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Neutrophil CD11b and serum procalcitonin as promising markers for early detection of neonatal sepsis

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Abstract

Although many biomarkers were used for diagnosing neonatal sepsis, none of them is conclusive alone. So, measuring multiple biomarkers were proposed to help in rapid diagnosis of neonatal sepsis. This study aimed to assess the potential role of measurement of neutrophil CD11b and serum procalcitonin (PCT) for early diagnosis of neonatal sepsis. This was a case control study, included 96 neonates admitted to Ain Shams University hospital. The neonates were divided into 3 Groups: Group A included proven sepsis Group with positive blood cultures (n=31), Group B, suspected Group with persistent clinical signs of sepsis but with negative blood cultures (n=36) and a control Group of normal newborns of matched age and sex (n=29). There was a statistically significant increase in expression of CD11b on neutrophils in Group A (median= 99.7) when compared with that of Group B (median= 99.4) and the control Group (median= 96.2) (p< 0.001), with increased mean fluorescence intensity in Group A (median= 21.3) when compared with that of Group B (median= 10.1) and control Group (median= 4.1) (p< 0.001). The receiver operating characteristic curve analysis was applied to assess the diagnostic performance of the tested markers. It showed that the serum PCT level can be used to discriminate between Group A and the control Group with 100% sensitivity, 100% specificity and CD 11b showed 100% sensitivity, 69% specificity. Moreover, to discriminate between Group A and Group B serum PCT showed 100% sensitivity, 88.9% specificity and CD 11b showed 87.1% sensitivity and 44.4% specificity. In conclusion, PCT and neutrophil CD11b are promising markers for diagnosis of early neonatal sepsis in preterm neonate.

Keywords: Neutrophil CD11b, Serum Procalcitonin, Neonatal Sepsis.

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Introduction

Neonatal sepsis is one of the critical problems that occurs early in neonatal life, although advances in neonatal care and suitable therapy, it remains one of the significant causes of morbidity and mortality.[1] Currently, there is no universally accepted definition of neonatal sepsis, and definitions vary widely across studies and hospitals.[2]

Diagnosis of neonatal sepsis is challenging as there is no specific clinical presentation, and the definitive method for diagnosis of bacterial sepsis is isolation of pathogen by performing blood culture, but this method is time-consuming and less sensitive than other methods.[3, 4]

This led to the overuse of empirical antimicrobial therapy, and it has been recognized that over exposure of neonates to antibiotics before establishing diagnosis of sepsis lead to poor outcomes and may result in emergence of antimicrobial resistance and severe complications of an imbalanced microbiome in neonates that may result in devastating complications like necrotizing enterocolitis. So, searching for reliable and specific biomarkers that can be helpful for the early diagnosis of neonatal sepsis is urgently needed.[4]

Several papers studied different biomarkers for early diagnosis of neonatal sepsis like acute-phase proteins, chemokines, components of complement system, adhesion molecules, cytokines, and cell surface biomarkers to determine their diagnostic value.[5, 6]

Neutrophil cluster of differentiation 11b (nCD11b) is a cell surface antigen, which is expressed normally on non-activated neutrophils surface but at very low levels. Its level of expression rapidly increases within minutes after neutrophil exposure to bacteria or its endotoxins and this is crucial for neutrophil migration to infection site.[7, 8]

CD11b, as a member of the β-integrin family of adhesion proteins, mediates inflammatory reaction by controlling adhesion and migration of leukocytes. Also, it plays a role in different processes like phagocytosis, chemotaxis, cell-mediated cytotoxicity, and the complement system. Also, it was noted that this marker was a highly useful for diagnosing early-onset neonatal sepsis.[9, 10]

Serum procalcitonin (PCT), a precursor of calcitonin, its circulating levels, was undetectable. However, it rises exclusively in response to bacterial infections, not to viral or other inflammatory diseases.[11] This specificity makes PCT a promising marker for identifying neonatal sepsis.[12]

Despite the introduction of numerous biomarkers for the diagnosis of sepsis, none of them showed definitive results in clinical practice. So, measuring multiple biomarkers was proposed and may assist in rapid diagnosis of neonatal sepsis. Consequently, this work aimed to assess the potential role of measuring neutrophil CD11b and serum procalcitonin for early diagnosis of neonatal sepsis.

Subjects and Methods

The study was performed in the Neonatal Intensive Care Unit (NICU) at Ain Shams University hospital. This case-control study included 96 neonates in their first 28 days of life, who were admitted to the NICU. The neonates were divided into 3 Groups. Group A, (n=31) included confirmed sepsis cases, with clinical symptoms and signs of sepsis and with positive blood cultures. Group B (n=36) included a suspected Group with persistent clinical signs of sepsis, elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) but of negative blood cultures and a control Group (n=29) of apparently healthy newborns of matched sex and age randomly selected from the follow up clinic. Exclusion criteria involved prematurity and systemic steroids administration.

All participants in the study were subjected to full history taking, clinical examination and laboratory work up. This included complete blood count using a hematology counter (Sysmex automated cell counter, USA), measurement of CRP by an immunoturbidimetric assay using an automated analyzer (Beckman Coulter AU Analyzer, Germany) and Blood cultures by an automated BACTEC microbial detection system (Biomerieux, France).

A venous blood sample (6 ml) was collected by venipuncture under complete aseptic condition. Of these, 2 ml of blood were collected in gel vacutainer tube; serum was separated by sample centrifugation at 2683g for 15 minutes and stored at –80°C until procalcitonin measurement by an enzyme-linked immunosorbent assay (ELISA). Another 2 ml of blood were added to blood culture

medium, and the last 2 ml of blood were collected in EDTA-K2 vacutainer tube and processed within 24 hours, as whole blood, by a flowcytometry for neutrophil CD11b expression.

Serum procalcitonin assay

Serum procalcitonin was assessed by using human procalcitonin ELISA kits (catalogue no.: E0977Hu, supplied by Bioassay Technology Laboratories, Shanghai Korain Biotech, China), according to the manufacturer's instructions. In brief, 50 µl of each sample and standard were added to the corresponding wells. Then 10 µl of anti-PCT antibody and 50 µl of the streptavidin-HRP conjugate were added to each well. The plate was then incubated at 37 °C for 60 minutes followed by washing of the wells 5 times using phosphate buffered saline (PBS). Then 50 µl of substrate solution A and 50 µl of substrate solution B were added to each well followed by incubation at 37 °C for 10 minutes in the dark. Then, 50 µl of the stop solution were added and the optical density of each well was determined immediately at 450 nm using a micro plate reader (Stat Fax 2100, Awareness Technology, USA). To plot a standard curve, 5 standards were used and the optical density values of the samples were converted into pg/mL by using the standard curve.

Assay for nCD11b expression by flow cytometry

The process started by staining of neutrophil cells, by adding 10 µl fluorescein isothiocyanate, (FITC) anti-CD11b monoclonal antibody (Beckman Coulter, Inc, USA) to 100 µl of the blood sample, mixed well, and then incubated at 4°C for 20 min in the dark. Red blood cells (RBCs) were lysed by adding 1 ml of the lysis solution to each tube, mixed, and incubated for 15 min at room temperature. The tubes were then centrifuged at 671g for 5 minutes, and the supernatant was discarded. The cells were washed twice with 2 ml PBS, with repeated centrifugation each time, followed by discarding of the supernatant. Data collection was performed using a flow cytometer (Navios flow cytometer, Beckman Coulter, Inc, USA), and analysis was conducted using the Kaluza software (SN: AV16127).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Science (SPSS, variant 27). Data are expressed as median and interquartile range for quantitative non-parametric measures and percentage for qualitative measures. A p value of <0.05 was considered statistically significant.

Results

This study included 96 neonates in the first 28 days of life who were divided into 3 Groups: Group A: included 31 neonates with confirmed sepsis who were diagnosed by clinical signs and symptoms with positive blood cultures. Group B: included 36 neonates with suspected sepsis who were clinically diagnosed with sepsis, but blood cultures showed negative results and a control Group which included 29 apparently healthy newborns with comparable age and sex.

Table 1 illustrates the demographic and clinical data of the three Groups in addition to the maternal history. It showed no statistically significant difference in gender, post-natal age, Apgar score, mode of delivery and maternal history of PROM between the three Groups. There was a statistically significant decrease in birth weight in Group A and Group B when compared to the control Group (p=0.038). Also, the results showed significant decrease of centiles in Group A and Group B when compared the control Group (p=0.007). Analysis of maternal history showed a significant increase in the number of mothers with history of urinary tract infection (UTI) in Group A when compared to Group B and the control Group.

Regarding the different interventions done, a statistically significant increase was observed in the number of neonates who were ventilated (invasive and non-invasive ventilation) in Group A when compared to Group B (p<0.001). And there was a statistically significant increase in number of neonates who received inotropic support in Group A when compared to Group B (p<0.001).

Table 1. Comparison of demographic data, maternal history, and clinical data in the studied Groups.

		Group A	Group B [N (%)]	Control Group	*p* value
Gender	Male	14 (45.2%)	19 (52.8%)	17 (58.6%)	NS^{X2}
	Female	17 (54.8%)	17 (47.2%)	12 (41.4%)	
Type of Delivery	Vaginal	4 (12.9%)	4 (11.1%)	5 (17.2%)	NS^{X2}
	CS	27 (87.1%)	32 (88.9%)	24 (82.8%)	
Maternal History	UTI	9 (29%)	0 (0%)	0 (0%)	$< 0.001^{X2}$
	PROM	6 (19.4%)	5 (13.9%)	0 (0%)	NS^{X2}
Non-invasive Ventilation		13 (41.9%)	6 (16.7%)		$< 0.001^{X2}$
Invasive Ventilation		12 (38.7%)	5 (13.9%)		$< 0.001^{X2}$
Inotropic Support		14 (45.2%)	3 (8.3%)		$< 0.001^{X2}$
			Median (IQR)		
Post-natal Age		33 (31 – 34)	32 (30 – 34)	33 (31.5 – 35)	NS^{KW}
Birth Weight		2 (1.6 – 2.5)	2 (1.65 – 2.4)	2.4 (1.96 – 2.8)	0.038^{KW}
Centile		25 (25 – 50)	25 (10 – 50)	50 (38 – 63)	0.007^{KW}
Apgar score (1^{st} Min)		5 (5 – 6)	5 (4.8 – 6)	6 (5 – 6.5)	NS^{KW}
Apgar score (5 Min)		8 (7 – 9)	8 (7.8 – 9)	8 (7 – 9)	NS^{KW}

CS: Cesarean section, UTI: urinary tract infection, PROM: premature rupture of membrane, X2: Chi-square test, KW: Kruskal-Wallis H Test, $p > 0.05$ is not significant (NS).

Analysis of blood culture results in Group A revealed that *Klebsiella spp* was the most isolated pathogen (n= 12, 38.7%) followed by *Staphylococcus aureus* (n=6, 19.4%). *Coagulase negative staphylococci, pseudomonas spp. and Candida spp.* were equally detected in 9.7% of patients while *Methicillin resistant Staph aureus (MRSA)* and *Escherichia coli* were equally detected in 6.5% of the studied patients.

Different laboratory parameters were assessed in the studied Groups. There was no statistically significant difference in total leukocytic count (TLC), hemoglobin (Hb) level and platelets count between the studied Groups. However, there was a statistically significant increase in procalcitonin (PCT) level in Group A when compared to Group B and the control Group. Also, a significant rise in the CD11b expression level on the surface of neutrophils was found with increased mean fluorescence intensity (MFI) among Group A patients when compared to Group B and the control Group as shown in Table 2.

Table 2. Comparison between studied laboratory data the study Groups.

		Group A (N =31)	Group B (N=36)	Control Group (N=29)	*p* $value^{KW}$
			Median (IQR)		
TLC ($\times 10^3/\mu l$)		9 (7 – 12)	10 (8.4 – 14.9)	13 (6.5 – 14.5)	NS
Hb (g/dl)		13 (11.5 – 16)	13 (11.6 – 17)	15.1(12.7–17.2)	NS
Platelet count ($\times 10^3/\mu l$)		201 (160 – 367)	248 (139 – 359)	314 (200 – 403)	NS
CRP (mg/dl)		24 (24 – 60)	6 (4 – 12)	5 (3 – 6)	<0.001
PCT (pg/ml)		1660 (1170 – 2100)	420 (355 – 713)	268 (211 – 441)	<0.001
CD11b expression	%	99.7 (98.7 – 100)	99.4 (98.2-99.8)	96.2 (96.1 – 98.7)	<0.001
	MFI	21.3 (18.8 – 30.2)	10.1 (9.3 – 17.1)	4.1 (3.4 – 6.7)	<0.001

TLC: total leukocytic count, Hb: hemoglobin, CRP: C-reactive protein, PCT: procalcitonin, MFI: Mean fluorescence intensity KW: Kruskal-Wallis H Test, $p > 0.05$ is not significant (NS).

The receiver operating characteristic (ROC) curve analysis was done to assess the diagnostic performance of PCT, nCD11b expression percentage and MFI in discriminating the different study Groups. Regarding differentiation between Group A and the control Group, the ROC curve analysis showed that serum PCT level can be used to differentiate between the two Groups at a cut-off level more than 717.5 pg/ml with 100% sensitivity, 100% specificity, 100% positive predictive value (PPV) and 100% negative predictive value (NPV) (area under the curve (AUC) of 1, p<0.001). It also showed that nCD11b expression can be used to discriminate between the same two Groups at a cut-off level more than 97.4 % with 100% sensitivity, 69% specificity, 77.5% PPV and 100% NPV (AUC = 0.905 & p< 0.001). While nCD 11b MFI can be used to differentiate between the same two Groups at a cut-off level more than 9.57 (MFI) with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1 & p < 0.001) as shown in Figure 1.

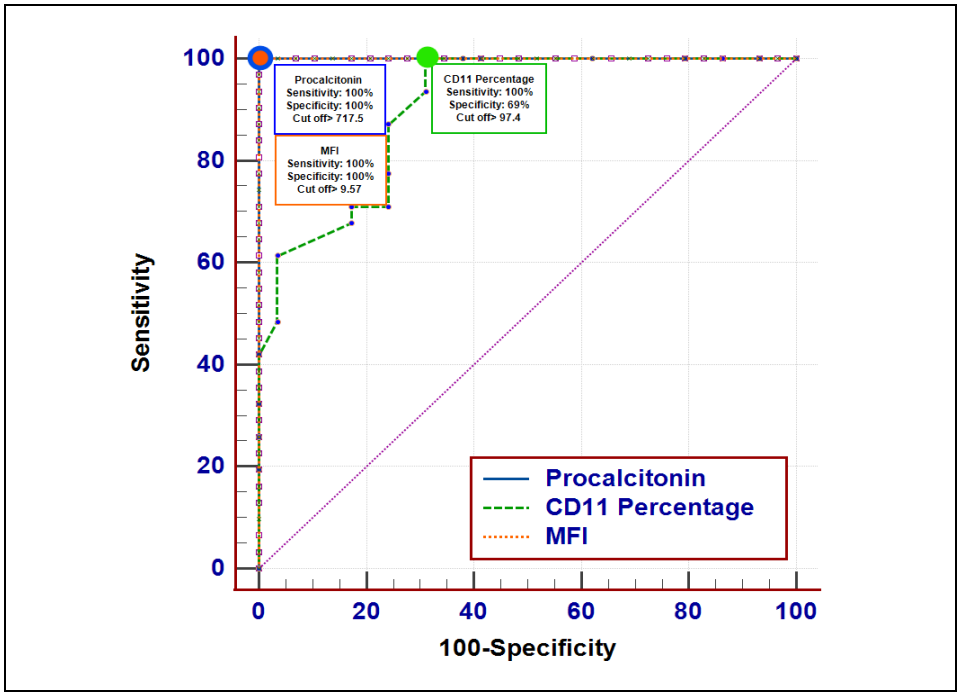

Figure 1. Receiver operating characteristic (ROC) curve analysis showing diagnostic performance of PCT and nCD11b expression in differentiating Group A from the control Group

For discrimination between Group B and the control Group, the ROC curve analysis revealed that serum PCT level can be used to differentiate between the two Groups at a cut-off level more than 268.1 pg/ml with 88.9% sensitivity, 51.7% specificity, 69.6% PPV and 78.9% NPV (AUC = 0.72 & p=0.001). While nCD11b expression can differentiate between the same two Groups at a cut-off level more than 97.4 % with 83.3% sensitivity, 69% specificity, 76.9% PPV and 76.9% NPV (AUC = 0.781& p< 0.001). In addition, it was found that nCD11b MFI can be used to differentiate between the same two Groups at a cut-off level more than 8 (MFI) with 91.7% sensitivity, 93.1% specificity, 94.3% PPV and 90% NPV (AUC = 0.975 & p< 0.001) as shown in Figure 2.

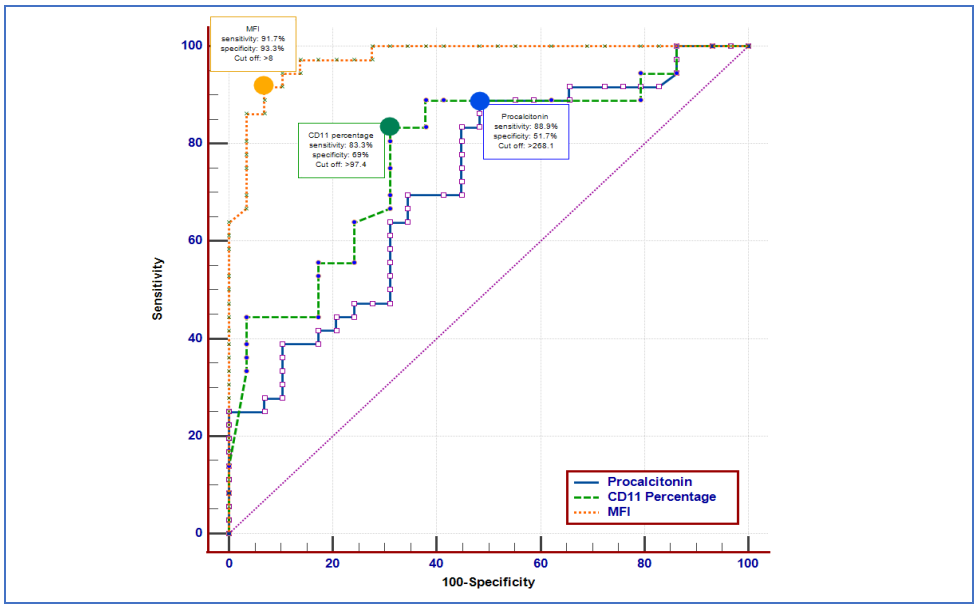

Figure 2. Receiver operating characteristic (ROC) curve analysis showing the diagnostic performance of PCT and nCD11b expression in differentiating Group B from the control Group.

Assessment of the diagnostic performance of PCT, nCD11b expression and MFI in the discrimination between Group A and Group B revealed that serum PCT level can be used to differentiate between the two Groups at a cut-off level more than 877.8 pg/ml with 100% sensitivity, 88.9% specificity, 88.6% PPV and 100% NPV (AUC = 0.947 & p <0.001). Also, nCD11b expression can differentiate between the two Groups at a cut-off level of > 98.54 % with 87.1% sensitivity, 44.4% specificity, 57.4% PPV and 80% NPV (AUC = 0.683, p=0.005). While its MFI can be used to differentiate between the two Groups at a cut-off level of >15.9 (MFI) with 96.8% sensitivity, 72.2% specificity, 75% PPV and 96.3% NPV (AUC = 0.86 & p< 0.001) as shown in Figure 3.

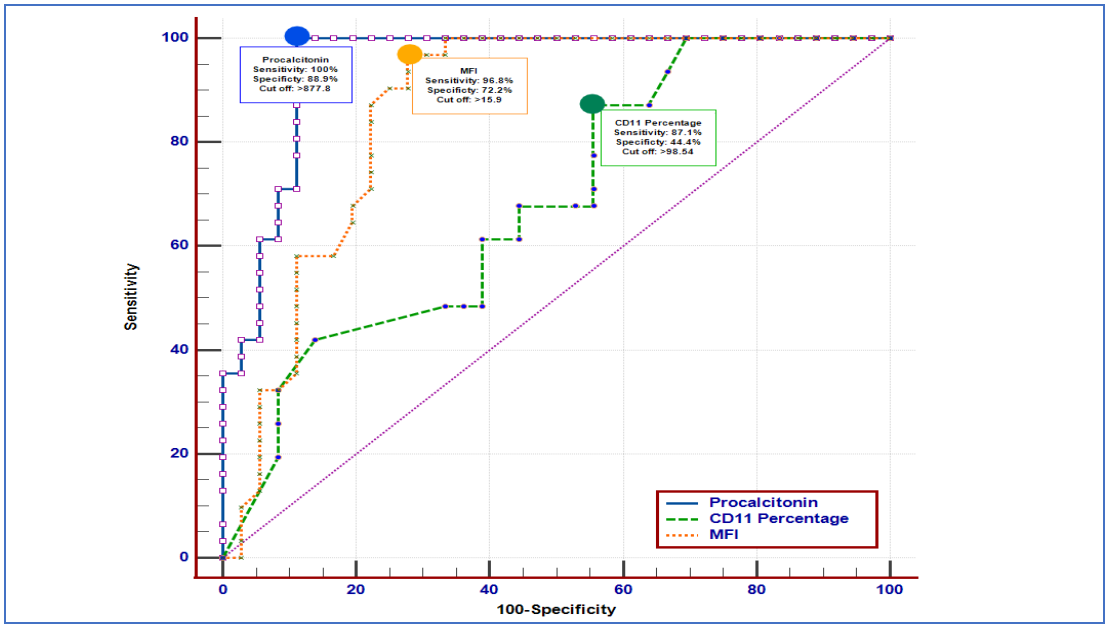

Figure 3. Receiver operating characteristic (ROC) curve analysis showing diagnostic performance of PCT and CD11b expression in differentiating Group A from Group B.

Correlation analysis was done between serum PCT level and the different laboratory parameters among all studied Groups. It showed no significant correlation in Group A. However, in Group B, there were a statistically significant positive correlation between PCT level and TLC (r= 0.498, *p*=0.002) and a statistically significant positive correlation was found between PCT level and CRP (r= 0.929, *p*<0.001).

Discussion

Neonatal sepsis is considered as a significant health problem because of the limited and non-specific clinical symptoms and signs that are frequently seen in other noninfectious diseases.[13] Blood cultures are the conventional gold standard for diagnosis; however, it exhibits a significant rate of false negative results. Several factors have been recognized as contributing to the decreased blood culture sensitivity which include low density and intermittent neonatal bacteremia, inadequate blood sample volume, and using several empirical antibiotics prior to withdrawal of blood culture samples.[14] The high morbidity and mortality rates of neonatal sepsis can be significantly lowered with prompt diagnosis and treatment. Furthermore, a precise diagnosis prevents needless antibiotic use that has been proven to be connected to emergence of drug-resistant strains of organisms and gram-negative bacterial colonization.[15]

This study included 96 neonates in the first 28 days of life who were divided into 3 Groups, the first Group (Group A) included 31 patients with neonatal sepsis who were diagnosed according to clinical signs and symptoms and their blood cultures workup were positive, the second Group (Group B) included 36 patients clinically diagnosed with sepsis, but blood cultures were negative. The last Group was the control Group of 29 apparently health newborns with matching age and sex.

Positive blood culture results isolated from Group A showed growth of Klebsiella spp. in 12 patients (38.7%), *S. aureus* in 6 patients (19.4%), coagulase negative staphylococci in 3 patients (9.7%), pseudomonas spp. in 3 patients (9.7%), Candida spp. in 3 patients (9.7%), MRSA in 2 patients (6.5%) and *E. coli* spp. in 2 patients (6.5%). Hence the difference between ours and other study results like El-Said et al., 2024, where E-coli was the most isolated organism (56%) followed by *S. aureus* and *Klebsiella pneumonia* (17% and 15% respectively).[16] Similarly, an Egyptian study by Yousif et al., 2018 found that the most isolated organism was also *E. coli*, followed by *Klebsiella pneumoniae and Staphylococci.*[17] Another study conducted by Mohamed et al., 2012 reported Gram-positive organisms as the most isolated organisms (69.2%) including *S. aureus* (30.7%), *Staphylococcus coagulase negative* (23.1%), *Streptococcus pneumoniae* (7.7%), and enterococci (7.7%). Whereas Gram-negative organisms were isolated in 30.8% of cases with *Klebsiella* isolated in 23.1% and *E. coli* isolated in 7.7% of cases.[18] In a study conducted by Shehab El-Din et al., 2015 Gram-positive bacteria were also the most encountered among neonatal sepsis patients followed by *Serratia marcescens and Klebsiella pneumoniae*.[19]

However, our results come in consistence with a study conducted by Shobowale et al., 2015 in Lagos university teaching hospital who found that the commonly isolated organisms were *Klebsiella pneumoniae* (36.5%), followed by *Staphylococcus aureus* (18.8%).[20] This was also found in a meta-analysis study that investigated the pathogens causing early onset neonatal sepsis in middle eastern countries. They found that in middle-income countries the commonest pathogens were *Klebsiella species* (26%), *S. aureus* (17%), and *E. coli* (16%) while in high-income countries, the predominant pathogens were Group B Streptococcus (26%), *E. coli* (24%), and *Klebsiella* (9%).[21]

In the present study, all subjects were subjected to testing different laboratory parameters and there was no statistically significant difference in TLC, HB and PLT count among the studied Groups. In opposite to our results, El said et al., 2024 found that there was a significant difference in hemoglobin level between sepsis Group and control Group[16] and, Yousif et al., 2018 reported that there was significant decrease in hemoglobin level and

platelets count in their sepsis Group compared to the controls.[17]

A study done by Misra et al., 2013 found that leukopenia was a better predictor for neonatal sepsis when compared to leukocytosis especially with gram negative infections (sensitivity of 87.5% versus 25%).[22] Otherwise, another study has reported that mature and immature leukocytes increased in severe neonatal infections possibly due to the release of cytokines and other growth factors that stimulate bone marrow.[23]

The study by Shahab El-Din et al., 2015 found that abnormalities in the complete blood cell count, were found in 66.8% of the septic Group of neonates with 6.9% having leucopenia (white blood cell [WBC] < 5,000/mm3), 22.3% leukocytosis having (WBC > 20,000/mm3), 23.2% with neutropenia, and 45.5% showing thrombocytopenia (platelets < 140,000/mm3).[19] A comparison of leukocyte classification and count showed that WBC and neutrophil counts in the bloodstream infection (BSI) Group were significantly lower than the control Group.[24]

The current study illustrated a statistically significant increase in CRP and procalcitonin levels in Group A patients when compared to Group B and the control Groups. Also, the results showed a statistically significant increase in the CD11b expression level on neutrophils in Group A patients when compared to Group B and the control Groups. This comes in concordance with Yousif et al., 2018 who reported presence of significant increase in the average CD11b expression level among sepsis Group (86.04± 11.58 pg/ml) when compared to control Group (15.43±10.9 pg/ml).[17] On the other hand, a study conducted by Elsaid et al., 2024 found that there was no significant different regarding CD11b expression level on both monocytes and neutrophils between sepsis Group and control Group. They also demonstrated that CRP was increased significantly in sepsis Group than in the control Group, where 88.8% had positive CRP in the sepsis Group.[16]

Regarding CRP, it was found to be of less sensitive for diagnosis of bacterial infection, but it showed high sensitivity and specificity when measured after establishing the diagnosis of infection. So, it can be helpful in monitoring the efficacy of antibiotic therapy and evaluating the prognosis of infection.[25]

It was found that the neutrophil CD64 is an excellent marker for diagnosing severe sepsis in the ICU ($p< 0.05$), and neutrophil and monocyte CD11b can also be used.[26] It was also reported that expression of both monocyte and neutrophil CD11b and CD64 were high in the start of infection and declined gradually over time. The expression level of CD11b was highly affected by preanalytical factors and analytical settings so, it may be higher than the reported levels.[27]

Similar results were reported by other studies that found an increase in monocyte CD11b expression among septic infants in comparison to non-septic infants.[28, 29] And Nuutila et al., 2009 reported that levels of neutrophil CD11b in sepsis were controversial, possibly because of pre-analytical and analytical factors.[30]

The expression of CD11b on neutrophils was significantly up regulated in the proven sepsis and suspected sepsis Groups than the control Group, and higher in the sepsis Group compared to suspected sepsis Group.[10] It increased markedly within minutes after encountering bacteria or their endotoxins. This sole property enables the use of CD11b as an early marker for the diagnosis of bacterial infection.[31]

Opposing findings were reported by Adib et al., 2007 who found that no significant difference regarding CD11b expression levels between sepsis, suspected sepsis and the control Groups ($p= 0.069$). However, the mean of nCD11b expression levels were higher in suspected and sepsis Groups compared to the control Group.[7] This inconsistency of results regarding nCD11b expression may be due to differences in the blood collection time in relation to the stage of infection.

The study by Tao et al., 2024 revealed that PCT level in the sepsis Group was significantly higher when compared to the control Group.[24] In the present study, ROC curve analysis revealed that the serum PCT level can be used at a cut of level of > 717.5 pg/ml as a tool to differentiate between neonatal sepsis patients and the control Group with 100% sensitivity,

100% specificity, 100% PPV and 100% NPV. In addition, CD11b percentage can also be used to differentiate between the two Groups at a cut of level of > 97.4 pg/ml with 100% sensitivity, 69% specificity, 77.5% PPV and 100% NPV. Furthermore, MFI showed that at a cut of level of >9.57 pg/ml it had 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1 & p< 0.001).

The study by Fouad et al., 2020, reported that PCT showed high sensitivity and specificity of 98.6% and 88.9%, respectively.[13] These findings align with previous studies indicating that PCT is a highly effective tool in the diagnosis of neonatal sepsis when compared to other common sepsis markers.[32, 33] Similar findings were found in a meta-analysis conducted in 2011 and demonstrated that PCT has a good diagnostic accuracy for neonatal sepsis, at an area under the curve of 0.87, the sensitivity and specificity were 81% and 79% respectively.[34] Lower sensitivity (52%) was reported by Aydin et al., 2017.[35]

Regarding CD64 and CD11b, it was found that they could be used for early diagnosis of sepsis with sensitivity and specificity of more than 97%. Also, both markers could be used for prediction of patients with positive blood culture with sensitivities of more than 97% and specificities of 93% and 98%; respectively.[17]

Aydin and coworkers, 2017 reported sensitivity and specificity for CD11b, of 72% and 68%, respectively which is lower than results of the present study.[35] While Fouad et al. 2020 showed that CD11b had 85% sensitivity and 93.3% specificity[13] and these results also were consistent with those reported in another study, which demonstrated a sensitivity of 86.3% and a specificity of 100%.[36] The difference between results might be attributed to the different inclusion criteria and patient Grouping in addition to variation in the sample size and the ethnic Groups. These factors can significantly influence the performance characteristics of the diagnostic markers across different research settings.

Diagnosis of sepsis is very important and dependence on one marker for diagnosis may be not conclusive. So, different studies tried to find combination of different markers to improve early diagnosis of sepsis. A combination of PCT, CD11b, and CRP was tested by Fouad et al., 2020.[13] Others as Genel et al., 2012 used a combination of CD11b, CD64 and CRP[37] while Yang et al., 2016 used combination of PCT, CD64, WBC, and CRP.[38]

Using combined markers has improved the discrimination power between different studied Groups, and this was reported by different studies. One of the studies demonstrated that the use of combination of CD11b, CD64, and CRP in the diagnosis of neonatal sepsis significantly improved the sensitivity and negative predictive value (1.0 for both) compared to using CD64 alone (0.81 and 0.75, respectively), CRP alone (0.81 and 0.77, respectively) or CD11b alone (0.66 and 0.61, respectively).[37] Additionally, using CD64 and PCT in combination increased the sensitivity to 90.9%, compared to 79.5% for CD64 alone and 68.2% for PCT alone.[38] Fouad et al., 2020 reported that using CRP, CD11b and, PCT biomarkers in combination and guided by clinical data could enhance the diagnostic accuracy of the traditional tests, leading to earlier diagnosis, treatment, and better prognosis.[13]

In a study done by Du et al., 2014 significantly higher nCD11b expression in neonatal sepsis was reported, with a sensitivity and specificity of 81% and 70%, respectively. These results are in line with the results provided by our study as nCD11b sensitivity, and specificity were 100% and 69% respectively.[39]

In a study done by EL Meneza et al., 2021, they concluded that the best cut of point of CD11b was >0.695 ng/ml; and this marker had a sensitivity, specificity, PPV, and NPV of 100% in the sepsis Group while CD11b showed 88% sensitivity and 80% specificity with a PPV of 81.5% and an NPV of 87% in the suspected sepsis Group.[10] Similar results were reported by another study, by Sharma, et al., 2018[6] while another study reported different values.[7] The controversial findings may be referred to the differences in methods of measurements of CD11b and gestational age of studied Groups.[40]

The present study showed a good discrimination performance of PCT to

differentiate between sepsis patients and the control Group. However, the clinical application of PCT has several limitations. Firstly, the predictive value of PCT in neonatal sepsis during the early neonatal period is affected by the physiological differences observed between preterm, late preterm, and term infants. Setting a cut of value for PCT in newborns with different gestational ages may increase its diagnostic accuracy of neonatal sepsis, but a recent research showed diverging opinions regarding PCT cut of values and there was no uniform conclusion.[24] Secondly, the heterogeneity in sample timing, cut of values, patient population, and definition of early onset sepsis or late onset sepsis can influence the outcomes of PCT. Lastly; PCT was not reliable to differentiate bacterial infections from other nonbacterial infection diseases. Therefore, PCT could not be used as a stand-alone test for neonatal sepsis diagnosis.[24]

A recent approach to improve the diagnosis of sepsis is to combine the results of multiple laboratory tests in an algorithm and this was applied in the diagnosis of adult sepsis and recently some studies were done in neonatal sepsis to prove this approach.[13]

In conclusion, serum PCT level and nCD11b expression are promising markers for the early diagnosis of sepsis. The combined usage of these biomarkers hand in hand with the clinical data and microbiology culture results would accelerate the management process. This would decrease mortality and morbidity rates and enhance a cost-effective process saving time, resources and shortening the period of hospital admission for this vulnerable Group of patients. The presented high sensitivity and specificity of these two biomarkers in the diagnosis of sepsis patients may necessitate the need for employing different biomarkers rather than relying to a single marker for accurate sepsis diagnosis.

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Author Contributions

SAA, RA and AMS; designed and approved the whole research protocol and contributed to paper writing. MAK and FMM; contributed to the protocol design, revised laboratory work, and contributed to paper writing. RA and AAM; monitored data collection process and the laboratory work, interpreted the data, contributed to paper writing, and critically revised the paper and approved the final version to be published. AMS; supervised sample collection according to inclusion criteria, revised clinical data, diagnosis, and patient classification and contributed to paper writing. AAM and FMM; supervised the laboratory work and analyzed it, carried out statistical analysis and drafted the paper. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU R101/2024, dated May 2024).

Informed consent

Parents of neonates participated in the study, provided informed consents before being included in the study.

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