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At Admission Evaluation of the Score for Neonatal Acute Physiology (SNAP II) and Estimation of Serum Procalcitonin Allow Early Diagnosis of Early-Onset Neonatal Sepsis

[1]Yehia H. Abdel Maqsoud MD, 1Ahmed A Sobeih MD, 1Effat Assar MD, [2]Deena A. Elshabrawy MD, [3]Manal Hassaan MD, [4]Raafat R. Mohammed MD

[1]Department of Pediatrics, Faculty of Medicine, Benha University
[2]Department of Clinical Pathology, Faculty of Medicine, Benha University
[3]Department of Medical Biochemistry, Faculty of Applied Medical Sciences, 6 October University
[4]Fellow and Researcher in Biochemistry, Clinical Pathology Department, Benha University, Benha, Egypt

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Keywords

- Early-onset neonatal sepsis
- Score for Neonatal Acute Physiology
- Procalcitonin
- Interleukin-6
- High-sensitivity
- C- reactive protein

Abstract

Objectives: The study aimed to evaluate the discriminative ability of, at admission, estimation of serum high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6) and procalcitonin (PCT) between neonates with various grades of early-onset sepsis (EOS) using the Score for Neonatal Acute Physiology (SNAP II). **Patients & Methods:** The study included 87 neonates with suspected EOS within the 1st week of life. All neonates were evaluated using the SNAP II and scores >40 indicate severe, 20-40 indicate moderate and score <20 indicate mild infection. At admission, venous blood samples were obtained for blood culture, total (TLC) and differential leucocytic count and ELISA estimation of serum hsCRP, IL-6 and PCT.**Results:** EOS neonates were categorized as Confirmed (n=44), Suspected (n=18) and EOS-free (n=25) according to severity of clinical sepsis and result of blood culture. Estimated laboratory parameters were significantly higher in patients than controls and in EOS than EOS-free neonates. Serum hsCRP and IL-6 levels could not, while PCT could differentiate between neonates with confirmed or suspected EOS. ROC curve analysis defined high serum PCT and IL-6, SNAP II score, neutrophil percentages, serum hsCRP and TLC as significant predictors for positive blood culture in decreasing order of significance, while Regression analysis defined high serum PCT as a persistently significant predictor for positive culture, followed by high serum IL-6 and high SNAP II score. Combined SNAP II scoring and serum PCT could define 61.4%, while combined estimation of serum hsCRP, IL-6 and PCT levels could define 52.3% of neonates with combined EOS. **Conclusion:** Combined estimation of hsCRP, IL-6 and PCT could increase the diagnostic yield of neonatal sepsis; however, clinical evaluation using SNAP II score and serum PCT did better and could define neonates with positive blood culture earlier so as to allow early treatment.

Corresponding author: Dr. Manal Hassan, MD , Medical Biochemistry, Faculty of Medicine, [6]October University Tel. (+20) 01001682254 E-mail address: 151239@o6u.edu.eg.

Introduction

Bloodstream infection among neonatal intensive care unit (NICU) infants is a frequent problem associated with poor outcomes (1). The genesis of the infection is divided into early-onset and late-onset types of the disease (2). Sepsis caused by Gram-positive bacteria mainly occurs in full-term infants, and most cases of sepsis caused by Gram-negative bacteria belong to the early-onset type (3).

Sepsis is an important cause of neonatal morbidity and mortality worldwide (4). However, its diagnosis and treatment is highly complicated (2) as it relies on clinical judgment of nonspecific manifestations (5), interpretation of nonspecific laboratory tests characterized by a high-false negative rate (4) and a delay in obtaining blood culture results (5).

Neonatal sepsis is characterized by uncontrolled inflammatory responses due to proven bacterial infection (6) and pro-inflammatory cytokine production is believed to play a central role in the pathogenesis of this disease (7). Evaluation of serum levels of inflammatory cytokine provided multiple advantages over traditional laboratory tests, as its levels were elevated in the early stage of neonatal sepsis (4) and are not affected by pre-natal antibiotic administered to the mother (8).

Hypothesis:

The study proposed that estimation of primary-phase reactants provides early discrimination between neonates with clinically suspected early-onset sepsis (EOS) prior to gaining the results of blood culture; thus could allow early intervention so as to reduce the frequency of additional morbidities and possible mortality.

Objectives :

The study aimed to evaluation of the discriminative ability of, at admission, estimation of serum C-reactive protein (CRP), interleukin-6 (IL-6) and procalcitonin (PCT) between neonates with various grades of EOS using the Score for Neonatal Acute Physiology (SNAP II).

Study Design:

Comparative double-blinded selective clinical trial

Setting :

University Hospital at Benha

Patients & Methods:

The study protocol was approved by the Local Ethical Committee and parents of enrolled neonates signed fully-informed consent concerning study participation and giving blood samples for assigned investigations. For comparative purpose, blood samples were collected from twenty age- and sex-matched healthy neonates free of all sepsis manifestations as control group.

All neonates with suspected EOS within the 1st week of life were evaluated for eligibility for inclusion in the study. Inclusion criteria included gestational age (GA) of 28-41 weeks and 5-min APGAR score of ≥7. Exclusion criteria included neonates with small for gestational age, chromosomal abnormality, multiple congenital anomalies, genetic syndromes, missed inclusion criterion, history of premature rupture of membranes, chorioamnionitis, neonatal asphyxia, and on refusal or lack of informed consent from the parents. All neonates were evaluated using the

SNAP II (Table 1) and scores >40 indicate severe, scores in range of 20-40 indicate moderate and score <20 indicate mild infection (9). At admission, venous blood samples were obtained for assigned investigations and blood culture.

Sepsis was defined by a positive blood culture and one of at least three of the six categories shown in table 2 (10, 11) and neonates were categorized regarding the presence of sepsis according to **Baltimore et al.** (12) into the following groups:

1- Confirmed EOS included neonates with evident clinical sepsis manifestations and had positive blood culture.

2- Suspected EOS included neonates with evident clinical sepsis manifestations and had negative blood culture

3- EOS-free included neonates with mild physiological derangement but no evident clinical sepsis manifestations, showed no deterioration till 72-hr and had negative blood culture.

Investigations:

All investigations were performed by a clinical pathologist who was blinded about the clinical diagnosis. At admission, venous blood samples were obtained and divided into three parts:

a. The 1st part was used for blood culture by incubating blood samples both aerobically and anaerobically, for 72 hours.

b. The 2nd part (20 µl) was used for complete blood count (Fyfmex, CC-800, TOA-medical electronic, USA).

The 3rd part (5 ml) was put in clean dry tube, allowed to clot and then serum was separated in clean dry Eppendorff tube to be stored at -80°C till assayed using sandwich ELISA technique for estimation of serum levels of high-sensitivity C-reactive protein (hsCRP) (Biosystem, Barcelona, Spain) (13), Interleukin-6 (IL-6) (Quantikine, USA) (14) and PCT (RayBio, Parkway, Norcross, USA) (15).

Table (1): *Score for neonatal acute physiology II (SNAP II) (9)*

Variable	Measure	Score
Lowest men ABP (mmHg)	>29	**0**
	20-29	**9**
	<20	**19**
Lowest temperature (^{o}C)	>35.6	**0**
	35-35.6	**8**
	<35	**15**
PO2/FiO2 ratio	>2.49	**0**
	1.0-2.49	**5**
	0.3-0.9	**16**
	<0.3	**28**
Lowest pH	>7.19	**0**
	7.10-7.19	**7**
	<7.10	**16**
Seizure	None	**0**
	Yes	**5**
Urine output (ml/kg/hr)	>0.9	**0**
	0.1-0.9	**5**
	<0.1	**18**

Table (2): *Diagnostic criteria for sepsis* (10, 11)

Categories	Manifestations
Temperature	**Temperature instability or central temperature >37.5°C lasting at least 4 hr**
Disturbed metabolism	**Metabolic acidosis with a pH < 7.25 or poor peripheral perfusion**
Cardiovascular	**Cyanosis, pallor, heart rate (HR) >180 beats/minute or <80 beats/minute, arterial blood pressure (ABP) below 2SD of the mean ABP for age and weight or poor peripheral perfusion**
Respiratory	**Respiratory distress or respiratory rate (RR) >60/ minute, subcostal and/or intercostals retractions, grunting or respiratory pause lasting >10 sec**
Abdominal	**Abdominal distension, feeding intolerance and/or vomiting;**
Neurological	**Lethargy, hypotonia or convulsions**

Statistical analysis:

Results were analyzed using One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (X^2 test). Receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) that was compared versus the null hypothesis that AUC=0.05 and Regression analysis (Stepwise method) were used for stratification of studied parameters as specific predictors. Statistical analysis was conducted using IBM SPSS (Version 23, 2015) statistical package. P value <0.05 was considered statistically significant.

Results:

One hundred and sixteen neonates were admitted to NICU for sepsis evaluation; 87 neonates were eligible for inclusion in the study; enrollment data showed non-significant difference versus control neonates. Clinical evaluation detected 92 morbidities in 62 neonates (EOS neonates) for a frequency of 1.5 morbidities per patient, while 25 neonates showed no evident morbidities (EOS-free). According to SNAP II scoring 23 neonates were graded as having severe, 40 neonates as moderate and 24 neonates as having mild EOS. Positive blood culture confirmed sepsis in 44 patients, while 43 patients had negative blood culture; thus neonates were categorized as Confirmed EOS (n=44), Suspected EOS (n=18) and EOS-free (n=25) as shown in figure 1. Clinical enrollment data of neonates after categorization showed non-significant difference between groups apart from SNAP II scores that were significantly ($p<0.05$) higher in neonates with EOS compared to EOS-free neonates, (Table 3).

Total leucocytic count (TLC), neutrophil percentage and serum hsCRP level were significantly ($p<0.05$) higher in patients than controls and in EOS neonates compared to EOS-free neonates with non-significantly ($p>0.05$) higher counts in neonates with confirmed than suspected EOS. Mean serum IL-6 and PCT were significantly ($p>0.05$) higher in patients as total and categorized according to presence of EOS than controls, but were significantly ($p<0.05$) lower in EOS-free neonates compared to EOS neonates. Mean serum hsCRP and IL-6 were non-significantly ($p>0.05$) higher in neonates with confirmed than neonates with suspected EOS, while mean serum PCT levels were significantly ($p<0.05$) higher in neonates with confirmed than neonates with suspected EOS (Table 4).

Table (3): *Enrolment data of studied neonates compared to control neonates*

Group Data		Patients				Controls
		EOS-Free	Suspected	Confirmed	Total	
Number		25 (28.7%)	18 (20.7%)	44 (50.6%)	87 (100%)	**20 (100%)**
Gestational age (weeks)		34.2±3.6	35.2±2.3	33.5±3.1	34.1±3.1	**35.1±3.1**
5-min APGAR score		8±0.7	8.1±0.6	7.9±0.8	7.3±1.3	**7.1±1.4**
Birth weight (gm)		3207.3±173.6	3110±241	3102.4±211	3124±210	**3115±230**
Head circumference (cm)		34.9±0.8	34.8±0.7	34.7±0.8	34.8±0.8	**34.6±1**
Age (days)		5.2±1.2	4.6±1.2	4.5±1.4	4.7±1.3	**5.6±1.2**
SNAP II score		9.7±2.8*†	26.9±14.3*	36.5±12.5	30.6±13.5	**0**
Provisional clinical diagnosis	Pneumonia	0	6 (33.3%)	32 (72.7%)	38 (43.7%)	**0**
	Enterocolitis	0	5 (27.8%)	9 (20.5%)	14 (16.1%)	**0**
	Pyoderma	0	4 (22.2%)	5 (11.4%)	9 (10.3%)	**0**
	Cholestatic hepatitis	0	1 (5.5%)	3 (6.9%)	4 (4.6%)	**0**
	Urinary tract infection	0	5 (27.8%)	4 (9.1%)	9 (10.3%)	**0**
	Upper respiratory tract infection	0	2 (11.1%)	8 (32%)	10 (11.5%)	**0**
	Phymosis with infective urinary obstruction	**0**	**2 (11.1%)**	**6 (24%)**	**8 (9.2%)**	**0**

Data are presented as mean±SD & numbers; percentages are in parenthesis; *: significant difference versus neonates with confirmed EOS; †: significant difference versus neonates with suspected EOS.

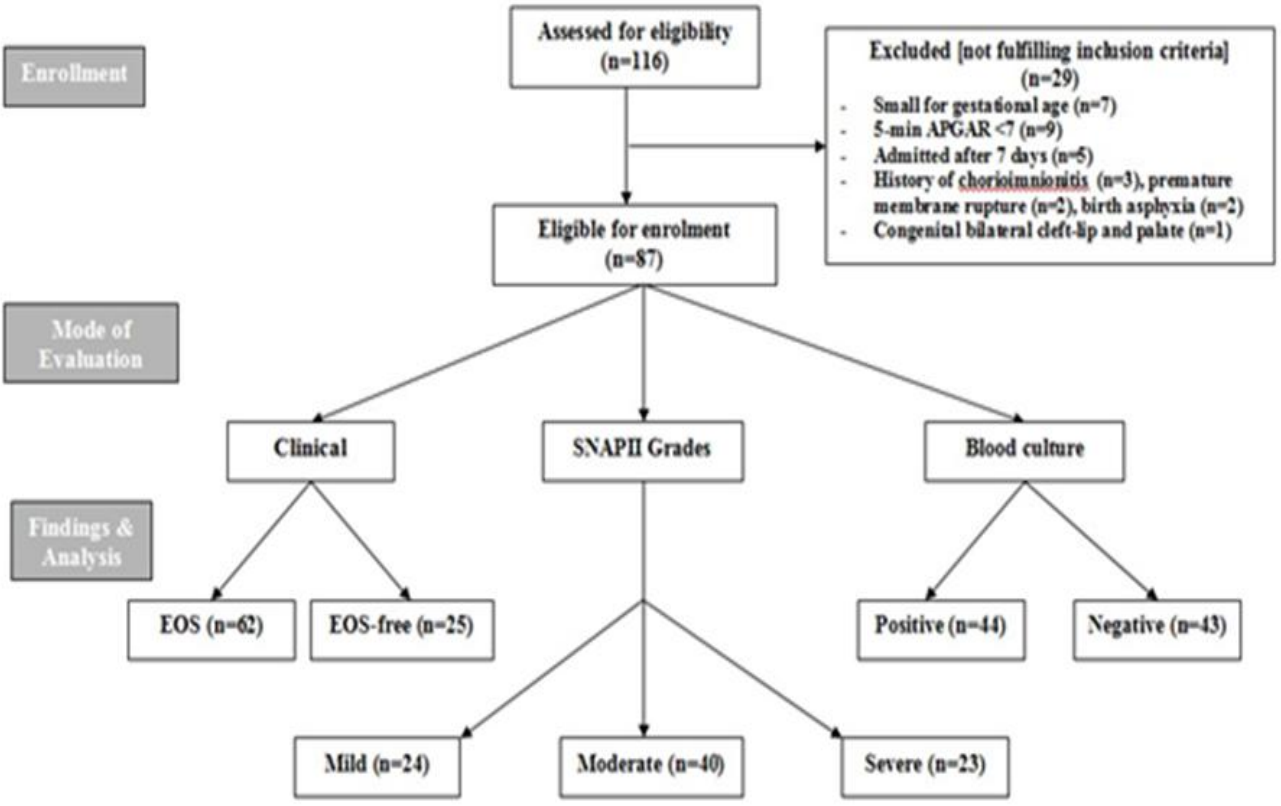

Fig. (1): Consort Flow Sheet

Table (4): Laboratory findings of EOS neonates compared to control findings

Group Parameter		Control	Patients			
			Free	Suspected	Confirmed	**Total**
Leucocytic count	Total (10^3)	6.86±0.61	8.47±3.3	16.1±3.1*†	16.9±2.4*†	14.07±4.7*
	Lymphocytes (%)	28.3±3.2	28.5±3.6	27.4±2.9	29±3.6	28.7±3.38
	Neutrophil (%)	54.6±4.4	52.6±3.9	61.7±3.7*†	61.6±3.9*†	59.6±5*†
hsCRP (mg/ml)		0.71±0.2	1.15±0.73	2.7±0.35*†	2.76±0.7*†	2.25±0.95*
IL-6 (mg/ml)		17.8±4.4	35.92±11.5*	75.5±23.8*†	84.4±29.2*†	68.6±32*
PCT (ng/ml)		**0.213±0.11**	**9.04±3.74***	**34.9±23.1*†**	**60.8±33.2*†‡**	**44.1±34.4***

Data are presented as mean±SD; hsCRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; PCT: Procalcitonin; *: significant difference versus control levels; †: significant difference versus EOS-free; ‡: significant difference versus suspected EOS.

ROC curve analysis for SNAP II scoring and laboratory findings for prediction of positive blood culture defined high serum PCT and IL-6, high SNAP II score, high neutrophil percentages, high serum hsCRP and high TLC as significant predictors for positive blood culture in decreasing order of significance (Fig. 2). Regression analysis of these parameters defined high serum PCT as a persistently significant predictor for positive culture, followed by high serum IL-6 and high SNAP II score (Table 5).

Table (5): *ROC curve and Regression analyses for SNAP II and laboratory parameters as predictors for positive blood culture*

ROC curve defined significant predictors			Regression analysis models for sequence of significant predictors				
Parameter	AUC (95% CI)	P		Parameter	β	t	**p**
SNAP II score	0.752 (0.648-857)	<0.001	Model 1	Serum PCT	0.307	2.988	**0.004**
TLC	0.699 (0.585-814)	0.001		Serum IL-6	0.280	2.837	**0.006**
Lymphocytes percentage	0.558 (0.437-679)	0.353		SNAP II score	0.215	2.259	**0.027**
Neutrophils percentage	0.735 (0.630-839)	<0.001	Model 2	Serum PCT	0.382	3.823	**<0.001**
Serum hsCRP	0.731 (0.623-0.838)	<0.001		Serum IL-6	0.315	3.153	**0.002**
Serum IL-6	0.789 (0.690-0.888)	<0.001	Model 3	Serum PCT	0.535	5.843	**<0.001**
Serum PCT	**0.851 (0.768-0.934)**	**<0.001**					

SNAP II: Score for Neonatal Acute Physiology; TLC: Total leucocytic count; hsCRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; PCT: Procalcitonin; AUC: Area under curve; CI: Confidence interval; β: Standardized coefficient

Considering median value for SNAP II scores and serum levels of studied markers, median value of serum PCT showed the highest sensitivity (79.6%) and specificity rates (83.7%) with high positive likelihood ratio for identification of neonates with possible positive blood culture (Table 6). Evaluation of diagnostic validity of high SNAP II score and serum PCT could define 61.4% of neonates with combined EOS, and high serum levels of hsCRP, IL-6 and PCT could define 52.3% of neonates with combined EOS.

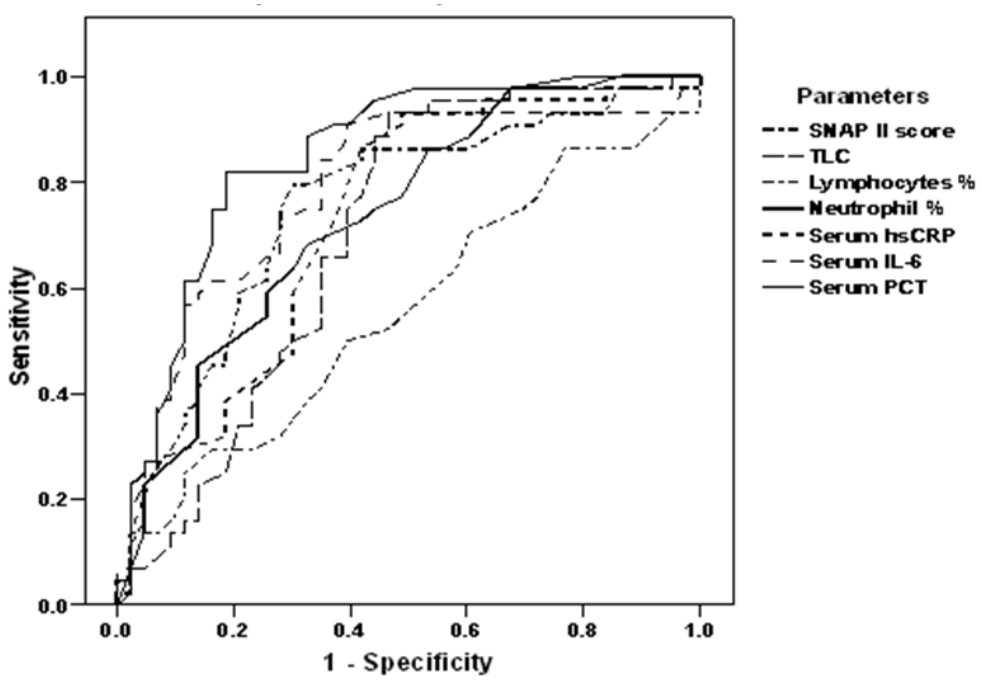

Fig. (2)*: ROC curve analysis for SNAP II and laboratory parameters as predictors for positive blood culture*

Table (5): *Validity characters of SNAP II and laboratory parameters as predictors for positive blood culture.*

	SNAP II	hsCRP	IL-6	PCT
Sensitivity	70.5%	61.4%	68.2%	79.6%
Specificity	69.8%	67.4%	69.8%	83.7%
Positive likelihood ratio	2.33	1.88	2.26	4.89
Negative likelihood ratio	**0.42**	**0.47**	**0.46**	**0.24**

SNAP II: Score for Neonatal Acute Physiology; hsCRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; PCT: Procalcitonin

Discussion:

The current study was based on selective basis through exclusion of all neonates with a possible risk to be immuno-compromised as those had prenatal chorioamnionitis, born after premature rupture of membrane, small-for-gestational age, had complicated delivery process, or had 5-min Apgar score of <7, so clinical evaluation of sepsis and its grading relied on the SNAP II score which is validated by **Dammann et al.** (16, 17) and **Carvalho et al.** (18) who documented that physiologic instability in early postnatal period could be identified by illness severity scores that allows evaluation of the risk of morbidities and mortalities among newborns.

In support of the reliance on SANP II score, statistical analyses defined high SNAP II score as a significant sensitive predictor for confirmed EOS diagnosis and can judge its severity. In line with these findings; **Lah Tomulic et al.** (19) documented that SNAP and 5-min Apgar scores are important indicators for neonatal outcome and can convey information concerning the benefits and

burdens of acute medical interventions. As another support,

recent comparative studies versus other scoring systems assured that SNAP II score override neonatal critical illness score (20) and the clinical risk index for babies scoring system (21) for early prediction of the severely morbid outcome and mortality risk in critically ill neonates.

Blood culture was considered as the gold standard for diagnosis and patients were considered to have confirmed EOS if blood culture was positive and suspected EOS if blood culture was negative. In support of such choice, **Shrestha et al.** (22) documented that clinical manifestation and laboratory test were insufficient to distinguish between neonatal infections, hence blood culture is mandatory and both culture positive and negative cases should be treated promptly and equally. Moreover, **Liu et al.** (23) found 16S rRNA PCR increased the sensitivity in detecting bacterial DNA in newborns with signs of sepsis, but concluded that blood culture is irreplaceable.

At admission TLC and neutrophil percentage were significantly higher in EOS neonates but could not differentiate between neonates with confirmed and suspected EOS and despite of the wide AUC for both parameters as predictors of positive blood culture, Regression analysis excluded both as significant predictors. Thus, at admission TLC could be used only for differentiation between sepsis patients and controls. In line with these findings, multiple previous studies (24, 25, 26) found high white blood cell count and absolute neutrophil count were associated with increasing odds of infection, but did not possess adequate sensitivity to reliably rule out late-onset neonatal sepsis (27).

The predictability of high, at admission, serum levels of hsCRP, IL-6 and PCT for differentiation between sepsis grades and for prediction of positive blood culture were variable; the three parameters could differentiate between patients and controls and between neonates with suspected or confirmed EOS from those of EOS-free neonates. However, high serum levels of IL-6 and PCT could differentiate between controls and EOS-free neonates, while high serum hsCRP could not, and only high serum PCT levels could differentiate between neonates with suspected and confirmed EOS. Moreover, statistical analyses defined high serum IL-6 and PCT levels as significant predictors for positive blood culture. These findings points to a possible role of inflammatory cytokines for early prediction of EOS.

Multiple recent studies supported these findings and assumption where **Ganesan et al.** (28) found IL-6 is a highly sensitive and CRP is more specific markers for diagnosis of neonatal sepsis, so its combination is better for prediction of neonatal sepsis and **Wu et al.** (29) documented that IL-6 and IL-8 are correlated to the severity of neonatal infection, but the value of IL-6 is higher than that of IL-8 in its diagnosis.

In 2017, **Ye et al.** (4) detected significantly elevated serum CRP, IL-6, IL-10 and IL-6/IL-10 levels in neonatal sepsis than control levels, but IL-6 and IL-6/IL-10 outperformed CRP to diagnose neonatal sepsis with IL-6 was the most sensitive predictor of neonatal sepsis. Also, **Fattah et al.** (30) reported that despite of higher PCT, IL-6,

TNF-α, and CRP estimated levels in infected new-

controls, PCT should be used as a part of sepsis screening for all suspected neonates. Moreover, **He et al.** (31) using an array of IL-27, IL-6, TNF-α, HSP 70, MMP-8, PCT, and CRP found all were significantly predictive of EOS, whereas both IL-27 and PCT were independent predictors of EOS in the multivariate model.

Evaluation of diagnostic validity of combined parameters showed that high SNAP II score and serum PCT could define 61.4% and high serum levels of hsCRP, IL-6 and PCT could define 52.3% of neonates with combined EOS. In line with the high diagnostic value of estimation of multiple parameters, **Wu et al.** (29) documented that the combined detection of IL-6 and IL-8 can improve the accuracy of the diagnosis of neonatal septicemia and **Fattah et al.** (30) found that IL-6, TNF-α, and CRP should be measured in combination for more

diagnostic accuracy. Also, **Al-Zahrani et al.** (32)

reported that for sepsis diagnosis, the combination of hs-CRP, PCT, and IL-6 is better than single marker, IL-6 was better for diagnosis of neonatal infection, but PCT had greater diagnostic value than did hs-CRP and IL-6.

Conclusion

Elevated serum inflammatory markers could identify EOS patients and differentiate between its severity grades. Combined estimation of hsCRP, IL-6 and PCT could increase the diagnostic yield of neonatal sepsis; however, clinical evaluation using SNAP II score with estimation of serum PCT did well than estimation of the three markers and could define neonates with positive blood culture earlier so as to allow early treatment.

born babies and neonates with clinical sepsis than

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