybean-Casein Digest Broth with Resins (BD Bactec Ped Plus /F bottles) Becton Dickinson, USA. Procedure was carried out according to manufacturer: 2 ml of blood were injected into the Bactec culture vial under complete aseptic conditions. The inoculated Bactec culture vials were placed in the Bactec 9050 fluorescent series instrument (Becton Dickinson, USA) as soon as possible for incubation and monitoring. Vials entered into the instrument will be automatically tested every 10 minutes for a difference in the fluorescence denoting presence of microorganism. Positive vials were Gram stained and subcultured on sabouraud agar, nutrient agar, blood agar and MacConkey agar and incubated in appropriate temperature and atmospheres according to established methods. Full identification of organisms was done with standard bacteriological and biochemical methods.

- **Complete Blood Count (CBC)**

CBC with differential was measured by automated cell counter system Coulter T680. The differential leucocytic counts were performed manually on leishman stained blood films. Neutrophils were classified as immature (band) forms when width of the nucleus at any constriction was not less than one third of its widest portion and hematological sepsis scoring

was calculated according to Rodwell's hematological sepsis score.

- **Hematological Sepsis Scoring System (HSS) (Rodwell *et al.*, 1988)**

The previously validated hematologic criteria were used as indicators for hematological sepsis scoring system: (1) Abnormal total leucocyte (TLC) count  $<5000$  or  $>30.000/\text{mm}^3$ , (2) Abnormal total neutrophil count, (3) Elevated immature neutrophil count, (4) Elevated immature to total neutrophil ratio  $>0.2$  (I/T), (5) Immature to mature neutrophil (I/M) ratio  $>0.3$ , (6) Platelet count less than or equal to  $150.000/\text{mm}^3$  (7) Pronounced degenerative changes in total neutrophil count. The higher the score the greater was the likelihood of sepsis. With score  $\leq 2$  the likelihood that sepsis was absent was 99 %.

### Statistical Analysis

The collected data were tabulated and analyzed using SPSS version 16 soft ware (Spss Inc, Chicago, ILL Company). Continuous variables were tested for normality using Kolmogorov-Smirnov test, using ANOVA test if normally distributed, or Man Whitney U test and Krauskal Wallis test if not normally distributed. ROC curve was used to determine cutoff values of presepsin and CRP with optimum sensitivity and specificity in prediction of sepsis using the blood culture as the gold standard.. The accepted level of significance in this work was stated at  $P < 0.05$ .

## Results

Three groups were included in this work:

- 1) Sepsis group ( $n=22$ ) with gestational age of  $38.5 \pm 0.9$  weeks and birth weight of  $3235.7 \pm 411\text{g}$ .
- 2) Non-infectious SIRS group ( $n=28$ ) with gestational age of  $38.2 \pm 1.1$  weeks and birth weight of  $3324.3 \pm 377.8\text{g}$ .
- 3) Control group ( $n=20$ ) chosen to be matched with patient groups in gestational age ( $38.4 \pm 1.2$  weeks). Their mean birth weight ( $3191 \pm 421.3\text{g}$ ).

Blood culture in the sepsis group revealed 14 Gram positive bacteria (7 Coagulase Negative Staphylococci, 4 Staphylococcus aureus, 2 Streptococcus pneumoniae, 1 Enterococcus faecalis) and 8 Gram negative bacteria (2 Pseudomonas aeruginosa, 3 Klebsiella, 3 Escherichia coli). There was no

significant difference in presepsin level between patients with Gram positive and patients with Gram negative bacteria ( $P=0.41$ ) (Table 1)

In sepsis group ( $n=22$ ), there were 9 neonates diagnosed as early-onset sepsis (EOS) (Sepsis occurring before 72 hours of

life) and 13 neonates as late-onset sepsis (LOS) (Sepsis occurring after 72 hours of life). There was no significant difference in presepsin level between EOS and LOS groups ( $P=0.89$ ) (Table 1).

Table 1. Presepsin level (pg/ml) according to onset of sepsis and type of bacterial growth on blood culture among the sepsis group ( $n=22$ ).

Variable	Median	Range	<i>P</i> value
<u>Onset of sepsis:</u>			
EOS (N=9)	1439	766 – 2108	NS
LOS (N=13)	1320	957 – 1887	
<u>Type of growth:</u>			
Gram positive organisms (N=14)	1386	823 – 2108	NS
Gram negative organisms (N=8)	1315	766 – 1925	

$P>0.05$  is non-significant (NS)

As reported in Table (2) & Figure (1, 2):

-When comparing sepsis group with controls, both plasma presepsin and serum CRP levels were found to be significantly higher in sepsis group than controls ( $P < 0.001$  for both biomarkers).

-When comparing non-infectious SIRS with controls, presepsin level showed non statistically significant difference between the two groups ( $P=0.43$ ), however, CRP was

significantly higher in non-infectious SIRS group than controls ( $P < 0.001$ ).

- When comparing sepsis group with non-infectious SIRS group, although the level of presepsin was significantly higher in sepsis group than non-infectious SIRS group ( $P < 0.001$ ), CRP showed no statistically significant difference between the two groups ( $P=0.086$ ).

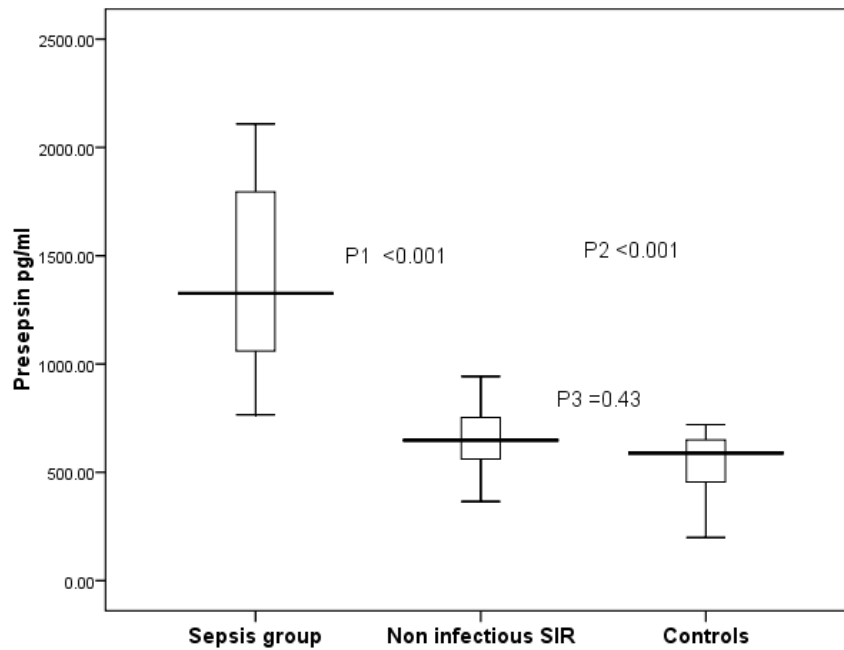
Table 2. Comparison of blood presepsin and CRP median levels and ranges between sepsis, non-infectious SIRS and control groups

Variable	Sepsis group (N=22)		Non-infectious SIRS group (N=28)		Control group (N=20)		<i>P</i> value
	Median	Range	Median	Range	Median	Range	
Presepsin (pg/mL)	1326.5†‡	766-2108	648.5	365-943	588.5	199-720	<0.001
CRP (mg/L)	39.75†	10.4-69	30.3†	5.8-61	3.3	0.6-8.1	<0.001

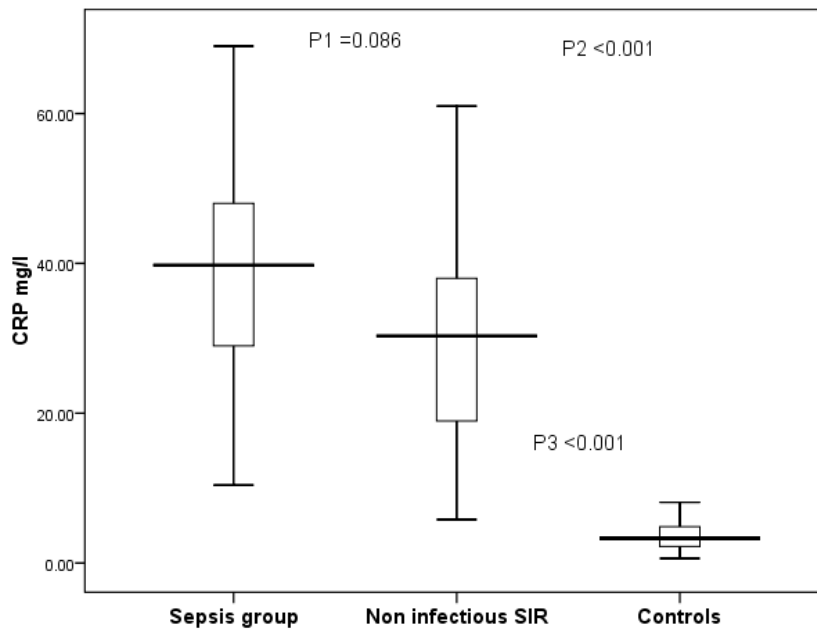
†→Significant in comparison with controls

‡→ Significant in comparison with non-infectious SIRS group

$P < 0.05$  is significant



**Figure 1.** Comparison of plasma presepsin levels (median and ranges) between sepsis, non-infectious SIRS and control groups.  $P_1$ → between sepsis group and non-infectious SIRS group,  $P_2$ → between sepsis group and controls,  $P_3$ → between non-infectious SIRS group and controls

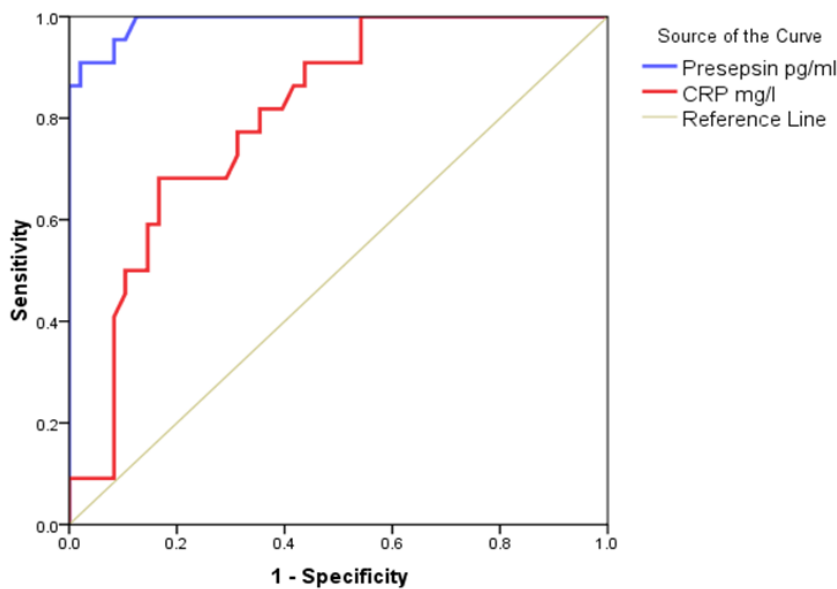


**Figure 2.** Comparison of serum CRP levels (median and ranges) between sepsis, non-infectious SIRS and control groups.  $P_1$ → between sepsis group and non-infectious SIRS groups,  $P_2$ → between sepsis group and controls,  $P_3$ → between non-infectious SIRS group and controls

Sensitivity and specificity of presepsin plasma levels for diagnosing sepsis in patients were determined (using the blood culture as gold standard) and Receiver-operating characteristic (ROC) curve was constructed. In a comparative study, of non-infectious group (controls and non-infectious SIRS group) and sepsis group, with CRP, the area

under the curve (AUC) of presepsin was 0.990, which was higher than the AUC of CRP (0.804) (Figure 3).

The ROC analysis showed that at the cut-off value of 812 pg/ml, presepsin may be able to discriminate between patients with and without sepsis with sensitivity and specificity of 95.5% and 91.7%, respectively (Table 3).



**Figure 3.** ROC curve for the diagnostic performance of presepsin and CRP in discrimination between patients with and without sepsis.

Table 3. Performance of the studied biomarkers in the diagnosis of sepsis.

Variable	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	AUC	95%CI	P value
Presepsin	95.5%	91.7%	84%	97.8%	92.8%	0.990	0.97-1.0	<0.001
CRP	81.8%	64.6%	51.4%	88.6%	70%	0.804	0.7-0.91	<0.001

*P* < 0.05 is significant.

## Discussion

In NICU the diagnosis of systemic infection and sepsis is often inconsistent. Accordingly, all episodes of SIRS are assumed to be of infectious etiology until microbiological confirmation, leading to a number of adverse

effects: firstly, an unnecessary and inappropriate use of antibiotics for all the babies without a bacterial infection; secondly, an unnecessary prolonged stay in NICU, leading to the exposure to further risk factors potentially compromising baby's outcome. On

the other hand, clinical course of neonatal sepsis may suddenly progress toward shock, disseminated intravascular coagulation (DIC), and death within very few hours from the onset of the disease (Mussap *et al.*, 2015). Consequently, the need of an early and accurate diagnosis of neonatal sepsis and the need to accurately discriminate non-infectious SIRS from sepsis is crucial in neonatology (Piantino *et al.*, 2013). Soluble CD14-ST presepsin was proposed as a new very sensitive biomarker of sepsis; unfortunately, very few studies published in the literature investigated the clinical value of sCD14-ST presepsin in neonatology (Mussap *et al.*, 2012).

Our study is one of a few studies that investigate the possible role of presepsin in the diagnosis of sepsis in neonates. Our results clearly show that presepsin blood level is significantly higher in babies with sepsis than that in controls. This result come in agreement with previous studies in adults (Shozushima *et al.*, 2011, Endo *et al.*, 2012, Vodnik *et al.*, 2013) as well as with previous studies in neonates (Poggi *et al.*, 2015, Topcuoglu *et al.*, 2015, Mussap *et al.*, 2015), all reported that presepsin level is significantly higher in septic patients than healthy controls.

Also, in agreement with previous studies (Liu *et al.*, 2013, Vodnik *et al.*, 2013), we recorded a significant increase in presepsin level in sepsis group when compared with non-infectious SIRS. However, there was a mild elevation of presepsin level in the non-infectious SIRS group, with no significant difference with that in controls. These results confirm the specific elevation of presepsin in patients with sepsis. In contrast, Mussap *et al.*, (2015), in their study, found no significant difference between septic newborns and non-bacterial SIRS newborns ( $p = 0.730$ ) and, in addition, they found that in patients with non-bacterial SIRS, presepsin blood level is

significantly higher than that in controls. They explained the elevation of presepsin level in non-bacterial SIRS group by: First, inclusion of premature babies in their study, and they suggest the hypothesis that, blood presepsin concentration can significantly increase in the course of various non-infectious conditions, commonly affecting premature babies which may be due to the monocyte/macrophage activation during the inflammatory process associated with preterm delivery. However, this explanation come in contrast to the results reported by pugni *et al.*, 2015 who measured the level of presepsin in large groups of non-septic term and preterm infants and they found non significant difference between the two groups. Second, patients included in the group of SIRS may developed bacterial infection, even without dissemination.

In our study, we tried to overcome these pitfalls and excluded the possibilities that may affect the results. First, all patients and controls enrolled in our study were full term babies. Second, we excluded any source of local or systemic infections in all patients included in our non-infectious SIRS group.

In the current study, there was no significant difference in presepsin level between EOS and LOS groups ( $P= 0.89$ ) which come in agreement with the result of Mussap *et al.*, (2015). In addition, our results confirm the results previously reported by (Endo *et al.*, 2012, Topcuoglu *et al.*, 2015) that found no relation between the presepsin level and type of growth in blood culture, also, we found no significant difference in presepsin level between Gram positive and Gram negative organisms ( $P=0.41$ ).

In the healthy group of our study, median presepsin level was 588.5 pg/ml which is comparable with that (603.5 pg/ml) reported by Pugni *et al.*, 2015, the first study that provide reference ranges of presepsin in a large group of healthy term and preterm neonates.

Although CRP is widely used to diagnose sepsis and monitor the response to antibiotic treatment in newborns, its utility is questionable because its value can increase after non-infective inflammatory events (Prashant *et al.*, 2013). Similarly, our results found non-significant increase in the level of CRP in sepsis group than in non-infectious SIRS groups ( $P = 0.68$ ).

A ROC analysis was performed for each of presepsin and CRP and their diagnostic performance for sepsis was compared. The ROC curves for these biomarkers in sepsis group and the non-infectious group (non-infectious SIRS and controls) are shown in Figure 3. The AUC of presepsin (0.990) was found to be higher than that of CRP (0.804). When the cut-off value of presepsin was set at 812pg/ml, its clinical sensitivity was 95.5% and its clinical specificity was 91.7%. The ROC curve showed that the presepsin concentration was a significantly sensitive indicator of sepsis than CRP.

This result come in concordance with that of Abdelaziz. 2013 who found that AUC of presepsin (0.97) at time of enrollment was better than that of CRP (0.68) and thereby, he concluded that presepsin (cut-off value 781pg/ml) is a more sensitive and specific sepsis marker rather than CRP and can effectively differentiate between bacterial and non-bacterial SIRS in neonates.

Similarly, our result come in agreement with that of Mussap *et al.*, 2015, their ROC analysis revealed that presepsin has a better diagnostic accuracy than CRP in discriminating septic newborns from controls with an AUC of 0.995 greater than that of CRP (0.827). Also, Poggi *et al.*, 2015, in their study, reported a high area under the curve for presepsin (0.972), indicating that presepsin is an accurate test for the diagnosis of sepsis in infants. The cutoff value with the best accuracy in their study was 885 pg/ml; at

which the specificity was 100%, and sensitivity for diagnosis of sepsis was 94%.

Based on high negative predictive value of presepsin reported by our study (97.8%) as well as by other authors (Poggi *et al.*, 2015), measurement of presepsin is most useful for excluding neonatal sepsis and hence, protect large numbers of patients from the exposure to the risk of empirical antibiotic therapy. Also, serial monitoring of this biomarker in select category of high-risk patients allows clinicians to withhold antibiotic when it becomes negative.

In contrast to blood cultures, presepsin measurement can be performed quickly and easily, not only in laboratories but also in critical care centers and intensive care units. Availability of a fully automated assay, the PATHFAST, that allows the measurement in a short time (17 minutes), will contribute to its introduction in clinical practice (Vodnik *et al.*, 2013).

In conclusion, presepsin is a sensitive, accurate and rapid biomarker and is useful as a diagnostic tool for the diagnosis of neonatal sepsis. A more extensive studies and more information are needed about its reference range in this category of population.

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