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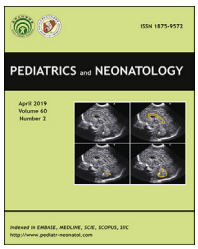

Original Article

Validity of biomarkers in screening for neonatal sepsis — A single center — hospital based study

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Key Words
high sensitivity CRP;
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neonatal sepsis;
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Background: The diagnosis of neonatal sepsis still considered to be a challenge for both clinicians and the laboratory due to the non-specific clinical presentations. The present study aimed to compare and assess the diagnostic & prognostic values of C-reactive protein (CRP), high sensitivity CRP (hsCRP), presepsin, interleukin-6 (IL-6) and procalcitonin (PCT) in neonatal sepsis separately and in combination.
Methods: This hospital-based cross-sectional study has been conducted on 168 neonates recruited from the neonatal intensive care unit (NICU) of Qena University Hospitals, Upper Egypt. Measurements of CRP using latex agglutination test, hsCRP, presepsin, IL6 and PCT assays using commercially available ELISA assay kits were done to all included neonates.
Results: There were significantly higher serum levels of CRP among late onset versus early onset sepsis group with significantly higher serum levels of hsCRP and presepsin among early onset compared with the late onset sepsis group ($p < 0.05$ for all). There were significantly higher hsCRP, presepsin and PCT serum levels in proven versus probable sepsis group ($p < 0.05$ for all). Significantly higher serum levels of presepsin and PCT were noted among survivors versus non survivors sepsis group ($p < 0.05$ for all). The cutoff value of the serum level of CRP >6 mg/dl showed lower sensitivity and specificity than that of hsCRP at cutoff >140 ng/ml in diagnosing neonatal sepsis. The cutoff value of presepsin >200 ng/ml showed equal sensitivity and specificity to IL-6 at cutoff >22 pg/ml. The cutoff value of PCT at > 389 pg/ml showed sensitivity and specificity approximate to that of hsCRP.

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Conclusions: CRP could be a helpful prognostic marker in late onset neonatal sepsis. hsCRP and PCT have higher diagnostic accuracy in neonatal sepsis in comparison to other studied markers. Both IL-6 and presepsin have equal diagnostic utility in neonatal sepsis, but presepsin could be helpful diagnostic marker in early onset neonatal sepsis.

1. Introduction

Neonatal sepsis can be defined as a generalized bacterial infection of the blood that is found during the first month of life.[1] It represents a catastrophic complication if it occurs in the neonatal period as it may begin with non-specific symptoms, but severe sepsis with rapid deterioration of the neonates, clinical condition may also occur. As the early detection of the neonates susceptible to have sepsis may augment the therapeutic range and lead to better outcomes, many sepsis biomarkers have been studied to determine their utility for early detection of sepsis.[2]

C-reactive protein (CRP) is generally considered a helpful marker for diagnosis of sepsis used in addition to blood culture, but sometimes it may be inadequate for sepsis diagnosis, so more rapid efficient markers are needed.[3] CRP is an acute phase protein synthesized in liver in the presence of infectious and/or inflammatory stimuli, correlating with the disease severity,[4] but its production significantly varied during the neonatal period,[5] with lower production in preterm compared to full term neonates.[6] The high sensitivity assays of CRP (hsCRP) could detect lower grade of inflammation.[7]

Cluster differentiation 14 (CD14) is the receptor for lipopolysaccharide-lipopolysaccharide binding protein (LPS-LBP) complexes.[8] CD14 has two types: mCD14 (membrane-bound) which is expressed on the cell surface of monocytes/macrophages and neutrophils; and sCD14 (soluble) which is present in the plasma, mediating the immune response and called presepsin.[9] Although plasma presepsin level may increase in response to sepsis, its increase has been reported in many other conditions such as liver cirrhosis, diabetes mellitus and heart failure.[10] Interleukin-6 (IL-6), one among the most important cytokines, is secreted in response to infection, inducing the B-lymphocytes to secrete antibodies and enhance cytotoxic T-cells differentiation.[11]

Procalcitonin (PCT) is formed of 116 amino acids and is the precursor of hormone calcitonin produced by thyroid gland under normal conditions,[12] but also by all tissues in response to cytokines, bacterial or fungal products.[13,14]

The diagnosis of neonatal sepsis still considered to be a challenge for both clinicians and the laboratory due to non-specific clinical presentations and insufficient research regarding standardized cutoff values for various sepsis markers,[15] so the present study investigated and compared the serum levels of the previously mentioned biomarkers in neonatal sepsis, separately and in combination, to determine the most useful marker/s regarding this issue.

2. Patients and methods

2.1. Study design

A hospital-based prospective case-control study was conducted on 168 neonates recruited from the neonatal intensive care unit (NICU) of Qena University Hospitals, Upper Egypt, who exhibited clinical signs and symptoms of sepsis at the time of admission or who developed sepsis during their hospital stay according to the inclusion criteria. The included patients were subjected to three classifications: the first classification was according to the result of the blood culture, divided into two subgroups (probable, if suspected sepsis with negative blood culture and proven, if with positive blood culture)[16]; Those with proven sepsis comprised two subgroups according to the onset of sepsis (early onset sepsis if suspected in the first week of neonatal life and late onset sepsis if suspected after the first week of neonatal life)[17]; and the third was based on the outcome of the proven sepsis group, subdivided into those who improved (survivors) and those who died (non-survivors). Patients were recruited according to the guidelines laid down in the declaration of Helsinki and all neonates, parents were informed about the aim of the study and their written consent was obtained, after approval of the University Hospital ethics committee.

2.2. Patients' selection criteria

Any neonate with signs or symptoms of suspected sepsis,[1] in the form of respiratory distress, apnea, oxygen dependence, feeding intolerance, poor feeding, hypotension, shock, poor peripheral perfusion, tachycardia, lethargy, temperature instability, seizures, altered mental status, skin mottling and unexplained acidosis. Those who had any of the previous manifestations, but with clearly apparent malformations, prematurity, Apgar score less than seven, on antibiotics treatment before the start of the study were excluded. Detailed history was taken and thorough clinical examination was made of all neonates included in the study.

2.3. Laboratory workup

Routine investigations were done for all included neonates in the form of complete blood count (CBC) (Cell Dyn 1800-Abbott diagnostics, Germany), complete liver and kidney functions, blood glucose, serum electrolytes (Cobas C311

Roche diagnostics, Germany), and arterial blood gases (GASTAT-1800 Series, Germany).

Blood culture was done on all included neonates with suspected sepsis (BACTEC™ 9050 automation system, Becton Dickinson, Ireland), where, 3 cc venous blood were inoculated into BACTEC Peds Plus/F blood culture bottle under complete aseptic conditions, and blood was placed in the BACTEC™ 9050 blood culture instrument within 2 h of collection; and subcultures were done in positive cases to identify the causative organism according to the standard methods.

Three cc samples of peripheral venous blood were drawn at admission into serum separator gel tubes, and samples were allowed to clot for 30 min at 37 °C before centrifugation for 15 min at 3000 rpm. Separated sera were aliquoted into 1 ml cryo-tubes and part of the separated sera of every included neonate was used for CRP analysis immediately at all sampling times and the remainder stored at −80 °C until time of hs-CRP, presepsin, IL-6 and PCT analysis. Serum CRP level was measured by the semi-quantitative latex agglutination test (AVITEX CRP kits; Catalog No. OD023; supplied by Omega Diagnostics, UK) according to the manufacturer's guideline. The AVITEX latex particles are coated by antibodies to human CRP; when the latex suspension was mixed with the serum containing high CRP levels on a slide, agglutination was seen within 2 min, and then serial dilutions of patient's serum in positive cases using isotonic saline were done to determine the CRP values. The samples were measured in a single assay to avoid repeated freeze—thaw cycles. Commercially available enzyme-linked immunesorbent (ELISA) assay kits were used for determination of hsCRP (Catalog No.: BEK1239), presepsin (Catalog No.: BYEK2096), IL-6 (Catalog No.: BEK1108) and PCT (Catalog No.: BYEK2006). All used kits were supplied by Chongqing Biospes Co., Ltd. Assays were done using micro plate ELISA reader (EMR-500,USA).

2.4. Statistical analysis

The normality of data were tested using the Anderson-Darling test and for homogeneity variances prior to further statistical analysis. Categorical variables were described by number and percent (n,%), whereas continuous variables were described by mean and standard deviation (mean, SD). Chi-square test and Fisher's exact test were used to compare categorical variables, while Student's *t*-test and analysis of variance (ANOVA) were used to compare continuous quantitative variables of parametric data. Spearman rank correlation coefficient was used to explore the relationship between quantitative variables. Medcalc Program was used to calculate sensitivity, specificity, positive and negative predictive values. A two-tailed test was considered significant when $p < 0.05$. All analyses were performed using the IBM SPSS version 20.0 software (IBM Corporation, Armonk, NY, USA).

3. Results

The mean gestational age of the included patients (91 males and 77 females) was 38.03 weeks ± 0.60 SD with age at admission of 9.34 days ± 2.69 SD. Poor oral feeding, tachypnea, chest retractions, tachycardia, delayed capillary refill, hepatomegally, dehydration, pallor and lethargy were the most frequent manifestations among the included neonates with sepsis.

The relative frequency of the causative microorganisms of the studied neonates with sepsis according to blood culture is presented in Fig. 1A. *Klebsiella pneumonia* was the most frequent bacteria. Fig. 1B shows the relative frequency of the sensitivity of the micro-organisms to various antibiotics according to culture and sensitivity test among the studied neonates with sepsis. The higher sensitivity was for amikacin.

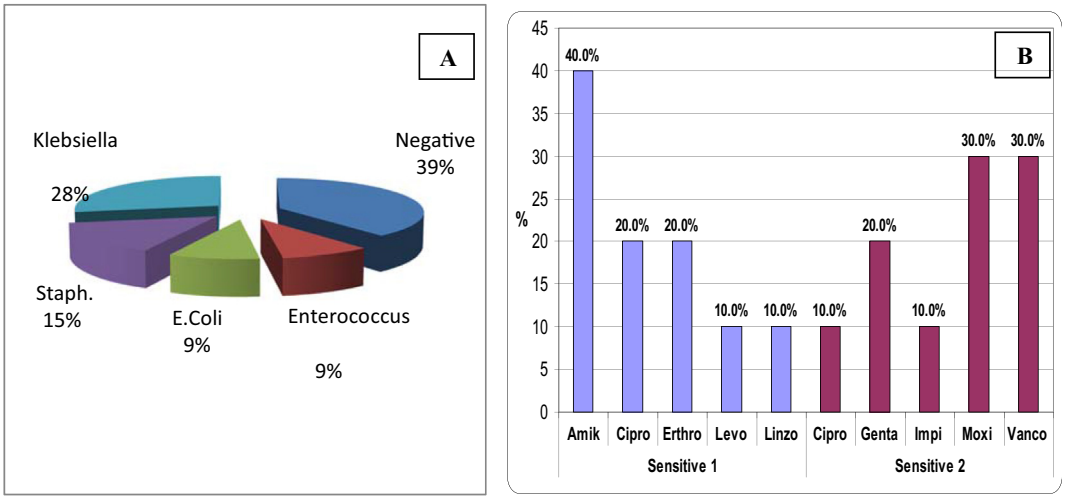

Figure 1 A) Relative frequency of the causative micro-organisms of the studied neonates with sepsis according to blood culture; B) Relative frequency of the sensitivity of the micro-organisms to various antibiotics according to culture and sensitivity test, among the studied neonates with sepsis. Abbreviations: Staph, staphylococous aureus; *E.coli*, *Eschereschia coli*; Amik, Amikacin; Cipro, Ciprofloxacin; Erthro, Erythromycin; Levo, levofloxacin; Linzo, Linezolid; Genta, Gentamycin; Impi, Imipenem; Moxi, Amoxicillin, Vanco, Vancomycin.

The mean ± SD of serum levels of the studied biomarkers among patients' subgroups according to the onset of sepsis, blood culture results and outcome of neonates with sepsis are presented in Table 1.

The significant correlations between the studied sepsis biomarkers are shown in Fig. 2, which revealed significant positive correlations between hsCRP with both presepsin ($r = 0.545$, $p < 0.05$) and procalcitonin ($r = 0.390$, $p < 0.05$). Also, there was a significantly positive correlation between presepsin and procalcitonin ($r = 0.415$, $p < 0.05$).

Receiver Operating Characteristics (ROC) Curves for the studied sepsis biomarkers are shown in Fig. 3A and B which revealed the following data: the cutoff value of CRP >6 mg/dl showed sensitivity of 79.41% and specificity of 93.33%, positive predictive value (PPV) 96.4, and negative predictive value (NPV) 66.7. Cutoff value of hsCRP >140 ng/ml showed sensitivity of 97.06% and specificity of 100.00%, PPV 100, and NPV 93.7, which showed significantly higher diagnostic accuracy than CRP ($p < 0.05$). Cutoff value of presepsin >200 ng/ml showed sensitivity of 82.35% and specificity of 100.00%, PPV 100, and NPV 71.4. Cutoff value of IL-6 > 22 pg/ml showed equal diagnostic accuracy to IL-6. Cutoff value of PCT >389 pg/ml showed sensitivity of 97.00% and specificity of 100.00%, PPV 100, and NPV 93.7 with significantly higher diagnostic accuracy of hsCRP and PCT versus CRP, IL-6 and PCT ($p < 0.05$) in neonatal sepsis diagnosis.

4. Discussion

Bacterial sepsis considered a life-threatening emergency with high mortality and morbidity in neonates.[15] Although blood culture was considered as the golden standard for diagnosis of neonatal sepsis, it is time consuming, with lower sensitivity and often high false-negative results.[18,19] In the present study, about 2/3 of the included neonates with clinical suspicion of sepsis were negative for blood culture, while, in positive blood culture cases, *K. pneumonia* was clearly the most common isolate among gram-negative bacteria. *Staphylococcus aureus* was the most common gram-positive isolate, and with the highest antibiotic sensitivity was for amikacin, amoxicillin and vancomycin. These findings were in agreement with many studies.[20–23] Although group B streptococci, which are commonly present in neonatal sepsis, weren't present in the present study, this could be explained by penicillin therapy after birth.

hsCRP (quantitative) is more sensitive than the conventional CRP (qualitative) as it can measure very low levels of CRP.[15] The findings of the present study revealed significantly higher serum levels of hsCRP and presepsin in neonates with early onset sepsis versus those with late onset sepsis, with significantly higher CRP serum levels in neonates with late onset sepsis versus those with early onset sepsis. Other studied markers revealed non-significant differences between the two subgroups. This clarifies the

Table 1 Mean ± SD of serum levels of the studied biomarkers among the patients' subgroups according to the blood culture results, the onset of sepsis and the outcome.

Studied biomarkers	Study group (n = 168)		*P*-value
	Probable sepsis (n = 66)	Proven sepsis (n = 102)	
CRP (mg/dl)	24.89 ± 11.37	24.97 ± 15.58	0.982
hsCRP (ng/ml)	1021.46 ± 467.74	1260.69 ± 478.67	0.008*
Presepsin (ng/l)	499.66 ± 150.59	598.42 ± 164.74	0.032*
IL-6 (pg/ml)	72.54 ± 27.68	83.40 ± 25.68	0.175
PCT (pg/ml)	788.22 ± 294.01	1105.81 ± 482.55	0.002*
Studied biomarkers	Proven sepsis group (n = 102)		*P*-value
	Early onset sepsis (n = 47)	Late onset sepsis (n = 55)	
CRP (mg/dl)	19.54 ± 12.02	30.95 ± 18.69	0.005*
hsCRP (ng/ml)	1224.53 ± 597.61	912.62 ± 241.67	0.010*
Presepsin (ng/l)	623.29 ± 109.67	521.06 ± 124.96	0.001*
IL-6 (pg/ml)	77.76 ± 60.25	83.58 ± 67.25	0.844
PCT (pg/ml)	1023.49 ± 378.43	1039.63 ± 550.35	0.507
Studied biomarkers	Proven sepsis group (n = 102)		*P*-value
	Survivors (n = 76)	Non- survivors (n = 26)	
CRP (mg/dl)	26.16 ± 21.86	27.00 ± 24.25	0.970
hsCRP (ng/ml)	504.57 ± 136.16	535.50 ± 125.98	0.381
Presepsin (ng/l)	261.41 ± 137.99	614.50 ± 183.02	0.002*
IL-6 (pg/ml)	42.53 ± 11.72	44.80 ± 14.78	0.504
PCT (pg/ml)	701.07 ± 615.81	1474.50 ± 305.42	0.017*

*P-value <0.05 considered significant.

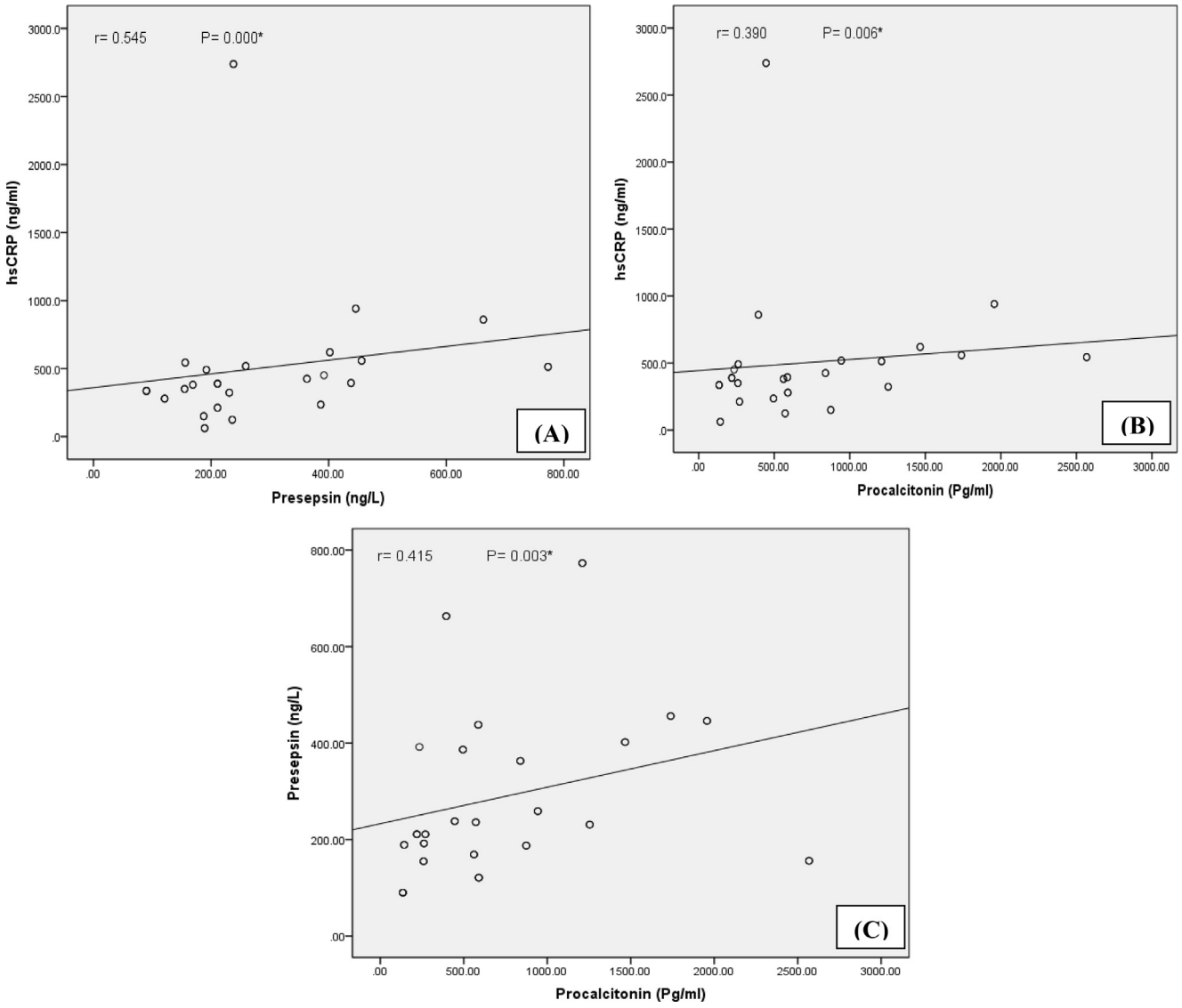

Figure 2 Correlations between the studied sepsis biomarkers among neonates with sepsis. Significant positive correlations between hsCRP with both presepsin (A) and procalcitonin (B). Significant positive correlation between presepsin and procalcitonin (C).

value of hsCRP and presepsin in diagnosing early onset neonatal sepsis (EONS) and the value of CRP in diagnosing late onset neonatal sepsis (LONS). These findings were in agreement with Abdollahi et al.,[24] Sharma et al.[25] and Spanuth et al.[26]

Regarding the utility of the studied biomarkers in combination in relation to the results of blood cultures, there were significantly higher serum levels of hsCRP, presepsin and PCT among the proven sepsis subgroup versus neonates with probable sepsis. These findings were in accordance with Boraey et al.,[21] Sharma et al.,[25] and Maurice et al.[27] The findings of the present study revealed no statistically significant difference in the CRP concentration between the 'probable sepsis' and the 'proven sepsis' subgroup, which was consistent with the finding of Khassawneh et al.[28]

The findings of the present study revealed significantly higher serum levels of PCT and presepsin among non-survivor neonates with sepsis versus survivor group with non-significant differences between the two groups as regard the other measured biomarkers, indicating that PCT and presepsin are the best prognostic markers for neonatal sepsis among the studied biomarkers. This was in agreement with many studies.[27–31]

The findings of the present study revealed significant positive correlations between PCT with both hs-CRP and presepsin and also between hsCRP versus presepsin. This was in accordance with Abdollahi et al.,[24] Spanuth et al.,[26] Maurice et al.,[27] and Sabry et al.[32]

To identify the most suitable serum marker for diagnosis of the neonatal sepsis, there need to be sufficient sensitivity and specificity and detection either in early or late stage of the disease. It is difficult to find a single marker for 3 criteria, so it is advisable to use a combination of inflammatory markers as a part of the diagnostic workup of neonatal sepsis.[24] In our study we searched for the cutoff

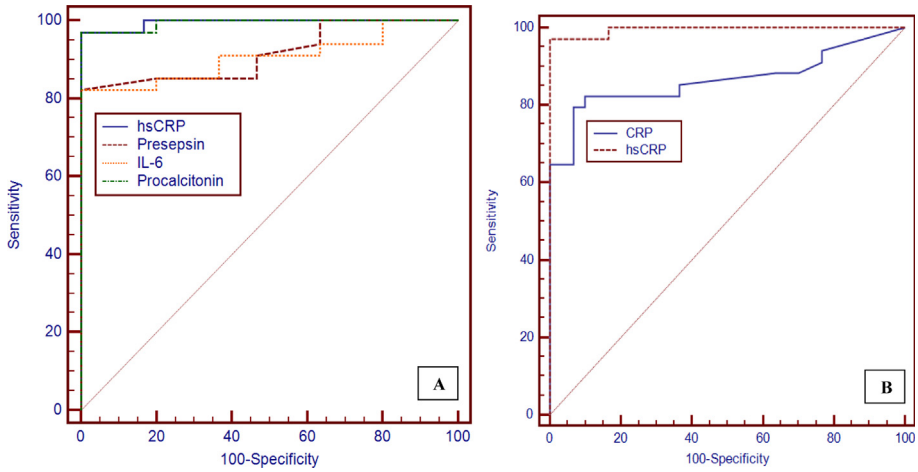

Figure 3 A) Receiver Operating Characteristics (ROC) Curves of hsCRP, presepsin, IL-6 and procalcitonin in diagnosing neonates with sepsis; B) Receiver Operating Characteristics (ROC) Curves of CRP and hsCRP in diagnosing neonates with sepsis. hsCRP vs. Presepsin-0.005*, hsCRP vs.IL-6 0.003*, hsCRP vs.Procalcitonin-0.878, Presepsin vs. IL-6-0.810, Presepsin vs. Procalcitonin-0.004*, IL-6 vs. Procalcitonin-0.001*, hsCRP vs. CRP-0.001*.

values for the studied biomarkers rather than predefining them. As regards the validity of CRP, hs-CRP, presepsin, PCT and IL-6 as calculated, in diagnosing neonatal sepsis in this study, the cutoff value of the serum level of CRP >6 mg/dl showed lower validity power than that of hsCRP at cutoff value of >140 ng/ml. The cutoff value of presepsin >200 ng/ml showed equal validity power to IL-6 at cutoff value > 22 pg/ml, with higher diagnostic utility than CRP and lower diagnostic utility than both hsCRP and PCT. The cutoff value of PCT at >389 pg/ml showed diagnostic utility approximate to that of hsCRP, so the combination of hs-CRP and PCT together with any IL-6 or presepsin (especially in EONS) will be better predictors for neonatal sepsis. These findings were in agreement with Abdollahi et al.,[24] who reported that simultaneous assays of hsCRP, IL-6 and PCT were more sensitive in diagnosing neonatal infections. Our findings were in accordance with other studies.[27,29,33–36] Al-Zahrani et al.,[22] concluded that PCT had a greater diagnostic utility than hsCRP and IL-6, and the combination of markers (hsCRP, IL-6 and PCT) was better than a single marker to diagnose neonatal sepsis. In contrast, a study done by Ganesan et al.,[15] reported that hs-CRP had very low specificity and positive predictive value compared to CRP, PCT and IL-6 in the diagnosis of neonatal sepsis, which could be explained by differences in the inclusion criteria of the patients.

5. Conclusion

The findings of the present study prove that hs-CRP, PCT and presepsin together were early, sensitive, specific diagnostic markers for neonatal sepsis. Both presepsin and IL-6 have equal diagnostic utility in neonatal sepsis. CRP was more valuable in late onset neonatal sepsis. None of the studied biomarkers (CRP, hsCRP, presepsin, IL-6, PCT) could be used individually to confirm or exclude neonatal sepsis and their combined use, together with other hematological markers (CBC and blood culture) in addition to clinical suspicion is important.

Conflicts of interest

The authors report no conflicts of interest in this work.

Acknowledgment

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