Original Article

Utility of cytokine, adhesion molecule and acute phase proteins in early diagnosis of neonatal sepsis

M. A. Fattah[1,2], Al Fadhil A. Omer[3], S. Asaif[4], R. Manlulu[5], T. Karar[1], A. Ahmed[6], A. Aljada[7], Ayman M. Saleh[8,9], Shoeb Qureshi[10], A. Nasr[7]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud Bin Abdulaziz University, Riyadh, Saudi Arabia, [2]College of Graduate Studies, Sudan University of Science and Technology, [3]Department of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan, [4]Department of Pediatrics, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences and King Abdullah International Medical Research Center (KAIMRC) National Guard Health Affairs, [5]Department of Pediatrics, King Fahad National Guard Hospital, National Guard Health Affairs, [6]Department of Epidemiology and Biostatistics, College of Public Health and Health Informatics, King Saud Bin Abdulaziz University for Health Sciences, [7]Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences and King Abdullah International Medical Research Center (KAIMRC) National Guard Health Affairs, [10]Department of Research, College of Applied Medical Sciences, King Saud Bin Abdulaziz University, [9]King Abdullah International Medical Research Centre (KAIMRC), National Guard Health Affairs, Riyadh, [8]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, KSAU-HS, Jeddah, Kingdom of Saudi Arabia

Address for correspondence:
Dr. Shoeb Qureshi, College of Applied Medical Sciences, King Saud Bin Abdulaziz University, P. O. Box 70819, Riyadh - 11577, Kingdom of Saudi Arabia. E-mail: qsab2002@yahoo.co.in

Abstract

Background and Aim: Neonatal infection, including bacterial sepsis, is a major health care issue with an annual global mortality in excess of one million lives. Therefore, this study aimed to evaluate the potential diagnostic value of C-reactive protein (CRP), E-selectin, procalcitonin (PCT), interleukins-6 (IL-6), and tumor necrosis factor-α (TNF-α) both independently and in combination for the diagnosis of neonatal sepsis in its earliest stages. **Materials and Methods:** A total of 320 subjects were included in this study. A prospective cross-sectional study was conducted among neonates admitted to Neonatal Intensive Care Unit at King Abdulaziz Medical City, Riyadh, KSA during January 2013 to August 2015, the study based on three study groups categorized according to clinical symptoms and blood culture result. Study groups include healthy control neonates ($n = 80$), clinical sepsis (CS) group ($n = 80$) with clinical signs of sepsis but their blood culture was negative, and sepsis group with clinical signs of sepsis and their blood culture was positive. **Results:** The study observed significant difference in plasma levels of CRP, IL-6, TNF-α, E-selectin, and PCT in patients group when compared with control group ($P < 0.001$). Furthermore, the levels are significantly different between patient groups including CS and neonatal sepsis group. Moreover, result observed significant difference in CRP and IL-6 in early onset sepsis (EOS) when compared with late onset sepsis (LOS) neonates ($P < 0.001$ and 0.01), respectively, while there were no significant difference in TNF-α, E-selectin, and PCT between EOS and LOS ($P = 0.44$, 0.27 and 0.24), respectively. Regarding biomarkers accuracy, the result showed that CRP has the best diagnostic accuracy with cutoff value of 3.6 ng/ml (sensitivity 78% and specificity of 70%). The best combination is shown with CRP and IL-6 in which sensitivity increased to 89% and specificity to 79%. **Conclusion:** It was concluded that infected new-born babies have a higher E-selectin, PCT, IL-6, TNF-α, and CRP compared with the neonates with CS and control. IL-6, TNF-α, and CRP should be measured in combination for mare diagnostic accuracy in neonatal sepsis. Likewise, PCT should be investigated as a part of sepsis screening for all suspected neonates.

Key words: C-reactive protein, cytokines, diagnostic accuracy, early onset sepsis, E-selectin, late onset sepsis, neonatal sepsis, procalcitonin

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.198362

How to cite this article: Fattah MA, Omer AA, Asaif S, Manlulu R, Karar T, Ahmed A, *et al*. Utility of cytokine, adhesion molecule and acute phase proteins in early diagnosis of neonatal sepsis. J Nat Sc Biol Med 2017;8:32-9.

INTRODUCTION

Bacterial sepsis and infection of neonates are considered major health care issues with an annual global mortality that exceeds one million lives. Indeed, the leading cause of mortality among infants in the 1st day of life is infection.[1,2] The rate of neonatal sepsis remains high in tertiary care Neonatal Intensive Care Unit (NICU) in Saudi Arabia and exceeds internationally reported rates.[3]

The neonatal sepsis is an infectious disease of various etiologies and is described as systemic inflammatory response syndrome associated with proven infection which decides degrees of inflammatory response and metabolic change.[4,5] The immune system of neonates is not completely developed, and thus they are more prone to bacterial infection. In neonatal period, innate immune cells including macrophages and dendritic cells (DCs) initiate an effective immune response. Cytokines produced by macrophages and DCs play an important role during host defense mechanisms in response to infectious pathogens.[6,7]

Blood culture test for bacteria often referred to as the gold standard is the most reliable diagnostic test of neonatal sepsis. However, it can take 48–72 h for the results to be released. As a result, treatment should often begin before the laboratory results are released. An additional challenge is the fact that the blood culture test can be false negative for one in five subjects with sepsis.[8,9] Thus, it is of critical importance to identify new biomarkers that will enable fast and reliable diagnosis using chemical and hematological scoring systems for sepsis in its earliest stages. An acute phase inflammatory marker such as C-reactive protein (CRP) does not accurately differentiate between the systemic inflammatory response and sepsis when measured individually.[10,11] Procalcitonin (PCT), peptide precursor of the hormone calcitonin, is an 116 amino acid protein secreted by parafolicullar C-cells of thyroid gland in normal healthy individual. However, the levels usually increase during septicemia, pneumonia meningitis, and urinary tract infection.[11,12] This biomarker is also produced by other cells such as macrophage, and monocyte of various organs in conditions of severe bacterial infection and sepsis.[13]

E-selectin, also known as CD62 antigen-like family member E (CD62E), is a cell adhesion molecule expressed only on endothelial cells activated by various cytokines. The unique role of selectins is represented by the direct leukocyte interaction with vascular endothelium by binding to carbohydrate epitopes on leukocyte or on endothelial cells.[14]

Other biomarker is tumor necrosis factor-α (TNF-α) which produce mainly by macrophage and monocyte. Release of TNF-α often occurs approximately 30 min after lipopolysaccharide injection, and the circulating level reaches the peak in approximately one and a half hour, with an estimated half-life of about 70 min.[15-17] TNF-α regulates the secretion of IL-1 β while high level of TNF-α is associated with severity of disease. There are evidence that TNF-α is found free in plasma concomitant with the appearance of clinical signs and symptoms of infection.[18-21]

Interleukin-6 (IL-6) is the most frequent studied cytokine in neonatal population. IL-6 is a cytokine with early response to infection, preceding the increase in CRP and followed by release of TNF-α. This cytokine is synthesized by mononuclear phagocytes, fibroblasts, endothelial cells, and trophoblast cells immediately after stimulation by microbial products.[17,22]

This study intends to evaluate the potential diagnostic value for common used biomarkers CRP, E-selectin, PCT, IL-6, and TNF-α both independently and in combination for the diagnosis of neonatal sepsis. In addition, the study aims to identify the optimal cutoff value for these biomarkers to assess whether these markers could be helpful in early discontinuation of unnecessary antibiotic in noninfected new-born.

MATERIALS AND METHODS

Study area

A prospective cross-sectional (hospital base) study was conducted among neonates admitted to NICU at King Abdulaziz Medical City (KAMC), Riyadh, KSA during January 2013 to August 2015.

Study population and sample size

Patients were selected based on the international criteria of center for disease control for diagnosis of neonatal sepsis as have postnatal signs of sepsis and a positive blood culture from sample of peripheral or central venous lines.[23]

The study based on three study groups is categorized according to clinical symptoms and blood culture results. Group I (control) is eighty healthy neonates who were born normally at KAMC without any abnormal signs or symptoms of infection. Group II is clinical sepsis (CS) that was recruited among eighty neonates who admitted to NICU suspected to suffer from neonatal infection due to the presence of clinical signs of sepsis but their blood culture was negative. Group III is subdivided into two subgroup. First is early onset sepsis (EOS) that includes eighty neonates admitted to NICU at KAMC with clinical signs of sepsis appeared in first or 2nd day of life, and

their blood culture was positives. Second subgroup is late onset sepsis (LOS) that includes eighty neonates admitted to NICU at KAMC with clinical signs of sepsis appeared after 3 days of life, and their blood culture was positives.

Inclusion criteria

Neonates with three or more of the following clinical signs were selected in patient group: (1) Respiratory manifestations, (2) bradycardia, (3) hypotonia or seizures, (4) poor skin color or capillary filling time >2 s, and (5) irritability or lethargy. The symptoms were recorded by the resident neonatologist at NICU, KAMC.

Exclusion criteria

Neonates diagnosed with congenital malformations, congenital infections associated with the TORCH complex, and refusal of parents were excluded from this study.

Sampling

A total of 5 ml of venous blood sample was obtained at respective time and separated into two tubes. First tube was used for blood culture and second tube for analysis of CRP, IL-6, TNF-α, PCT, and E-selectin. Plasma was separated within 30 min of collection and stored at −20 C for analysis.

Laboratory methods

Plasma CRP, IL-6, TNF-α, and E-selectin were determined by sandwich enzyme-linked immunosorbent assay kit (Abcam®, Cambridge, UK) following the manufacturer instructions. Unknown samples, standards, and control were analyzed in duplicate and mean value was determined for each test. The assay uses two antigen-specific monoclonal antibodies that bind to respective analyte (as an antigen) at different binding sites. One of these antibodies was biotinylated specific monoclonal antibodies and the other was coated in microtiter plate wells. Streptavidin – horseradish peroxidase was used as enzyme conjugate that binds to secondary antibodies. 3,3',5,5'-tetramethylbenzidine substrate was then added to each well to produce color reaction. A stop solution was added to reaction and plate was read using VeraMax reader.

An immunoluminometric assay (LIAISON BRAHMS, Germany) was used for the specific measurement of PCT in serum (detection limit 0.10 ng/ml) following the instructions of the manufacturer. The assay uses two antigen-specific monoclonal antibodies that bind PCT (as an antigen) at different binding sites (the calcitonin and katacalcin segments). One of these antibodies was luminescence-labeled (the tracer) and the other was coated on with magnetic particles (solid phase).

Statistical analysis

Descriptive and analyzing tests (Mann–Whitney rank-sum test, Student's *t*-test, Kruskal–Wallis H-test, Chi-square test, Pearson correlation, and Spearman rank correlation) were performed using SPSS software 16 for Windows (Chicago, Illinois, USA).

The reliability of analytes concentration for the diagnosis of sepsis was calculated by receiver operating characteristics (ROC) curves. ROC index (sensitivity + specificity - 1) was used for determination of optimal cutoff values of the diagnostic tests in the different postnatal periods. Sensitivity, specificity, and the likelihood ratio of positive and negative results with a 95% confidence interval were calculated. Statistical significance was set at $P < 0.05$. Variables are presented as mean ± standard error of mean. For comparison of serum CRP, PCT, E-selectin, IL-1B, and TNF-α between the groups, Kruskal–Wallis test were employed followed by *post-hoc* tests.

RESULTS

Results of the present study include 320 neonates divided into three groups, age and sex were matched between the three groups and showed no significant difference ($P = 0.9$). The mean ± standard deviation of weight in control group was 3610 ± 483 g and 1944 ± 560 g for patients group with significant difference between them ($P < 0.05$).

Clinical characteristics of patients groups

Comparing the clinical finding of patients groups, unstable temperature was significantly different ($P < 0.001$) between CS 2 (2%), EOS 2 (2%), and LOS 49 (61%). Unstable respiration signs were found more frequent in EOS group ($n = 61$, 76%) and less frequent in CS group ($n = 19$, 24%) with significant difference between groups ($P \leq 0.001$). Cases with rash or skin infection signs was found to be 29 (23%), 48 (60%), and 16 (20%) in CS, EOS, and LOS group, respectively ($P < 0.001$). No significance difference was observed in tachycardia, bradycardia, hypoglycemia, acidosis, or jaundice between patients group ($P > 0.05$ for all). Detailed clinical characteristics of patients group are summarized in Table 1.

Biomarkers in study population

In reference to Mann–Whitney U-test, results of the present study observed significant increase in plasma levels of CRP, IL-6, TNF-α, PCT, and E-selectin in patients group compared to healthy control group ($P < 0.001$ for all). As shown in Table 2, Kruskal–Wallis H-test indicates a significant difference in all biomarkers between patients groups ($P < 0.001$) with high level of significant difference for CRP indicated by high H value (H = 124). Comparing cultured proved sepsis patients, CRP and IL-6

showed a significant increase in LOS compared to EOS group ($P < 0.001$, $P = 0.01$, respectively). In contrast, no significant differences were found in TNF-α, E-selectin, and PCT between EOS and LOS group ($P = 0.44$, 0.27 and 0.24, respectively) [Table 3].

Causative bacteria in sepsis groups

Among 160 proved sepsis patients, *Escherichia coli* are isolated from 54 (68%) samples in EOS group and 18 (22%) samples in LOS group. Gram-positive Group B *Streptococcus* bacteria is associated with LOS in which 38 (48%) bacteria is isolated while only 5 (6%) are found in EOS group. Details in isolated bacteria are summarized in Table 4.

Table 1: Clinical data in patients groups

Clinical characteristics	Study groups (*n*=80) (%)			*P*
	CS	EOS	LOS	
Sex				
Male	39 (49)	41 (51)	40 (50)	0.951
Female	41 (51)	39 (49)	40 (50)	
Temperature				
Stable	78 (98)	78 (98)	31 (39)	<0.001*
Unstable	2 (2)	2 (2)	49 (61)	
Respiration				
Stable	61 (76)	19 (24)	39 (50)	<0.001*
Unstable	19 (24)	61 (76)	41 (50)	
Rash/skin infection				
No	51 (64)	32 (40)	64 (80)	<0.001*
Yes	29 (36)	48 (60)	16 (20)	
Bradycardia/tachycardia				
No	69 (86)	69 (86)	70 (87)	0.965
Yes	11 (14)	11 (14)	10 (13)	
Hypoglycemia				
No	72 (90)	72 (90)	73 (91)	0.953
Yes	8 (10)	8 (10)	7 (9)	
Acidosis				
No	71 (89)	66 (82)	66 (82)	0.450
Yes	9 (11)	14 (18)	14 (18)	
Jaundice				
No	76 (95)	76 (95)	77 (96)	0.909
Yes	4 (5)	4 (5)	3 (4)	

*Chi-square test is significant at *P*<0.05. CS: Clinical sepsis, EOS: Early onset sepsis, LOS: Late onset sepsis

Table 2: Biomarkers in patients group

Patients group	Mean±SEM*				
	CRP	IL-6	TNF-α	E-selectin	PCT
CS	3.7±0.08	788.1±43.5	21.7±1.4	148.9±7.9	3.9±0.4
EOS	3.8±0.21	928.6±31.9	27.6±1.4	177.1±3.5	5.6±0.4
LOS	7.3±0.22	1052.5±35.1	29.1±1.5	182.8±3.8	6.3±0.42
H	124	13	7	11	9
P	<0.001**	<0.001**	0.001**	<0.001**	<0.001**

*SEM: Standard error of mean, **Kruskal–Wallis H-test is significant at *P*<0.05. CRP: C-reactive protein, IL-6: Interleukin-6, PCT: Procalcitonin, TNF-α: Tumor necrosis factor-α, CS: Clinical sepsis, EOS: Early onset sepsis, LOS: Late onset sepsis

Table 3: Biomarkers in neonatal sepsis patients

Sepsis group	Mean±SEM*				
	CRP	IL-6	TNF-α	E-selectin	Procalcitonin
EOS	3.8±0.21	928.6±31.9	27.6±1.4	177.1±3.5	5.6±0.4
LOS	7.3±0.22	1052.5±35.1	29.1±1.5	182.8±3.8	6.3±0.42
P	<0.001**	0.01**	0.44	0.27	0.24

*SEM: Standard error of mean, **Mann–Whitney U-test is significant at *P*<0.05. CRP: C-reactive protein, IL-6: Interleukin-6, PCT: Procalcitonin, TNF-α: Tumor necrosis factor-α, EOS: Early onset sepsis, LOS: Late onset sepsis

Diagnostic accuracy of the biomarkers

Table 5 summarizes sensitivity, specificity, and likelihood ratios for the biomarkers and best combination revealed after analysis. ROC curve was used to define the optimal cutoff value for the five biomarkers. Comparison of each test using optimal cutoff value showed higher index for CRP (0.85) with sensitivity of 78% and specificity of 70%. IL-6 showed higher sensitivity (83%) but specificity was 68%. Best specificity (70%) for individual biomarker was showed by three biomarkers (CRP, TNF-α, and PCT). However, E-selectin showed the least diagnostic accuracy with ROC index of 0.74, sensitivity of 73%, and specificity of 60%. Youden index was used to calculate best combination between these biomarkers. CRP and IL-6 showed the best combination at cutoff values of 3.6 ng/ml and 83 ng/ml, respectively. Sensitivity was increased to 89% and specificity to 79% with ROC index of 0.89. CRP and TNF-α combination yields higher specificity (85%) with sensitivity of 79%. Relatively same result revealed using CRP and PCT combination. ROC curves for the mentioned combination are shown in Figure 1 as it clearly indicates increasing the area under the curve (AUC) in combination of two biomarkers. Diagnostic accuracy of the biomarkers in solo and combination is shown in Table 5.

DISCUSSION

Neonatal sepsis evaluations with positive blood culture were diagnosed as culture proven neonatal sepsis according to National Healthcare Safety Network definitions of laboratory-confirmed bloodstream infection.[23] Infant with positive clinical score of sepsis was categorized as CS.[24] This includes infant with another infectious diagnosis that is not accompanied by positive blood culture, such as pneumonia, urinary tract infection and necrotizing enterocolitis. Most studies in neonatal sepsis suggest that age and sex have no effect in categorizing infected and noninfected infants and this was supported by the current study ($P > 0.05$).

One of the methodological limitations in this study is the lack of some clinical data such as gestational age and mode of delivery; however, the findings are strengthen by relatively large number of investigated neonates, the presence of positive blood culture in all septic neonates, investigation of both EOS and LOS, and minimizing the interference by collecting the samples before administration of antibiotics. Since clinical signs of sepsis are nonspecific,

diagnostic laboratory method is needed for early diagnosis, prognosis, and effective treatment.

Due to high mortality and morbidity among neonatal sepsis, laboratory markers with 100% sensitivity (all infected neonates has positive test) and negative predictive value (negative test accurately rule out the disease) are required.

It is well-known that several biochemical and immunological markers increased in the plasma during neonatal sepsis, such as increased CRP, IL-6, TNF-α, PCT, and E-selectin.[9,25-27] Similarly, the results of the present study observe a significant increase in these biomarkers among proved-sepsis neonates compared to neonates with CS and health infants. The optimal cutoff value of the biomarkers for diagnosis of neonatal sepsis is not defined clearly, and there is divergence in many studies. Accordingly, we attempt to define the optimal cutoff value using ROC curve. The cutoff value of CRP, IL-6, TNF-α, E-selectin, and PCT is found to be 3.29 ng/ml, 705 ng/ml, 18.2 pg/ml, 168 ng/ml, and 2.7 ng/ml, respectively. These values were much closed to those recommended by manufactured in the package insert of each reagent kit (IL-6 <700, CRP <1.6, TNF-α <16, E-selectin <160, and PCT <3).

Among acute phase proteins, CRP is probably the most well-studied, but the findings regarding its diagnostic accuracy are mixed.[9,26] CRP is synthesized in liver in response to inflammation and its secretion is stimulated by IL-1, IL-6, and TNF-α. The level increased 6–8 h after exposure to infection, reach peak level at 24–48 h, and return to normal overtime as inflammation resolved.[28,29] Reviewing several studies in CRP, it has been reported that CRP level >1 mg/dl, as cutoff point, has sensitivity ranged between 70% to 93% and specificity ranged between 41% and 98%.[30,31] In this study, the most appropriate cutoff point using ROC curve was found to be 3.6 ng/ml. Using this value, sensitivity was 78%, specificity 70%, and AUC 0.85. However, increased CRP level in CS group (mean 3.7 ng/ml) thought to affect the specificity. In addition, the increase of CRP level is rather slow during the first 12–24 h of infection and this may negatively affect the sensitivity. The moderate sensitivity and specificity in this study and many other studies strongly suggest that CRP individually will not be sufficient for the clinician to rely on it. TNF-α has the same behavior of IL-6 and it has a good specificity and sensitivity. This was clearly demonstrated in this finding by very close results in IL-6 and TNF-α in solo and combination. In contrast, CRP reaches the peak level relatively later, and hence it is more specific for confirming infection. Therefore, an abnormal high level of CRP with normal plasma level of IL-6 proposes recent infection 24–48 h.

PCT, as an acute phase protein, has been intensively investigated as inflammatory marker in neonatal sepsis. Many studies reported that PCT increased 4 h after exposure to endotoxin, reached peak level 6–8 h after infection, and remained in high level for at least 24 h.[32] While PCT level is undetectable in healthy individual, the concentration increased gradually in relation to severity of inflammation in septic patients. As CRP, PCT synthesized by the liver and its production is stimulated by CRP and inflammatory mediator such as IL-6 and TNF-α. PCT remains in high level despite decrease of the stimulus mediator as inflammatory process is ongoing.[33] In previous studies, sensitivity of PCT was ranged between 83% and 100% and specificity ranged between 70% and 100%.[34-36] Hence, in this study, PCT was found to be in lower limit of accuracy (sensitivity 72 and specificity 70%). In addition, our results indicate less accuracy of PCT than CRP in the diagnosis of neonatal sepsis. However, Kocabas *et al.* reported that PCT is

Table 4: Causative bacteria according to onset of sepsis

Causative bacteria	EOS (*n*=80) (%)	LOS (*n*=80) (%)
Escherichia coli (Gram-negative)	54 (68)	18 (22)
Hemophilus influenza (Gram-negative)	21 (27)	2 (3)
Group B *streptococcus* (Gram-positive)	5 (6)	38 (48)
Staphylococcus aureus	0 (0)	22 (27)

EOS: Early onset sepsis, LOS: Late onset sepsis

Table 5: Sensitivity, specificity and likelihood ratios biomarkers

Cytokines	Cutoff	Sensitivity	Specificity %	ROC (95% CI)	LR^+	LR^-
CRP	3.6	78	70	0.85 (0.81-0.89)	2.29	0.44
IL-6	705	83			2.51	0.28
TNF-α	18.1	69	70	68	0.82 (0.76-0.87)	0.18
E-selectin	168	73	60	0.74 (0.68-0.79)	1.81	0.45
PCT	2.74	72	70	0.81 (0.75-0.86)	2.35	0.34
CRP/IL-6		89	79	0.89 (0.85-0.93)	2.41	0.38
CRP/PCT		78	85	0.88 (0.84-0.92)	2.36	0.37
IL-6/PCT		85	74	0.85 (0.81-0.88)	2.12	0.11
TNF-α/CRP		79	85	0.88 (0.84-0.92	2.01	0.32
TNF-α/IL-6		87	74	0.85 (0.80-0.89)	2.41	0.29
TNF-α/PCT		85	74	0.83 (0.79-0.88)	2.60	0.45

ROC: Receiver operating characteristic, CI: Confidence interval, CRP: C-reactive protein, IL-6: Interleukin-6, PCT: Procalcitonin, TNF-α: Tumor necrosis factor-α, LR: Likelihood ratio

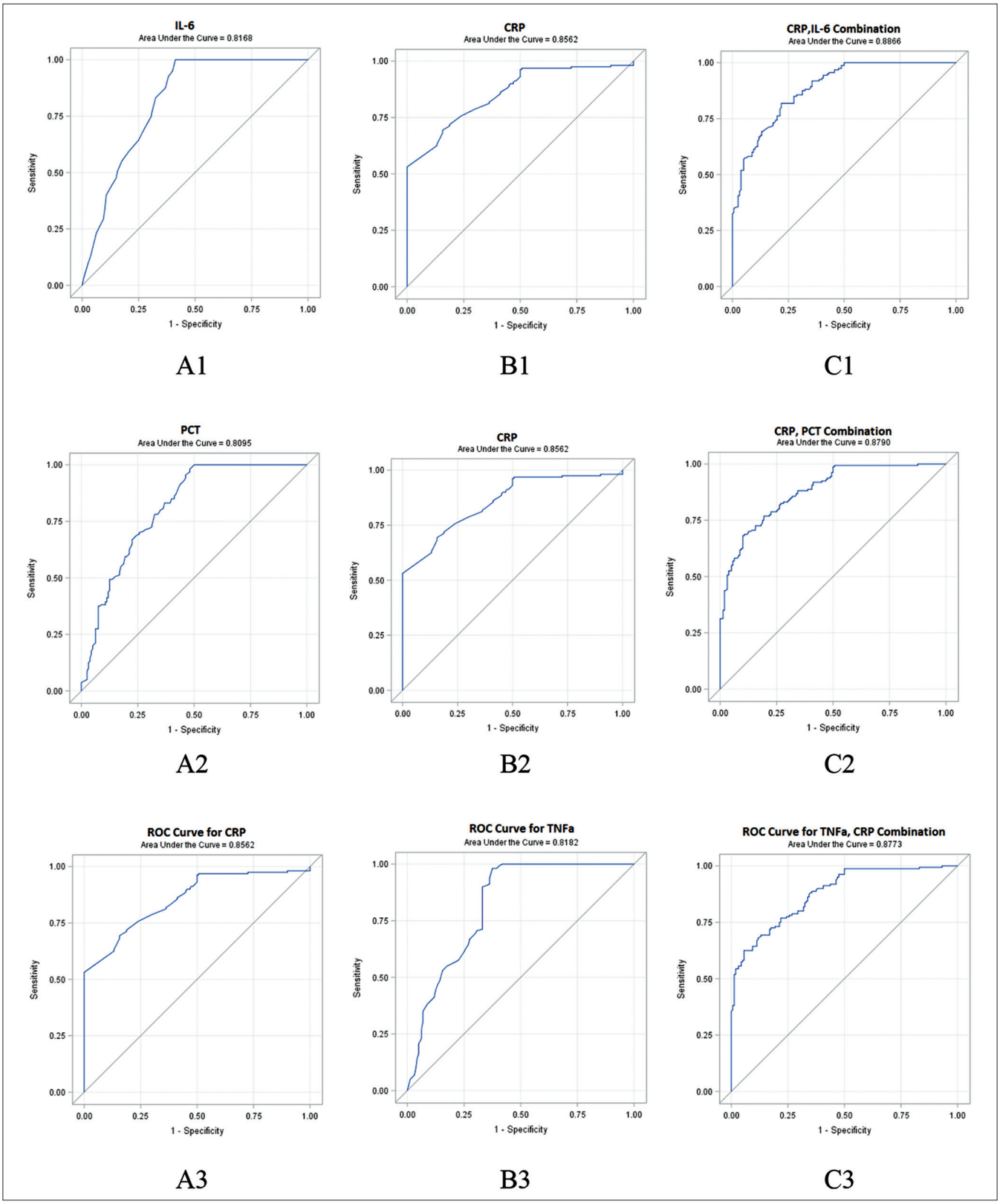

Figure 1: Receiver operating characteristics curve for biomarkers in solo and combination. (A1–C1) receiver operating characteristics curves for IL-6 and C-reactive protein individually and in combination, (A2–C2) receiver operating characteristics curves for procalcitonin and C-reactive protein individually and in combination, (A3–C3) receiver operating characteristics curves of C-reactive protein and tumor necrosis factor-α individually and in combination

more accurate than CRP.[37] Other studies reported that PCT is not better marker than CRP.[38-40] Diversity in the results suggests that physiological alterations observed in the 1st and 2nd day of life and the effect of prenatal and postnatal antibiotic administration will affect the PCT level and functions as confounder in relation between PCT and infection. Moreover, in the current finding, we observe no significant difference in PCT level between EOS and LOS and this suggests that PCT level does not affect by age at which onset of infection occurs.

Chiesa *et al.*, reported that increased PCT level in neonatal sepsis is caused by perinatal events rather than infection induction.[31] The accuracy of PCT depends on birth weight in which it shows more accuracy below 1500 g birth weight.[41] Other study by López Sastre *et al.* reports that PCT is not sufficient when measured individually in case of hospital-acquired neonatal sepsis.[42] It has been cautioned that PCT could not serve as individual marker for neonatal sepsis and should be a part of full sepsis work up.

IL-6 produced rapidly in the beginning of neonatal sepsis course.[43,44] This rapid elevation will be diminished shortly because of short half-life of IL-6. This specific property makes Il-6 useful as a very early indication for neonatal sepsis, but due to short half-life, clinician could not rely on IL-6 alone. A significant increase was observed in LOS when compared to EOS. This finding is supported by several studies[37,45] and this emphasizes the fact that IL-6 has a natural fluctuation immediately after postnatal period.

Best combination revealed by this study has been found in IL-6 (early and sensitive marker) and CRP (late and specific marker) in which AUC was the highest (0.89) with 89 and 79% for sensitivity and specificity, respectively. Similar results were reported by several studies in investigating diagnostic markers of neonatal sepsis.[46-48]

CONCLUSION

There is no doubt that inflammatory biomarkers such as cytokines, adhesion molecules, and CRP are increased in neonatal sepsis. However, these biomarkers should be used in proper pattern for best diagnosis to increase positive predictive value. PCT determination should be included in full sepsis screening test for all suspected neonates. A combination of IL-6, TNF-α, and CRP yield best diagnostic accuracy when used in two occasions during the course of the disease.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? Lancet 2005;365:891-900.
2. Sanchez-Schmitz G, Levy O. Development of newborn and infant vaccines. Sci Transl Med 2011;3:90ps27.
3. Alfaleh KM. Incidence of late onset neonatal sepsis in very low birth weight infants in a tertiary hospital: An ongoing challenge. Sultan Qaboos Univ Med J 2010;10:227-30.
4. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644-55.
5. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005;6:2-8.
6. Cloherty JP, Eichenwald EC, Hansen AR. Manual of Neonatal Care. Philadelphia, PA, USA: Wolters Kluwer Health/Lippincott Williams and Wilkins; 2012.
7. Hotoura E, Giapros V, Kostoula A, Spirou P, Andronikou S. Tracking changes of lymphocyte subsets and pre-inflammatory mediators in full-term neonates with suspected or documented infection. Scand J Immunol 2011;73:250-5.
8. Ganatra HA, Stoll BJ, Zaidi AK. International perspective on early-onset neonatal sepsis. Clin Perinatol 2010;37:501-23.
9. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol 2010;37:421-38.
10. Andrejaitiene J. The diagnostic value of procalcitonin in severe sepsis. Medicina (Kaunas) 2006;42:69-78.
11. Carrol ED, Thomson AP, Hart CA. Procalcitonin as a marker of sepsis. Int J Antimicrob Agents 2002;20:1-9.
12. Gendrel D, Bohuon C. Procalcitonin as a marker of bacterial infection. Pediatr Infect Dis J 2000;19:679-87.
13. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515-8.
14. Austgulen R, Arntzen KJ, Haereid PE, Aag S, Døllner H. Infections in neonates delivered at term are associated with increased serum levels of ICAM-1 and E-selectin. Acta Paediatr 1997;86:274-80.
15. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 1989;169:333-8.
16. Shannon E, Noveck R, Sandoval F, Kamath B, Kearney M. Thalidomide suppressed interleukin-6 but not tumor necrosis factor-alpha in volunteers with experimental endotoxemia. Transl Res 2007;150:275-80.
17. Fan Y, Yu JL. Umbilical blood biomarkers for predicting early-onset neonatal sepsis. World J Pediatr 2012;8:101-8.
18. de Bont ES, Martens A, van Raan J, Samson G, Fetter WP, Okken A, *et al.* Diagnostic value of plasma levels of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) in newborns with sepsis. Acta Paediatr 1994;83:696-9.
19. Silveira RC, Procianoy RS. Evaluation of interleukin-6, tumour necrosis factor-alpha and interleukin-1beta for early diagnosis of neonatal sepsis. Acta Paediatr 1999;88:647-50.
20. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1987;1:355-7.
21. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest 1993;103:565-75.
22. Tasci Y, Dilbaz B, Uzmez Onal B, Caliskan E, Dilbaz S, Doganci L, *et al.* The value of cord blood interleukin-6 levels for predicting chorioamnionitis, funisitis and neonatal infection in term premature rupture of membranes. Eur J Obstet Gynecol Reprod Biol

2006;128:34-9.
23. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309-32.
24. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 1988;112:761-7.
25. Bhandari V, Wang C, Rinder C, Rinder H. Hematologic profile of sepsis in neonates: Neutrophil CD64 as a diagnostic marker. Pediatrics 2008;121:129-34.
26. Mannan MA, Shahidullah M, Noor MK, Islam F, Alo D, Begum NA. Utility of C-reactive protein and hematological parameters in the detection of neonatal sepsis. Mymensingh Med J 2010;19:259-63.
27. López Sastre JB, Pérez Solís D, Roqués Serradilla V, Fernández Colomer B, Coto Cotallo GD, Krauel Vidal X, *et al.* Procalcitonin is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. BMC Pediatr 2006;6:16.
28. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.
29. Ballou SP, Kushner I. C-reactive protein and the acute phase response. Adv Intern Med 1992;37:313-36.
30. Caksen H, Kurtoglu S, Hallaç IK, Ustünbas HB, Uzüm K, Kiliç H. The relationship between scoring systems and cytokine levels in neonatal sepsis. Ann Acad Med Singapore 2003;32:418-20.
31. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, *et al.* C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: Influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clin Chem 2003;49:60-8.
32. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, *et al.* Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994;79:1605-8.
33. van Rossum AM, Wulkan RW, Oudesluys-Murphy AM. Procalcitonin as an early marker of infection in neonates and children. Lancet Infect Dis 2004;4:620-30.
34. Gendrel D, Raymond J, Coste J, Moulin F, Lorrot M, Guérin S, *et al.* Comparison of procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differentiation of bacterial vs. viral infections. Pediatr Infect Dis J 1999;18:875-81.
35. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: A systematic review and meta-analysis. Clin Infect Dis 2004;39:206-17.
36. Prat C, Domínguez J, Rodrigo C, Giménez M, Azuara M, Blanco S, *et al.* Use of quantitative and semiquantitative procalcitonin measurements to identify children with sepsis and meningitis. Eur J Clin Microbiol Infect Dis 2004;23:136-8.
37. Kocabas E, Sarikçioglu A, Aksaray N, Seydaoglu G, Seyhun Y, Yaman A. Role of procalcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor-alpha in the diagnosis of neonatal sepsis. Turk J Pediatr 2007;49:7-20.
38. Monneret G, Labaune JM, Isaac C, Bienvenu F, Putet G, Bienvenu J. Procalcitonin and C-reactive protein levels in neonatal infections. Acta Paediatr 1997;86:209-12.
39. Lapillonne A, Basson E, Monneret G, Bienvenu J, Salle BL. Lack of specificity of procalcitonin for sepsis diagnosis in premature infants. Lancet 1998;351:1211-2.
40. Janota J, Stranák Z, Belohlávková S, Mudra K, Simák J. Postnatal increase of procalcitonin in premature newborns is enhanced by chorioamnionitis and neonatal sepsis. Eur J Clin Invest 2001;31:978-83.
41. Auriti C, Fiscarelli E, Ronchetti MP, Argentieri M, Marrocco G, Quondamcarlo A, *et al.* Procalcitonin in detecting neonatal nosocomial sepsis. Arch Dis Child Fetal Neonatal Ed 2012;97:F368-70.
42. López Sastre JB, Coto Cotallo D, Fernández Colomer B; Grupo de Hospitales Castrillo. Neonatal sepsis of nosocomial origin: An epidemiological study from the "Grupo de Hospitales Castrillo". J Perinat Med 2002;30:149-57.
43. Girardin EP, Berner ME, Grau GE, Suter S, Lacourt G, Paunier L. Serum tumour necrosis factor in newborns at risk for infections. Eur J Pediatr 1990;149:645-7.
44. de Bont ES, Martens A, van Raan J, Samson G, Fetter WP, Okken A, *et al.* Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 plasma levels in neonatal sepsis. Pediatr Res 1993;33 (4 Pt 1):380-3.
45. Chiesa C, Signore F, Assumma M, Buffone E, Tramontozzi P, Osborn JF, *et al.* Serial measurements of C-reactive protein and interleukin-6 in the immediate postnatal period: Reference intervals and analysis of maternal and perinatal confounders. Clin Chem 2001;47:1016-22.
46. Ng PC, Cheng SH, Chui KM, Fok TF, Wong MY, Wong W, *et al.* Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule, and C-reactive protein in preterm very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 1997;77:F221-7.
47. Messer J, Eyer D, Donato L, Gallati H, Matis J, Simeoni U. Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996;129:574-80.
48. Doellner H, Arntzen KJ, Haereid PE, Aag S, Austgulen R. Interleukin-6 concentrations in neonates evaluated for sepsis. J Pediatr 1998;132:295-9.