Detection of Presepsin and Surface CD14 as a Biomarker For Early Diagnosis of Neonatal Sepsis

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Abstract: Objective: To evaluate the value of Presepsin and surface CD14 in the discrimination between infectious and noninfectious inflammation in comparison to conventional laboratory parameters as total leucocytic count (TLC), C-reactive protein (CRP). **Design and setting:** Prospective, observational study in three neonatal intensive care units. **Patients:** Forty-nine neonates with suspected sepsis (according to clinical suspicion or recent laboratory evidence that supports infection) and twenty-one apparently healthy neonates serving as healthy control group. The suspected sepsis group was further classified according to blood culture result into blood culture positive and blood culture negative patients. Another classification was based on the onset of sepsis into early onset sepsis (EOS) and late onset sepsis (LOS). **Interventions and measurements:** plasma Presepsin level, flow cytometric CD14 expressed in two ways: percent and mean fluorescent intensity (MFI), CRP, blood culture and complete blood count (CBC). **Results:** As for presepsin, CD14 MFI and CRP, their levels showed statistically significant increase when the comparison was held between: suspected sepsis group and control group, blood culture positive patients and controls, clinically septic patients and controls, patients with EOS and controls, patients with LOS and controls. The area under the curve (AUC) was the biggest for CD14 MFI (0.802), followed by presepsin (0.784), then CRP (0.659) and finally CD14% (0.608). The combination of presepsin and CD14 MFI achieved a better diagnostic performance (AUC= 0.883) than CD14 MFI did solely. More interestingly, the addition of measuring Hb level to the combination of presepsin and CD14 MFI showed the best diagnostic performance (AUC= 0.922). **Conclusion:** presepsin and CD14 MFI may help clinicians to make a decision regarding the use of antimicrobial therapy in early stages of sepsis when conjugated with clinical judgment. However, none of the studied markers could be used alone in diagnosing sepsis in an earlier and more specific way but rather the use of combination biomarkers is a more applicable mean to achieve this goal.

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1. Introduction

Sepsis syndrome is systemic inflammatory response syndrome (SIRS) caused by infectious agents. On the other hand, SIRS could be caused by other non-infectious causes like severe trauma and burns [1].

Diagnosing sepsis in neonates can be difficult as most early symptoms and clinical findings are often confusing with many other pathological conditions that may occur at this age period as prematurity and failure to thrive [2,3]. In addition, neonates have immature humoral and cellular immune systems which make them unable to generate a sufficient inflammatory response [4-7]. The role of the laboratory investigations becomes crucial in early diagnosing sepsis and differentiating between infectious and non-infectious causes in order to implement an early targeted therapy and improving the prognosis of patients [8-10]. Although blood culture is considered the gold standard for identifying the microbial agents in suspected septic neonates [11], long time is needed to obtain results. It also has low sensitivity due to small sample volume often taken from neonates and maternal antibiotic treatment. Furthermore, the possibility of presence of contamination by skin flora as coagulase negative staphylococcus is a problem in many cultures [12,13]. C-reactive protein (CRP) is a nonspecific acute phase reactant that rises in both infectious and other non-infectious inflammatory conditions as meconium aspiration syndrome and perinatal asphyxia [14]. It also undergo a physiological rise 3 days after birth [15,16]. It also shows lowest sensitivity in the early stages of infection in comparison to later stages [17]. CD14 is a multifunctional cell surface glycoprotein that exists either in a glycosylphosphatidylinositol (GPI) anchored membrane form or in a soluble form (sCD14). Presepsin is the N-terminal 13 kDa truncated fragment of soluble CD14 [18]. CD14 is expressed on the surface of various cells, including monocytes,

macrophages, neutrophils, chondrocytes, B cells. It plays an important role in mediating cell activation stimulated by bacterial cell wall component. It is a specific high-affinity receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein (LBP) and may modulate LPS-triggered apoptosis [19]. It also can recognize other bacterial elements as peptidoglycans, the major cell wall component of gram-positive bacteria [8].

Upon binding of the LBP complex to CD14, it activates the toll-like receptor 4 (TLR4) specific proinflammatory signaling cascade thereby starting the inflammatory reaction of the host against infectious agents [20]. Then the complex LPS-LBP-CD14 is released into circulation by shedding of CD14 from the cell membrane yielding the soluble form (sCD14) which is also directly secreted by hepatocytes leading to the conclusion that sCD14 should be considered as a minor acute-phase protein [8]. sCD14 is cleaved by cathepsin D and other proteases in plasma to produce sCD14-ST presepsin[21].

2. Methods

Patients and setting

This prospective observational study was conducted in three neonatal intensive care units between October 2013 and May 2014. Informed consents from the parents were taken and the study was approved by the Ethics committee of our institution (no 13113).Forty-nine consecutive neonates with SIRS and suspected infections (based on clinical suspicion or recent laboratory evidence that supports infection) were eligible for enrollment. A first follow up was done after 3-5 days from the initial workup for 19 patients from the suspected sepsis group and a second one was done after another 5 days for 9 patients from the same group. The study also included twenty one apparently healthy neonates serving as healthy control group taken from either the maternity ward or admitted to NICU for non-septic causes with proved normal laboratory findings and no illness subsequently detected.

Inclusion criteria:

Neonates were enrolled into the suspected sepsis group according to either:

1- Clinical suspicion of sepsis based on the presence of two or more of the following clinical symptoms or signs of infection:

- Respiratory compromise: respiratory rate of >60 breaths per minute, or cessation of respiration for ≥20 seconds, occurring at a rate of ≥2 times per hour, or pulse oxymeter readings of ≤ 85%.
- Cardiovascular compromise: heart rate of <100 beats per minute, pallor or hypotension.
- Metabolic changes: hypothermia (rectal temperature of <36^{o}C), fever (body temperature of >38^{o}C), feeding intolerance, glucose instability (blood glucose level of <45 mg/dL or >125 mg/dL), or metabolic acidosis (pH<7.25).
- Neurologic changes: lethargy or decreased activity [22].

2- Recent laboratory results providing evidence of probable sepsis like positive CRP (>6mg/l) [16].

All newborns were subjected to Detailed history taking from patient medical records including**:** Birth weight (BW), Gestational age (GA), use of mechanical ventilation, presence of associated genetic disorder in addition to Thorough clinical examination**.**

Sample collection and measurements:

Two milliliters (ml) of peripheral venous blood samples were aseptically withdrawn from each patient. Each sample was divided on 2 tubes: sterile plain tube from which serum was separated afterwards for CRP measurement and ethylene diamine tetra-acetate (EDTA) tube from which 100 µl fresh blood taken for flow cytometric analysis. Samples were processed within 24 hours of collection or stored refrigerated at (4°C) for processing in a period not exceeding 48 hours. The remaining amount was centrifuged to separate plasma and the yielded plasma was stored at –70°C until used for the assay of presepsin(Mitsubishi Chemical Europe GmbH Willstätter Str. 30, 40549 Düsseldorf, Germany). Frozen samples were allowed to thaw and brought to room temperature only before analysis. Before processing samples were gently mixed and then centrifuged at 2500 – 3000 x g for 10 minutes according to instructions given by presepsin manufacturer kit pamphlet.

Methods:

1) Complete blood count using COLUTER LH 750 analyzer (Beckman Coulter Inc, USA.). The following hematologic criteria were used as indications for Sepsis:

a. Absolute neutrophil count (ANC):

- <1,800 or >6,500/µL at birth,
- <7,000 or >12,000/µL at 3 to 24 hours of age,
- <4,000 or >9,000/µL at 25 to 48 hours of age,
- <2,000 or >7,000/µL beyond 48 hours of age.

b. Absolute band count of >1,500/µL.

c. Immature to total neutrophil ratio (IT- ratio) of >0.16.

d. White blood cells (WBC) count of >18,000/µL or <6,000/µL.

e. Platelet count of <150,000/µL[22].

2) C-reactive protein:

CRP was measured by high sensitive-CRP reagent (Synchron Clinical System, Beckman Coulter Inc., Brea, CA, USA) using the Automated Chemistry Analyzer (UniCelDxC 800, Beckman Coulter Inc.) and if not available by using latex immunoassay (Plasmatec, Dhanmondi, Dhaka - Bangladesh).

3) Blood cultures:

All blood cultures were collected using standard sterile techniques. The Pediatric Blood Culture System Medium 8 ml (Salix) and if available the Bactec Microbial Detection System (Becton Dickinson, Parsipanny, NJ, USA) were used to detect positive blood cultures, subcultures were done on chocolate agar plates and if there is growth detected, cultures on both blood and Maconkey plates were done.

4) Presepsin level measurement:

Presepsin concentration was measured with PATHFAST (Mitsubishi Chemical Europe GmbH Willstätter Str. 30, 40549 Düsseldorf, Germany) compact automated immunoanalyzer based on a chemiluminescent enzyme immunoassay (CLEIA) supplied by Mitsubishi Chemical Medience[23].

5) Flow cytometric analysis for surface expression of CD14:

Analysis of monocyte and granulocyte CD14 surface marker was done using CD14 monoclonal antibodies (MoAbs) labelled by fluorescineisothiocyanate (FITC) using BD (**Becton, Dickinson and Company, USA.**) Accuri™ C6 Cytometer, USA. CD14 expression was measured as mean fluorescent intensity (MFI) and percentage of cells expressing CD14 (CD14%)[24,25].

Statistical Analysis:

Data were analyzed using IBM SPSS ver. 16.0 program. Description of qualitative data was carried out by using frequency and percentage. Description of quantitative data was carried out by using mean and standard deviation for normally distributed results or median & IQR (interquartile range) for skewed results.

Comparison between quantitative parametric variables was carried out by using Student's t-test (t). Comparison between two quantitative, nonparametric variables was carried out by using Mann-Whitney's U-test. Comparison between two independent groups as regards the categorized data was carried out using Chi-square test. Correlation analysis assessing the strength of association between two non-parametric variables was done by Spearmann's correlation study. The correlation coefficient denoted symbolically rs, defines the strength and direction of the linear relationship between each of variables. Significance level (p) is expressed as $p > 0.05$ is non-significant (NS), $p < 0.05$ is significant (S) and $p < 0.01$ or < 0.001 are highly significant (HS). Receiver-operting characteristic (ROC) curves were constructed to obtain the most sensitive and specific cutoff value for each examined variable. To evaluate the diagnostic accuracy area under the ROC curve (AUC) was also calculated. Combined ROC curves were done to detect the combination of laboratory parameters that achieved the best diagnostic performance and to assess whether combining laboratory markers has statistically better diagnostic value than usage of a single marker or not [26].

3. Results:

A total of 49 neonates with suspected sepsis were included in the study along with 21 non infected controls. The demographic data of patients and controls regarding sex, weight, gestational age and age during study is shown in **(table 1).** The clinical and laboratory background of patients of the suspected sepsis group (group 1) is demonstrated in **(Table 2).** The difference between neonates of both groups regarding presepsin levels, CD14 MFI, CD14 %, CRP, TLC, platelets and Hbdemonstrated in (**Table 3)**.

Table (1): Demographic data of study population

	suspected sepsis group (n=49)	control group (n=21)	Test statistics	p
Age (days)				
Median	8	1		0.000
Range	44	44	Z*=5.285	HS
IQR	11	1		
Sex [No. (%)]				
Male	23 (46.9)	10 (47.6)		0.958
Female	26 (53.1)	11 (52.4)	X^{2*}=.003	NS
Weight [No. (%)]				
Normal	20 (40.8)	18 (85.7)		
LBW	16 (32.7)	2 (9.5)		0.010
VLBW	11 (22.4)	1 (4.8)	Z*=2.559	S
Macrosomia	2 (4.1)	0 (0)		
Gestational age (weeks)				0.000
Mean ± SD	43.46 ± 3.6	38.09 ± 2.34	t*=4.995	HS

t*: analysis using independent student's t-test. X^{2*}: analysis using Pearson chi-square test.

Z*: Data were presented as median and IQR and compared using Mann-Whitney test

NS = non-significant S = significant HS = highly significant

In a trial to find out any association between levels of measured variables and blood culture results, we classified group 1 into cases with positive blood culture (n=31) and those with negative blood culture (n=14) and we compared between them and between each of these subgroups and the control group in **tables (4, 5 and 6)**. According to type of sepsis, we classified suspected sepsis patients into neonates with early onset sepsis (EOS) (n=28) and those with late onest sepsis (LOS) (n=21). **Table (*7*)** shows the difference in measured variables among patients who had EOS and those who had LOS. **Table (*8* and *9*)** shows the difference in measured variables once between patients with EOS and control group and and then between patients with LOS and control group, respectively.

Table 10 shows the correlation between different parameters among study population at initial workup. A significant negative correlation was found between presepsin and Hb as well as between CRP and Hb. No other significant correlations could be detected.

Table (11) shows area under ROC curves and the cut off value that produced best sensitivity and specificity for CRP, presepsin, CD14 MFI and CD14% as predictors of sepsis. Figures 1-4 show ROC curves illustrating AUC of CRP, presepsin, CD14 MFI and CD14%, respectively.

Results of combined Roc curves analysis of the combinations of parameters that showed the best diagnostic performance are shown in **table (12).**

Table (2): Clinical and laboratory background the suspected sepsis group (n=49):

Variable	No	%
1- PDA	5	10.2
2- Respiration		
• **Free**	23	46.9
• **Distressed**	26	53.1
3- Diagnosis		
• **Prematurity**	21	42.9
• **RDS**	11	22.4
• **Operative causes**	7	14.3
• **Congenital anomalies**	6	12.2
• **Others**	4	8.2
4- Blood culture		
• **No growth**	14	28.7
• **Klebsiella**	10	20.4
• **Acinetobacter**	1	2
• **Coaulase –ve staph**	5	10.3
• **Staph aureus**	1	2
• **MRSA**	1	2
• **Streptococcus**	2	4.1
• **Fungal**	1	2
• **Polymicrobial**	8	16.3
• **Enterococcus**	1	2
• **Bacillus**	1	2
• **Missing**	4	8.2

5- Length of hospital stay (days)	Median	Range	IQR
	15.5	88	28

Table (3): Comparison between suspected sepsis group and control group regarding the different laboratory parameters:

Variable	Group 1 [suspected sepsis] (n=49)	Group 2 [control group] (n=21)	Z*	P
presepsin (pg/ml)			3.197	0.001 HS
Median	2879	686		
Range	10534	19823		
IQR	3217	2018.75		
CD14 MFI			3.635	0.000 HS
Median	2019.07	264.87		
Range	9732.02	5775.03		
IQR	2725.62	464.02		
CD14 %			1.303	0.193 NS
Median	43.84	55.84		
Range	95.36	59.77		
IQR	43.39	28.35		
CRP (mg/L)				
Median	6	0	2.213	0.027
Range	101	103.7		S
IQR	24	103.7		
TLC ($\times 10^3/\mu l$)				
Median	15.1	16.8	0.417	0.677
Range	63.9	22.7		NS
IQR	10.55	5.8		
Platelets ($\times 10^3/\mu l$)				
Median	220	235	1.025	0.305
Range	532	319		NS
IQR	220.5	178		
Hb (g/dl)				
Median	13.4	18.4		0.000
Range	10.1	12.7	4.064	HS
IQR	4.5	5		

Z*: Data were presented as median and IQR and compared using Mann- Whitney test
NS = non-significant; S = significant; HS = highly significant

Table (4): Comparison between blood culture positive and blood culture negative patients regarding different parameters studied:

Variable	Blood culture positive (n=31)	Blood culture negative (n=14)	Z*	P
Presepsin (pg/ml)			**0.782**	**0.435 NS**
Median	**2879**	**2624**		
Range	**5986**	**8757**		
IQR	**3288.5**	**2740.25**		
CD14 MFI			**0.316**	**0.752 NS**
Median	**2120.24**	**2326.45**		
Range	**9355.68**	**6170**		
IQR	**2593.3**	**4143.69**		
CD14 %			**1.086**	**0.277 NS**
Median	**36.29**	**47.38**		
Range	**66.42**	**95.36**		
IQR	**36.16**	**62.78**		
CRP (mg/l)				
Median	**10**	**6**	**1.240**	**0.215**
Range	**101**	**96**		**NS**
IQR	**24**	**12**		
TLC ($\times 10^3/\mu l$)				

Median	**14.3**	**18.05**		**0.309**
Range	**63.9**	**44.6**	**1.018**	**NS**
IQR	**11.5**	**10.85**		
Platelets ($\times 10^3$/µl)				
Median	**214**	**198.5**	**0.466**	**0.641**
Range	**532**	**494**		**NS**
IQR	**192**	**303**		
Hb (g/dl)				
Median	**13.4**	**11.9**	**0.879**	**0.379**
Range	**10.1**	**9.5**		**NS**
IQR	**4.3**	**4.6**		

Z*: analysis using Mann Whitney test
HS = highly significant; NS = non-significant; S = significant

Table (5): Comparison between blood culture positive patients and control group regarding different parameters studied:

Variable	Blood culture positive (n=31)	Control group (n=21)	Z*	P
Presepsin (pg/ml)			2.704	0.007 HS
Median	2879	686		
Range	5986	19823		
IQR	3288.5	2018.75		
CD14 MFI				
Median	2120.24	264.87	3.819	0.000 HS
Range	9355.68	5775.03		
IQR	2593.3	464.02		
CD14 %			1.809	0.070 NS
Median	36.29	55.84		
Range	66.42	59.77		
IQR	36.16	28.35		
CRP (mg/l)				
Median	10	0		
Range	101	103.7	2.117	0.034
IQR	24	103.7		S
TLC ($\times 10^3$/µl)				
Median	14.3	16.8		
Range	63.9	22.7	0.69	0.490
IQR	11.5	5.8		NS
Platelets ($\times 10^3$/µl)				
Median	214	235	1.359	0.174
Range	532	319		NS
IQR	192	178		
Hb (g/dl)				
Median	13.4	18.6		
Range	10.1	12.5	3.985	0.000
IQR	4.3	4.93		HS

Z*: anal
ysis using Mann Whitney test
HS = highly significant; NS = non-significant; S = significant

Table (6): Comparison between blood culture negative patients and control group regarding different parameters studied:

Variable	Blood culture negative (n=14)	Control group (n=21)	Z*	P
Presepsin (pg/ml)			1.428	0.153
Median	2624	686		NS
Range	8757	19823		
IQR	2740.25	2018.75		
CD14 MFI			2.868	0.004
Median	2326.45	264.87		HS
Range	6170	5775.03		
IQR	4143.69	464.02		
CD14 %			0.499	0.618
Median	47.385	55.84		NS
Range	95.36	59.77		
IQR	62.78	28.35		
CRP (mg/l)				
Median	6	0		
Range	96	103.7	1.165	0.244
IQR	12	103.7		NS
TLC ($\times 10^3/\mu l$)				
Median	18.05	16.8		
Range	44.6	22.7	0.583	0.560
IQR	10.85	5.8		NS
Platelets ($\times 10^3/\mu l$)				
Median	198.5	235	0.765	0.444
Range	494	319		NS
IQR	303	178		
Hb (g/dl)				
Median	11.9	18.6	3.568	0.000
Range	9.5	12.5		HS
IQR	4.6	4.93		

Z*: analysis using Mann Whitney test; HS = highly significant; NS = non-significant; S = significant

Table (7): Comparison between EOS and LOS patients regarding different parameters studied:

Variable	EOS (n=28)	LOS (n=21)	Z*	P
Presepsin (pg/ml)			1.183	0.237
Median	2489	3647		NS
Range	5336	10534		
IQR	1711.5	4199		
CD14 MFI				
Median	1747.33	2801.16	1.059	0.290
Range	9355.68	8166.76		NS
IQR	2809.62	3945.47		
CD14 %			0.640	0.522
Median	44.02	43.425		NS
Range	78.1	91.90		
IQR	42.05	40.54		
CRP (mg/l)				
Median	6	10	1.660	0.097
Range	96	101		NS
IQR	17	42		
TLC ($\times 10^3/\mu l$)				

Median	14.3	18	1.529	0.126
Range	26.9	61.4		NS
IQR	12.1	8.5		
Platelets ($\times10^3$/µl)				
Median	210	274	1.423	0.155
Range	475	530		NS
IQR	178	383		
Hb (g/dl)				
Median	14.1	12.4	1.223	0.221
Range	10.1	9.7		NS
IQR	5.05	4.5		

EOS: early onset sepsis; LOS: late onset sepsis; Z*: analysis using Mann Whitney test.
HS = highly significant; NS = non-significant; S = significant

Table (*8*): Comparison between EOS and control group regarding different parameters studied:

Variable	EOS (n=28)	Control group (n=21)	Z*	P
Presepsin (pg/ml)			2.583	0.010
Median	2489	686		S
Range	5336	19823.00		
IQR	1711.5	2018.75		
CD14 MFI				
Median	1747.33	264.87	3.267	0.001
Range	9355.68	5775.03		HS
IQR	2809.62	464.02		
CD14 %			1.627	0.104
Median	44.02	55.84		NS
Range	78.1	59.77		
IQR	42.05	28.35		
CRP (mg/l)				
Median	6	0	1.553	0.120
Range	96	103.7		NS
IQR	17	103.7		
TLC ($\times10^3$/µl)				
Median	14.3	16.8	1.044	0.297
Range	26.9	22.7		NS
IQR	12.1	5.8		
Platelets ($\times10^3$/µl)				
Median	210	235	1.718	0.086
Range	475	319		NS
IQR	178	178		
Hb (g/dl)				
Median	14.1	18.6	3.392	0.001
Range	10.1	12.5		HS
IQR	5.05	4.93		

EOS: early onset sepsis; Z*: analysis using Mann Whitney test. HS = highly significant
NS = non-significant; S = significant

Table (9): Comparison between LOS and control group regarding different parameters studied:

Variable	LOS (n=21)	Control group (n=21)	Z*	P
Presepsin (pg/ml)			2.794	0.005 HS
Median	3647	686		
Range	10534	19823		
IQR	4199	2018.75		
CD14 MFI			3.103	0.002 HS
Median	2801.16	264.87		
Range	8166.76	5775.03		
IQR	3945.47	464.02		
CD14 %			0.726	0.468 NS
Median	43.425	55.84		
Range	91.9	59.77		
IQR	40.54	28.35		
CRP (mg/l)				
Median	10	0	2.712	0.007
Range	101	103.7		HS
IQR	42	103.7		
TLC ($\times 10^3/\mu l$)				
Median	18	16.8	0.657	0.511
Range	61.4	22.7		NS
IQR	8.5	5.8		
Platelets ($\times 10^3/\mu l$)				
Median	274	235	0.102	0.919
Range	530	319		NS
IQR	383	178		
Hb (g/dl)				
Median	12.4	18.6	3.684	0.000
Range	9.7	12.5		HS
IQR	4.5	4.93		

LOS: late onset sepsis; Z*: analysis using Mann Whitney test
HS = highly significant; NS = non-significant; S = significant

Table (10): Correlation between different parameters among study population at initial workup:

Correlation between	r_s	p	Significance
Presepsin (pg/ml) and CD14 MFI	o.222	0.192	NS
Presepsin (pg/ml) and CD14 %	o.182	0.289	NS
Presepsin (pg/ml) and Hb (g/dl)	o.350*	0.018	S
Presepsin (pg/ml) and TLC	o.134	0.392	NS
Presepsin (pg/ml) and Neutrophils	0.060	0.744	NS
Presepsin (pg/ml) and Lymphocytes	0.137	0.412	NS
Presepsin (pg/ml) and Monocytes	0.023	0.900	NS
Presepsin (pg/ml) and CRP	0.215	0.156	NS
CD14 MFI and CD14 %	0.097	0.455	NS
CD14 MFI and Hb (g/dl)	0.172	0.185	NS
CD14 MFI and TLC	0.012	0.931	NS
CD14 MFI and Neutrophils	0.007	0.964	NS
CD14 MFI and Lymphocytes	0.125	0.378	NS
CD14 MFI and Monocytes	0.090	0.556	NS
CD14 MFI and CRP	0.10	0.434	NS
CD14 % and Hb (g/dl)	0.113	0.385	NS
CD14 % and TLC	0.198	0.133	NS

CD14 % and Lymphocytes	0.052	0.716	NS
CD14 % and Monocytes	0.022	0.886	NS
CD14 % and CRP	0.080	0.538	NS
Hb (g/dl) and CRP	0.302*	0.011	S
TLC and CRP	0.081	0.509	NS

Hb: hemoglobin TLC: total leucocytic count
PLT: platelet count r_s: Spearman's correlation coefficient.
HS = highly significant; NS = non-significant; S = significant

Table (11): Area under ROC curves (AUC) and the cut off value that produced best sensitivity and specificity for CRP, presepsin, CD14 MFI and CD14%:

	AUC	Cutoff value	Sensitivity %	Specificity %
CRP (mg/l)	**0.659**	**2**	**65.3 %**	**71.4 %**
		6	**63.3%**	**71.4%**
Presepsin (pg/ml)	**0.784**	**1807.5**	**85.2 %**	**72.2 %**
CD14 MFI	**0.802**	**539.49**	**90.9 %**	**64.7 %**
CD14 %	**0.608**	**43.84**	**50 %**	**70.6 %**

Table (12): Diagnostic value of best combinations yielded by combined ROC curve. Area under the curve (AUC), odds ratio (OR), positive predictive value (PPV), negative predictive value (NPV)

Parameter	AUC	Sensitivity %	Specificity %	OR	PPV	NPV
Presepsin (pg/ml) with CD14 MFI	**0.883**	**90.9 %**	**78.6 %**	**36.667**	**87.00%**	**84.60%**
CD14 MFI with Hb (g/dl)	**0.840**	**95.5 %**	**76.5 %**	**68.250**	**91.30%**	**86.70%**
Presepsin (pg/ml) with CD14 MFI with Hb (g/dl)	**0.922**	**90.9 %**	**85.7 %**	**60.000**	**90.9 %**	**85.7 %**

4. Discussion

Neonates especially premature ones who are admitted to the NICU are at higher risk of developing sepsis due to their immature immune system, exposure to nosocomial pathogens and the invasive life saving medical interventions that they could go through. So, finding more recent ways to early diagnose and treat neonatal sepsis became of great importance in lowering the morbidity and mortality rates in NICUs.

Presepsin and surface CD14 are potential possible biomarkers in comparison to other conventional methods (CRP and blood culture) in early diagnosis in sepsis.

Comparative median values of presepsin, CD14 MFI and CD14% between the suspected sepsis group and the control group revealed a significant increase of presepsin and CD14 MFI while CD 14% was insignificantly decreased in group 1 when compared to control group .

In the current study, the detection of significant increase in median values of presepsin and the insignificant decrease in median values of CD14% when neonates with suspected sepsis were compared to controls goes in line with what was previously hypothesized that LPS derived from gram-negative bacteria induces the shedding of CD14 molecules from the surface of monocytes and consequently CD14 expression on circulating monocytes is down-regulated in the acute phase of gram-negative sepsis, while the plasma soluble CD14 levels is increased[27].

The decrease in median values of CD14%, being insignificant may further explains why there was no significant correlation in our study between membrane (cellular) expression of CD14 evidenced by flow cytometer (whether CD14 MFI or CD14%) and soluble CD14 measured as presepin concentration.

In a trial to find a biomarker that can discriminate between blood culture positive and blood culture negative neonates who were suspected clinically to be septic, we couldn't detect a significant difference in presepsin,CD14 MFI and CD14% between those two groups. This may denote that either these biomarkers, especially presepsin, were not necessarily increased in bacterial infection or that blood culture has low sensitivity to detect bacterial infections [13]&[28]. However, we might propose that the first possibility could be excluded based on the fact that presepsin levels showed a highly significant

increase when we compared it between blood culture positive neonates and the control group.

There was no significant difference in the median values of presepsin, CD14 MFI and CD14% between patients with EOS and those with LOS. However, the values of presepsin and CD14 MFI were higher in patients with EOS than control group, as well as they were higher in patients with LOS than in control.. These findings indicate that elevated presepsin levels during sepsis are not affected by age although neonatal age is known to be continuously characterized by abrupt changes in homeostasis, particularly in preterm newborns [20].

We couldn't detect significant correlation between levels of CRP and presepsin or between CD14 MFI and CD14%.

The diagnostic performance of the studied parameters measured through ROC curves showed that The AUC was the biggest for CD14 MFI followed by presepsin then CRP and lastly CD14%. The cut off values that produced the best sensitivity and specificity for CRP was 2 mg/L at this value the sensitivity was 65.3% and specificity was 71.4%. However, when we assessed sensitivity and specificity at the currently used cut off for CRP in our laboratories which is 6 mg/L, the sensitivity didn't differ a lot from that revealed at 2 mg/L while the specificity remained the same.Other studies emphasize on the use of adjusted cut-off values for CRP according to gestational age and postnatal age which would subsequently give a better reflection if neonatal pathology [14,16].

Plasma presepsin showed a sensitivity of 85.2% and a specificity of 72.2% at a cut off value of 1807.5 pg /ml. Many authors detect lower cut off levels of presepsin. This difference may be due to different methods and different samples (plasma versus whole blood) used in the determination [20,29,28,30]. CD14 MFI showed a sensitivity of 90.9% and a specificity of 64.7 % at a cut off value of 539.49 MFI. CD14% showed the least sensitivity (50%) and it showed a specificity of 70.6% at a cut off value of 43.84%. Unfortunately, there is no unique cut off or reference range established in any paper for CD14 MFI or CD14% but rather they depend in their judgment on comparison between cases and controls.

When combined Roc curves analysis was done, the combination of Presepsin and CD14 MFI achieved better diagnostic performance than each of them alone in all aspects (AUC, sensitivity and specificity). Moreover, the addition of Hb measurement to this previous combination further improved the diagnostic performance.

Limitations

Our study had some limitations; the first limitation was the indeterminism of the clinical diagnosis at the initial presentation which made it somewhat difficult to determine the category of patient whether SIRS, sepsis or other conditions that could be easily confused with sepsis. Also, faced the problem of getting samples from healthy controls whom their age was matched with the septic group (due to difficulty in getting a consent from the parents). So, most of samples taken from neonates in the control group were in day 1 after delivery (as it was more feasible to get a consent from parents).

Conclusion:

Neonatal sepsis is associated with an increase in the levels of presepsin and CD14 MFI and they appear to be valuable and promising markers in the diagnosis of neonatal sepsis. However, none of the parameters studied is able to be the one parameter in the diagnosis of neonatal sepsis in a sufficiently early and specific way. Based on our results, the combination of multiple parameters is rather a wiser mean to achieve this goal. As regards presepsin, it may help influence a decision to be made regarding the use of antimicrobial therapy or not when conjugated with clinical judgment and it seems to have the potential to complement the existing combination of CRP, CBC and blood culture in the diagnosis of neonatal sepsis as its measurement is more feasible and not technically demanding as CD14 especially in NICUs where the time taken to obtain results is crucial. We also assume that none of the currently available biomarkers can be used to definitely determine whether a patient is infected or not, that's why the search for an ideal biomarker should continue.

Recommendations:

Further studies are needed to investigate the value of combining additional new biomarkers with conventional biomarkers and their effect on improving the diagnostic performance and accuracy of sepsis diagnosis. This also could improve neonatologists' ability to differentiate patients with bacterial infections from those with systemic inflammation of non-bacterial origin when they are admitted which would be of great importance in patients in whom diagnosis is not clinically clear-cut, as is often the case in NICUs.

Ethical approval:

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

References:

1. Ramphal R (2007) Sepsis syndrome. In: Southwick FS (ed) Infectious diseases: A clinical short course. McGraw-Hill USA, pp 57-59. doi:10.1036/0071477225.
2. Jackson GL, Engle WD, Sendelbach DM, Vedro DA, Josey S, Vinson J, Bryant C, Hahn G, Rosenfeld CR (2004) Are complete blood cell counts useful in the evaluation of asymptomatic neonates exposed to suspected chorioamnionitis? Pediatrics 113 (5):1173-1180.
3. Groselj-Grenc M, Ihan A, Pavcnik-Arnol M, Kopitar AN, Gmeiner-Stopar T, Derganc M (2009) Neutrophil and monocyte CD64 indexes, lipopolysaccharide-binding protein, procalcitonin and C-reactive protein in sepsis of critically ill neonates and children. Intensive Care Med 35 (11):1950-1958. doi:10.1007/s00134-009-1637-7.
4. Marodi L (2006) Neonatal innate immunity to infectious agents. Infect Immun 74 (4):1999-2006. doi:10.1128/IAI.74.4.1999-2006.2006.
5. Hotoura E, Giapros V, Kostoula A, Spirou P, Andronikou S (2011) Tracking changes of lymphocyte subsets and pre-inflammatory mediators in full-term neonates with suspected or documented infection. Scand J Immunol 73 (3): 250-255. doi:10.1111/j.1365-3083.2010.02499.x.
6. Levy O (2007) Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol 7 (5):379-390. doi:10.1038/nri2075.
7. Edmond K, Zaidi A (2010) New approaches to preventing, diagnosing, and treating neonatal sepsis. PLoS Med 7 (3):e1000213. doi:10.1371/journal.pmed.1000213.
8. Mussap M, Noto A, Fravega M, Fanos V (2011) Soluble CD14 subtype presepsin (sCD14-ST) and lipopolysaccharide binding protein (LBP) in neonatal sepsis: new clinical and analytical perspectives for two old biomarkers. J Matern Fetal Neonatal Med 24 Suppl 2:12-14. doi:10.3109/14767058.2011.601923.
9. Volker NU, Stephan D, Thorsten WO (2013) Flow Cytometry in the Detection of Neonatal Sepsis. International Journal of Pediatrics 2013:1-6.
10. Kuhl DA (2002) Current strategies for managing the patient with sepsis. Am J Health Syst Pharm 59 Suppl 1:S9-13.
11. Resch B, Hofer N, Müller W (2012) Challenges in the Diagnosis of Sepsis of the Neonate. In: Azevedo L (ed) Sepsis - An Ongoing and Significant Challenge. InTech. doi:10.5772/2958.
12. Shozushima T, Takahashi G, Matsumoto N, Kojika M, Okamura Y, Endo S (2011) Usefulness of presepsin (sCD14-ST) measurements as a marker for the diagnosis and severity of sepsis that satisfied diagnostic criteria of systemic inflammatory response syndrome. J Infect Chemother 17 (6):764-769. doi:10.1007/s10156-011-0254-x.
13. Terrin G, Passariello A, Manguso F, Salvia G, Rapacciuolo L, Messina F, Raimondi F, Canani RB (2011) Serum calprotectin: an antimicrobial peptide as a new marker for the diagnosis of sepsis in very low birth weight newborns. Clin Dev Immunol 2011:291085. doi:10.1155/2011/291085.
14. Hofer N, Müller W, Resch B (2013) The Role of C-Reactive Protein in the Diagnosis of Neonatal Sepsis. In: Resch B (ed) Neonatal Bacterial Infection. InTech, Croatia, pp 55-46
15. Chiesa C, Signore F, Assumma M, Buffone E, Tramontozzi P, Osborn JF, Pacifico L (2001) 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 47 (6):1016-1022.
16. Chiesa C, Natale F, Pascone R, Osborn JF, Pacifico L, Bonci E, De Curtis M (2011) C reactive protein and procalcitonin: Reference intervals for preterm and term newborns during the early neonatal period. Clinica Chimica Acta 412 (11–12):1053-1059. doi:http://dx.doi.org/10.1016/j.cca.2011.02.020.
17. Chirico G, Loda C (2011) Laboratory aid to the diagnosis and therapy of infection in the neonate. Pediatr Rep 3 (1):e1. doi:10.4081/pr.2011.e1.
18. Yaegashi Y, Shirakawa K, Sato N, Suzuki Y, Kojika M, Imai S, Takahashi G, Miyata M, Furusako S, Endo S (2005) Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. J Infect Chemother 11 (5):234-238. doi:10.1007/s10156-005-0400-4.
19. Bas S, Gauthier BR, Spenato U, Stingelin S, Gabay C (2004) CD14 is an acute-phase protein. J Immunol 172 (7):4470-4479.
20. Mussap M, Puxeddu E, Burrai P, Noto A, Cibecchini F, Testa M, Puddu M, Ottonello G, Dessi A, Irmesi R, Gassa ED, Fanni C, Fanos V (2012) Soluble CD14 subtype (sCD14-ST) presepsin in critically ill preterm newborns: preliminary reference ranges. J Matern Fetal Neonatal Med 25 (Suppl 5):51-53. doi:10.3109/14767058.2012.717462.
21. Zou Q, Wen W, Zhang Z (2014) Presepsin as a novel sepsis biomarker. World J Emerg Med 5 (1):16–19.
22. Choo YK, Cho HS, Seo IB, Lee HS (2012) Comparison of the accuracy of neutrophil CD64 and C-reactive protein as a single test for the

early detection of neonatal sepsis. Korean J Pediatr 55 (1):11-17. doi:10.3345/kjp.2012.55.1.11.
23. Novelli G, Morabito V, Ferretti G, Pugliese F, Ruberto F, Venuta F, Poli L, Rossi M, Berloco PB (2013) Pathfast presepsin assay for early diagnosis of bacterial infections in surgical patients: preliminary study. Transplantation proceedings 45 (7):2750-2753. doi:10.1016/j.transproceed.2013.07.021.
24. Rahman F (2006) Introduction to flowcytometry. J Clin Invest 45:345–361.
25. Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J (1993) Guideline for flow cytometric immunophenotyping: a report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. CYTOMETRY-NEW YORK- 14:702-702.
26. Haker S, Wells WM, Warfield SK, Talos I-F, Bhagwat JG, Goldberg-Zimring D, Mian A, Ohno-Machado L, Zou KH (2005) Combining Classifiers Using Their Receiver Operating Characteristics and Maximum Likelihood Estimation. Medical image computing and computer-assisted intervention : MICCAI International Conference on Medical Image Computing and Computer-Assisted Intervention 8 (0 1):506-514.
27. Takeshita S, Nakatani K, Tsujimoto H, Kawamura Y, Sekine I (2000) Detection of circulating lipopolysaccharide-bound monocytes in children with gram-negative sepsis. J Infect Dis 182 (5):1549-1552. doi:10.1086/315884.
28. Endo S, Suzuki Y, Takahashi G, Shozushima T, Ishikura H, Murai A, Nishida T, Irie Y, Miura M, Iguchi H, Fukui Y, Tanaka K, Nojima T, Okamura Y (2012) Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. J Infect Chemother 18 (6):891-897. doi:10.1007/s10156-012-0435-2.
29. Shozushima T (2011) Presepsin (sCD14-ST) as a new diagnostic biomarker of sepsis: development of diagnostic tools using the whole blood. Critical Care 15 (3):1-24. doi:10.1186/cc10372.
30. Okamura Y, Yokoi H (2011) Development of a point-of-care assay system for measurement of presepsin (sCD14-ST). Clin Chim Acta 412 (23-24):2157-2161. doi:10.1016/j.cca.2011.07.024.

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