Neutrophils CD 11b Expression as Biomarker in Early Diagnosis of Neonatal Sepsis

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ABSTRACT

Background: Neonatal sepsis is a major contributor to both mortality as well as mortality in newborns. It is crucial to diagnose and treat the neonate with suspected sepsis at an early stage in order to prevent life-threatening complications.

Aim: To evaluate the value of neutrophils CD11b for the early diagnosis of neonatal infection and its relation with other laboratory markers such as CBC, hematological scoring system, and CRP and its role as a predictor for outcome in neonatal sepsis.

Patients and methods: This cross-sectional comparative research included 75 newborns at NICUs of the Pediatric Department, Al-Azhar University Hospitals which carried out from October 2023 to August 2024. **Neonates divided into 3 groups: Group 1:** patients with sepsis (n=25), **Group 2:** patients with Suspected sepsis (n=25) and **Group 3:** control group (n=25), **Results:** Neutrophils CD11b, neutrophils, bands, lymphocytes, as well as CRP, exhibited a statistically significant positive correlation, whereas there was a significant negative correlation among neutrophils CD11b, HB, as well as platelets. Furthermore, a statistically significant positive correlation was observed among neutrophils CD11b and birth weight, Neutrophils CD11b had sensitivity of 98% and specificity of 96% with highly significance for diagnosis of neonatal sepsis., **Conclusion:** Based on our finding we conclude that, CD11b may be a reliable, rapid and correct biomarker for the early neonatal sepsis recognition and significantly associated with poor risk and prognostic factors of neonatal sepsis. Furthermore, large, multicenter, prospective studies are warranted to support our findings.

Key words: Neutrophils CD 11b، Diagnosis, Neonatal Sepsis

INTRODUCTION

Neonatal sepsis is a significant contributor to mortality as well as morbidity. Identification and intervention of neonates with suspected sepsis at an early stage are essential to prevent life-threatening complications.[1]

Over forty percent of global fatalities among children under five occur during the neonatal period, leading to 3.1 million newborn deaths annually.[2]

The majority of these fatalities typically transpire in low-income nations.[3]

Neonatal sepsis is a clinical illness that is diagnosed in a newborn who is twenty-eight days old or fewer and is characterized by systemic infection symptoms and the identification of a bacterial pathogen in the bloodstream.[4] Neonatal sepsis is associated with two patterns of disease: early-onset (occurring within seven days of delivery) and late-onset (occurring after seven days).[5]

The diagnosis and management of sepsis present a significant challenge for neonatologists in neonatal intensive care units. Initial warning signs and symptoms are frequently varied and non-specific. Furthermore, there exists the challenge of differentiating the clinical presentation of newborn sepsis from noninfectious etiologies.[6]

CD11b is a neutrophil cell surface antigen that is typically expressed at a low level on non-activated cell surfaces. However, within minutes of coming into contact with bacteria or endotoxins, the expression on the surface of neutrophil cells increases significantly.[7]

This research aimed to evaluate the value of neutrophils CD11b for the early diagnosis of neonatal infection and its relation with other laboratory markers such as CBC, hemological scoring system, and CRP and its role as a predictor for outcome in neonatal sepsis.

PATIENTS AND METHODS

Ethical consideration: the data that were obtained from participants where identified by name in any report or publication concerning this study, the purpose and nature of the study as well as risk – benefit assessment was explained to the parents and informed consent was obtained .

Sample size: The sample size was calculated using the G*Power software (version 3.0.10), F-test MANOVA using within and between interaction effects was selected. Power is 0.80, α level of 0.05 and mean CD11b was 284.31±66.36, 144.52±31.81 and 112.18±13.82 in patients with Proven sepsis, Clinical Sepsis and control groups respectively. a generated sample size of at least 66 subjects. Adding 9 subjects (as drop out), so total sample size is 75 subjects, 25 subjects in each group.[8]

Inclusion criteria: Patients aged from 1 day to 28 days, term and preterm infants and neonates diagnosed with sepsis according to Tollner clinical sepsis scores were suggestive of sepsis. [9]

Exclusion criteria: Neonates with congenital anomalies and congenital infection and neonates with other diseases such as hypoxic-ischemic encephalopathy, birth injury, metabolic disorder, or surgical problems.

Study procedure:

All patients were subjected to the following:

Full history taking: personal history, Obstetric history includes prior sibling mortality and past admission to the NICU. Prenatal history involves diabetes mellitus, maternal fever above 38 degrees Celsius, maternal urinary tract infection (UTI), and maternal antibiotic treatment; natal history includes premature rupture of membranes (PROM) lasting over 18 hours, and protracted second stage of labor. The patient's postnatal history includes a low Apgar score at one to five minutes, intensive resuscitation, respiratory distress, cyanosis, as well as fever, in addition to a present history that is comprised of the most common symptoms of sepsis.

Full clinical examination: Gestational age assessment using new Ballard score[10], birth weight measurement, **Vital signs:** respiratory rate, heart rate, temperature and blood pressure, neonatal reflexes (moro, grasping, sucking), Apgar score at 1.5 min, and detection of clinical signs of sepsis.

Laboratory investigations: C.B.C. with DLC, C-reactive protein (C.R.P.), Blood culture and flow cytometric determination of neutrophil CD11b (Human Integrin alpha M/CD11b PE-conjugated antibody).

Statistical analysis

Data analysis packages was SPSS version 21. Qualitative data presented by number and percentage; quantitative data presented by mean, standard deviation. Tests of significant will be done (chi square for qualitative, ANOVA test for quantitative analysis) and level of significance was set at p equal to or below 0.05.

Results

According to maternal characteristics, there was no statistically significant difference between the studied groups as regard maternal age, gestational age and mode of delivery p>0.05.(Table 1)

Table (1): Distribution of maternal characteristics between the studied groups.

Variables	Sepsis group N=25	Suspected group N=25	Control group N=25	p-value
Maternal age (years) Mean± SD	34.24±7.7	31.84±5.6	31.24±3.53	0.17
Gestational age (weeks) Mean± SD	31.7±3.02	32.3±2.9	32.5±2.25	0.573
Pre term	17 (68%)	13 (52%)	9 (36%)	0.043
Term	8 (32%)	12 (48%)	18 (64%)	

Mode of delivery				
NVD	6 (24%)	5 (20%)	7 (28%)	0.8
CS	19 (76%)	20 (80%)	18 (72%)	

SD: Standard Deviation, P-value > 0.05: Non-significant; P-value ≤ 0.05: Significant; P-value ≤ 0.001: Highly significant

According to this table, there was no statistically significant difference between the studied groups as regard maternal age, gestational age and mode of delivery. While there was statistically significant difference between the studied groups as regard pre term and term labor.

Table (2): Distribution of neonatal characteristics between the studied groups.

Variables	**Sepsis group N=25**	**Suspected group N=25**	**Control group N=25**	**p-value**
Neonatal age (days) Mean± SD	14.4±8.5	14.28±9.01	14.24±7.68	0.99
Neonatal sex				
Male	10 (40%)	9 (36%)	10 (40%)	0.95
Female	15 (60%)	16 (64%)	15 (60%)	
Onset				
Early	16 (64%)	-	-	-
Late	9 (36%)	-	-	-
Birth weight (kg) Mean± SD	2.91±0.32	3.08±0.51	4.01±0.33	<0.001 P1<0.001 P2<0.001 P3<0.001
APGAR 1 min Mean± SD	5.64±1.61	5.6±1.63	7.28±0.79	<0.001 P1= 0.99 P2<0.001 P3<0.001
APGAR 5 min Mean± SD	7.6±1.2	7.84±1.14	8.24±0.78	0.1
Premature rupture of membranes (PROM)				
Yes	19 (76%)	8 (32%)	0 (0%)	<0.001
No	6 (24%)	17 (68%)	25 (100%)	P1= 0.001 P2<0.001 P3=0.002
Maternal fever				
Yes	10 (40%)	4 (16%)	0 (0%)	0.001

No	15 (60%)	21 (84%)	25 (100%)	P1= 0.058 P2<0.001 P3= 0.037
UTI				
Yes	6 (24%)	3 (12%)	0 (0%)	0.033
No	19 (76%)	22 (88%)	25 (100%)	P1= 0.269 P2=0.009 P3= 0.074

SD: Standard Deviation, p1: Group 1 vs Group 2, p2: Group 1 vs Group 3, p3: Group 2 vs Group 3, P-value > 0.05: Non-significant; P-value ≤ 0.05: Significant; P-value ≤ 0.001: Highly significant

There was no statistically significant difference between the studied groups as regard neonatal age, neonatal sex and AGAR 5 min, while there was highly statistically significant difference between the studied groups as regard birth weight, AGAR 1 min, PROM, Maternal fever and UTI. in Sepsis group 16 patients (64%) were early onset and 9 patients (36%) were late onset. (Table 3)

Table (3): Distribution of laboratory investigations between the studied groups.

Variables	Sepsis group N=25	Suspected group N=25	Control group N=25	p-value
HB (g/dl) Mean± SD	11.8±1.6	12.4±1.7	13.6±1.4	<0.001 P1= 0.29 P2<0.001 P3= 0.04
Platelets (x10^3 /ul) Mean± SD	58.9±15.7	47.8±11.2	296.5±67.0	<0.001 P1= 0.59 P2<0.001 P3<0.001
WBCs (x10^3 /ul) Mean± SD	19.4±5.0	18.7±4.3	10.1±2.4	<0.001 P1= 0.82 P2<0.001 P3<0.001
Neutrophil (%) Mean± SD	63.5±4.1	62.1±7.0	40.9±5.1	<0.001 P1= 0.67 P2<0.001 P3<0.001
Band (%) Mean± SD	17.7±1.4	16.1±1.9	4.1±2.2	<0.001 P1= 0.01

				P2<0.001 P3<0.001
Lymphocyte (%) Mean± SD	18.5±4.1	18.0±6.9	52.7±9.7	<0.001 P1= 0.97 P2<0.001 P3<0.001
CRP (mg / l) Mean± SD	76.9±47.2	22.5±11.3	4.4±1.9	<0.001 P1<0.001 P2<0.001 P3=0.04
Neutrophils CD11b)FU) Mean± SD	288.1±53.7	148.8±27.0	11.3±5.2	<0.001 P1<0.001 P2<0.001 P3=0.001
Blood culture				
Positive	25 (100%)	0 (0%)	----	<0.001
Negative	0 (0%)	25(100%)	----	

SD: Standard Deviation, p1: Group 1 vs Group 2, p2: Group 1 vs Group 3, p3: Group 2 vs Group 3, P-value > 0.05: Non-significant; P-value ≤ 0.05: Significant; P-value ≤ 0.001: Highly significant

According to this table, there was highly statistically significant difference between the studied groups as regard HB, platelets, WBCs, neutrophil, band, lymphocyte, CRP, neutrophils CD11b and blood culture. (Table 4)

Table (4): Correlation between Neutrophils CD11b and laboratory data.

Variables	**Neutrophils CD11b**	
	r	**P**
HB (g/dl)	-0.324	**0.02**
Platelets (x10^3 /ul)	-0.493	**<0.001**
WBCs (x10^3 /ul)	0.226	0.12
Neutrophil (%)	0.490	**<0.001**
Band (%)	0.285	**0.045**
Lymphocyte (%)	0.493	**<0.001**
CRP	0.425	**0.002**

r: Pearson correlation, P-values < 0.05 statistically significant.

According to this table, there was statistically significant positive correlation between Neutrophils CD11b, neutrophil, Band, lymphocyte and CRP, while there was statistically significant negative correlation between Neutrophils CD11b, HB and platelets. (Table 5)

Table (5): Correlation between Neutrophils CD11b and neonatal outcomes.

Variables	Neutrophils CD11b	
	r	**P**
Birth weight	.283	**0.047**
APGAR 1min	-.241	0.09
APGAR 5min	-.266	0.06

r: Pearson correlation, P-values < 0.05 statistically significant.

There was statistically significant positive correlation between Neutrophils CD11b and birth weight, while there was no statistically significant correlation between Neutrophils CD11b and APGAR at 1min and 5min. (Table 6)

Table (6): ROC analysis for Neutrophils CD11b for diagnosis of neonatal sepsis.

Variables	Area	Cut off point	Sensitivity	Specificity	PPV	NPV	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
									Lower Bound	Upper Bound
Neutrophils CD11b	0.989	17	98%	96%	82.7%	97.9%	0.01	0	0.969	1

This table shows that Neutrophils CD11b had sensitivity of 98% and specificity of 96% with highly significance for diagnosis of neonatal sepsis.

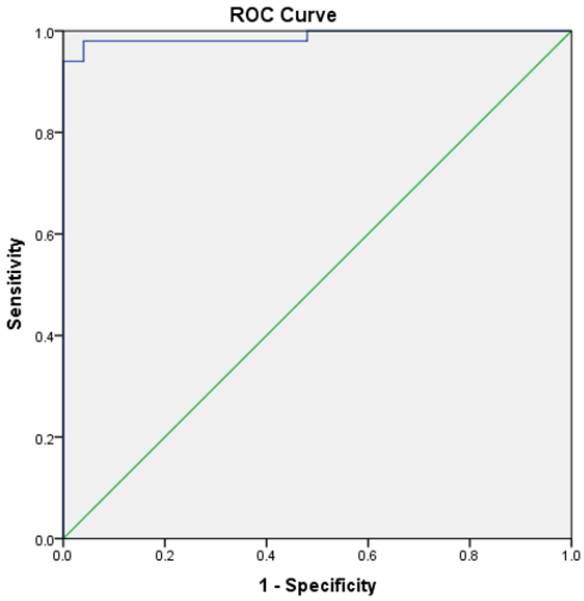

Figure (1): ROC curve for Neutrophils CD11b for diagnosis of neonatal sepsis

DISCUSSION

There are two primary kinds of sepsis in newborns: early-onset and late-onset. Both types can induce respiratory distress, although the first usually appears within the first 72 hours of life while the second usually causes septicemia after 72 hours.[11]

The main results of this study were as follows:

According to maternal characteristics, we revealed that there was no statistically significant difference between the studied groups as regard maternal age, gestational age and mode of delivery p>0.05.

Our findings in agreement with **ELMeneza et al.,**[12] who aimed to evaluate the diagnostic value of neutrophil CD11b in the early diagnosis of sepsis in full-term newborn infants. The patients were classified into three groups: sepsis group (n=25); suspected sepsis group (n=25), and control group (n=25). They reported that no significant intergroup differences in gestational age (GA), sex, or postnatal age among the study groups.

As well, our results in concordance with **MOHAMED et al.,**[13] who aimed to evaluate the usefulness of estimation of neutrophil expression of CD64 and CD11b in bacterial neonatal sepsis. They reported that there was no statistically significant difference between groups as regard gestational age.

According to neonatal characteristics, we reported that there was no statistically significant difference between the studied groups as regard neonatal age, neonatal sex, AGAR 5 min and PROM p>0.05, while there was highly statistically significant difference between the studied groups as regard birth weight and AGAR 1 min p<0.001.

According to laboratory investigations, we reported that there was highly statistically significant difference between the studied groups as regard HB, platelets, WBCs, neutrophil, band, lymphocyte, CRP,

neutrophils CD11b and blood culture p<0.001.

As well, our results in concordance with **Dabour et al., [14]** who aimed to assess the value of neutrophils CD11b for the early diagnosis of neonatal infection. A total of 90 newborns [30 neonates with proven sepsis clinically and positive blood culture (patient group), 30 clinical symptoms of sepsis and negative blood culture (suspected group) and 30 healthy newborns (control group)] were enrolled in the study. They found that there was highly statistically significant difference between the studied groups as regard HB, and neutrophils CD11b.

In addition, our results in concordance with **Sheneef et al., [15]** who aimed to evaluate the value of peripheral blood neutrophils CD11b and CD64 expression levels as early diagnostic markers of neonatal sepsis. They reported that the laboratory investigations showed that, CRP level was significantly higher in cases (36.1± 31.7) than in controls (5.3±12.0). There was a significant increase in the neutrophils CD11b expression levels in cases (57.1±2.5) than their levels in the controls (11.8±7.2) (P <0.001)

In our study we revealed that there was statistically significant positive correlation between neutrophils CD11b, neutrophil, band, lymphocyte and CRP, while there was statistically significant negative correlation between neutrophils CD11b, HB and platelets p<0.05.

Our results in concordance with **Weirich et al.,[16]** who stated that CD11b levels correlated with peak CRP ($r^2 = 0.76$, $p < 0.0001$)

In our study we revealed that there was statistically significant positive correlation between neutrophils CD11b and birth weight p<0.05, while there was no statistically significant correlation between Neutrophils CD11b and APGAR at 1min and 5min p>0.05.

In contrast, our findings disagreed with **Weirich et al., [16]** who stated that no relationships were found between Apgar scores, birth weights, or gestational ages and CD11b.

Unlike the present study also, **Nupponen et al., [17]** who reported that there were no associations between birth weight or gestational age and the levels of CD11b expression.

In our study we reported that neutrophils CD11b had sensitivity of 98% and specificity of 96% with highly significance for diagnosis of neonatal sepsis.

Our results in concordance with **Sheneef et al., [15]** who revealed that neutrophils CD11b had sensitivity of 95% and specificity of 95% with highly significance for diagnosis of neonatal sepsis.

Conclusion

Based on our finding we conclude that, CD11b may be a reliable, rapid and correct biomarker for the early neonatal sepsis recognition and significantly associated with poor risk and prognostic factors of neonatal sepsis. Furthermore, large, multicenter, prospective studies are warranted to support our findings.

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