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Diagnostic Value of Neutrophil CD64, CD11b and Hematological Scoring System in Neonatal Sepsis

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Abstract

Introduction: Suspected sepsis is one of the most frequently challenging diagnoses in neonates, the clinical signs of infection are not specific and late. Blood culture is the golden standard but time consuming in neonatal sepsis diagnosis provides late information.

Aim of work: Evaluation of the Rodwell scoring system, Cluster of Differentiation 64 (CD 64), and Cluster of Differentiation 11b (CD11b) expression as markers in cases with culture proven sepsis.

Patients and methods: 107 patients with culture proven sepsis were enrolled in the cases group and 118 patients were enrolled in the control group. Both groups were examined for monocytic and neutrophilic CD11b and CD64 expressions analyzed by flowcytometry. Complete blood count (CBC) was analyzed and Rodwell scores (hematological scoring system) were calculated for both groups.

Results: CD64 on both monocytes and neutrophils was significantly higher among patients with culture proven sepsis compared to those in the control group with sensitivity of 94.9% and 76.2% (P< 0.001) and specificity of 59% and 83% (P< 0.001), respectively. Rodwell score was significantly higher in cases group compared to the control group with sensitivity of 87.85% and specificity of 94% (P<0.001).

Conclusion: Rodwell scoring system is a simple screening test for early diagnosis of neonatal sepsis. It may help clinicians in identifying sepsis and to institute proper anti-biotic therapy.

Key words: CD64, CD11b, hematological scoring system, Rodwell score, neonatal sepsis

Introduction:

Newborn sepsis mortality rate varies enormously depending on the hospital and across countries. Worldwide it is estimated that 1.3 million cases of neonatal sepsis happen annually with consequence of 203,000 deaths per year births. Changing this reality is a difficult challenge, but one must be undertaken. Preventive measures, early diagnosis, and establishment of efficient management are the only ways to avoid septic shock, multiple organ failure, and consequently death. Early diagnosis of neonatal sepsis provides physicians with time to determine the cause/causative organism which helps to provide more efficient treatment in a more adequate time preventing the occurrence of further complications **(Mahmoud et al., 2023).**

In Egypt, neonatal sepsis is considered a big problem due to inadequate infection control measures and nursing staff **(Salim et al., 2019).** A study done at neonatal intensive care units (NICU) of Cairo University showed that the overall incidence of suspected sepsis was 32.9% **(Mohsen et al., 2017),** In Banha university the mortality rate in outborn neonatal sepsis was (42%) (**Salim et al., 2019).**

Timely diagnosis of sepsis in neonates is critical as the illness can be rapidly progressive and fatal, referring to immaturity of the immune system in neonates, and also the use of empirical broad-spectrum antibiotics has been found to result in detrimental effects on neonatal intestinal microbial diversity and richness, leading to intestinal dysbiosis **(Majumdar et al., 2013) (Stylianou-Riga et al, 2024),** Then inability to adequately exclude the diagnosis of neonatal sepsis can result in unnecessary and prolonged exposure to antibiotics. Thus laboratory tests that can assist the clinician in the diagnosis of neonatal sepsis are of utmost importance **(Khair et al, 2010).** Blood culture is considered the gold standard for diagnosing bacteremia, but it is time consuming**.** Also, false negative results are common due to the low-density, transient and intermittent bacteremia **(Ince, 2014).**

Rodwell score is a hematological scoring system used in early diagnosis of neonatal sepsis. It has the advantage of being easy to use and is applicable to all infants, including those who have received antibiotics. The higher the score, the greater the certainty that sepsis is present thus simplifying the interpretation of hematological profiles **(Ghosh et al, 2001).** Also, cell surface antigens that have been studied in recent years as potentially promising infection markers include CD11b, CD64 and CD69 **(Turunen et al., 2005).**

The migration and recruitment of leukocytes is essential for the normal immune response to injury and infection and for various inflammatory and autoimmune disorders **(Maiguel et al., 2011).** CD11b is an α subunit of the β2 integrin adhesion molecule, and is expressed on neutrophils, monocytes, natural killer cells, and a subset of lymphocytes **(Kawai et al., 2005).** Its density can increase markedly within a few minutes of exposure to microbial products and thus may potentially be used as an 'early' warning marker. study by **Turunen et al., 2005** suggested that daily measurement of neutrophil or monocyte CD11b/CD18 can identify cases before the arousal of clinical suspicion.

Human CD64 is a trans-membrane glycoprotein (72-kDa), which is expressed on monocytes and macrophages and can also be induced on neutrophils with IFN-γ and G-CSF. Cytokine stimulation induces rapid clustering of CD64 on the cell membrane to facilitate rapid binding and transmission of the immune complexes. This leads to an inflammatory response by triggering release of TNF-α and IL-6, superoxide production, antigen presentation to T cells or lysis of antibody coated cells **(Akinrinmade et al., 2017).** The substantial increase in CD64 expression in response to bacterial infection in preterm and term infants is similar in magnitude compared to older children and adults, suggesting it might be useful in neonatal and adult

infections as a marker for infection. CD64 had the highest sensitivity (95–97%) and specificity (88–90%) at the onset and up to 24 h after the initial clinical presentation for diagnosing late-onset bacterial infection and necrotizing enterocolitis (NEC).

Herein, this study aims at the evaluation of Rodwell score (hematological scoring system HSS) and the plasma membrane expression of circulating neutrophils and monocytes for CD64 and CD11b as diagnostic markers for patients with neonatal sepsis.

Patients and methods

Trial design and participants

A prospective randomized controlled trial was conducted from July 2017 till July 2018 at the neonatal intensive care unit (NICU) at Cairo University hospitals and Almaza district hospital. 107 neonates with culture proven sepsis were enrolled in the cases group and 118 neonates with negative cultures were enrolled in the control group.

The current study was approved by the Institutional Review Board (IRB) of the cairo university hospitals, and the guardian consent was obtained before enrollment in the current study. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice norms, and local and national regulatory requirements.

Eligibility criteria

All neonates admitted to neonatal intensive care unit (NICU) at Cairo University hospitals and Almaza district hospital with culture proven sepsis were enrolled in this study. Neonates who are severely jaundiced and those with multiple congenital anomalies were excluded.

Outcome evaluation

Laboratory investigations of complete blood count with differential white blood cell count were conducted at the time of sepsis evaluation for cases group and also for the control group. Samples were collected on EDTA anticoagulated vaccutainer tubes. In addition, Rodwell score was applied using the CBC parameters (hemoglobin, total leucocyte count, platelets count, total polymorph-nuclear leukocytes, immature & mature polymorphonuclear cell count and degenerative changes in neutrophils). C- reactive protein and blood cultures were collected.

Rodwell scoring

criteria	abnormality	score
Total WBC count	≤5000/µl	1
	≥25000/µl at birth	1
	≥30000 at 12-24 hours	1
	≥21000 from day 2 onwards	1
Total PMN count	No mature PMN seen	2
	Increased/ decreased	1
Immature PMN count	increased	1
I:T PMN ratio	increased	1
I:M PMN ratio	≥0.3	1
Degenerative changes in PMN	Toxic granules/ cytoplasmic vacuoles/ dohle bodies	1
Platelets count	150000/µl	1

The normal values are; Total PMN count: 1800- 5400, Immature PMN count < 600, Immature: total PMN ratio: 0.12, Immature: mature ratio- > or= 0.3

Interpretation If ≤2 so sepsis is unlikely, from 3 to 4 sepsis is possible, ≥5 so sepsis is very likely. (Rodwell et al, 1988), (Supreeth et al., 2015).

CD11b & CD64 assay technique

Flow cytometry was done for assessment of cell surface expression of CD11b & CD64 on neutrophils and monocytes using Flowcytometer (Beckman Coulter Epics XL, France), Automatic pipette, Centrifuge, Cooling ultracentrifuge, Vortex stirrer with the following reagents Fluorescein-isothiocyanate (FITC) antihuman CD11b mouse monoclonal (IgG2b subclass) antibodies purchased from Miltenyi Biotec (USA) (Cat No: 130- 098-085) and Phycoerythrin (PE) and antihuman CD64 mouse monoclonal (IgG1k subclass) antibodies from Miltenyi Biotec (USA) (Cat No: 130- 100-415).

Sample preparation for flowcytometric assay

Sample preparation was done by two ml of venous blood was withdrawn on EDTA vaccutainer tubes. Samples were processed within 12 hours of collection. After warming up the argon laser for 30 minutes, the full alignment procedures were performed using standard immunocheck alignment fluorospheres for adjusting forward scatter, side scatter, photomultiplier tubes (PMT) 2,3,4, for green, orange and red adjustments respectively. Monoclonal antibodies tagged with fluorescene isothiocyanate (FITC) were analyzed on PMT2, while those tagged with phycoerythrin were analyzed on PMT3. The auto control tube was then introduced, the laser scatter was received on both forward and side scatter detectors (PMTl) showing the cell population on a basic histogram and adjustment of the auto-fluorescence on the corresponding PMT. From each EDTA anticoagulated blood tube, 100 ul of blood were used, subjected to three washes using 2 ml PBS. Supernatant from the last wash was discarded and then 1ml of lysis solution was added and incubated at room temperature for 10 minutes then washed. Ten ul of each of CD11b & CD64 antibodies were added followed by a 20 minute incubation in the dark at room temperature. Finally 0.5 ml PBS was added to each tube. Data analysis was performed by using light scatter gating to define the neutrophil and monocyte populations. CD64 and CD11b values were quantified as mean fluorescence intensity using Beckman Coulter Epics XL software.

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 25. Qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges. The comparison between two groups with qualitative data were done by using Chi-square test and/or Fisher exact test was used instead of Chi-square test when the expected count in any cell was found less than 5 **(Chan, 2003a) & (Chan, 2003b).**

Results

In the current study, 107 subjects were enrolled in the cases group and 118 subjects were enrolled in the control group. Table 1 shows the comparison of demographic data of the patients . females accounted for 45.8% in the patient group and 49.2% of subjects in the control group, while males accounted for 54.2% of subjects in the patient group and 50.8% in control group.

66% of cases group were preterm below 37 weeks and 33% were more than or equal 37 weeks, while the control group included 27% below 37 weeks and 72% were more than or equal 37 weeks. It was also shown that the demographic data of patients classified according to onset of sepsis were 47 patients was classified as EOS with 29 males and 18 females, 60 patients was classified as LOS with 29 males and 31 females, with no significant difference between the 2 groups.

Table 1. Comparison between cases group and control group regarding demographic data

Variable		Cases group (A) No. = 107	Control group (B) No. = 118	Test value	P-value
Age (days)	Median(IQR) Range	10.00 (5 - 21) 1.00 – 50.00	5.00 (3 - 8) 1.00 – 53.00	-5.807**	0.000
Weight	Mean±SD Range	1.99 ± 0.77 0.86 – 4.5	2.54 ± 0.72 0.6 – 4.42	-5.580**	0.000

***P-value <0.05 Significant (S); **P-value< 0.01**

Table 2. Risk factors for sepsis in cases and control groups

Risk factors		Cases group No. = 107	Control group No. = 118	Test value	P-value
IDM	No Yes	88 (82.2%) 19 (17.8%)	112 (94.9%) 6 (5.1%)	9.124**	0.003
PROM	No Yes	55 (51.4%) 52 (48.6%)	114 (96.6%) 4 (3.4%)	61.349**	0.000
Pre-eclampsia	No Yes	85 (79.4%) 22 (20.6%)	100 (84.7%) 18 (15.3%)	1.081	0.298
TORCH	No Yes	104 (97.2%) 3 (2.8%)	117 (99.2%) 1 (0.8%)	1.230	0.267
UTI	No Yes	85 (79.4%) 22 (20.6%)	118 (100.0%) 0 (0.0%)	26.891*	0.000
Mode of delivery	NVD CS	24 (22.4%) 83 (77.6%)	51 (43.2%) 67 (56.8%)	10.915*	0.001
APGAR 1 min	Mean±SD Range	6.00 ± 1.05 3 – 8	6.97 ± 0.75 5 – 8	-7.827**	0.000
APGAR 5 minute	Mean±SD Range	8.12 ± 0.77 6 – 9	8.63 ± 0.52 7 – 9	-5.589**	0.000
Gestational age (weeks)	Mean±SD Range	33.65 ± 3.12 28 – 41	36.20 ± 2.87 27 – 40	-6.384**	0.000
Others	Antipartum hemorrhage HCV or HBV SLE Pulmonary HTN Rheumatic HTN Oligohydraminous	5 (4.7%) 1 (0.9%) 0 (0.0%) 1 (0.9%) 0 (0.0%) 1 (0.9%)	0 (0.0%) 1 (0.8%) 2 (1.7%) 0 (0.0%) 1 (0.8%) 0 (0.0%)	12.559	0.128

	Meconium	1 (0.9%)	0 (0.0%)
	Cholengitis	0 (0.0%)	1 (0.8%)

***P-value <0.05 Significant (S); **P-value< 0.01**

Table 2 shows highly significant increase in risk factors for sepsis between cases group and control group especially IDM, PROM, UTI, APGAR score, C.S and prematurity.

Table 3. Laboratory data for studied subjects

Laboratory tests		Cases group N= 107	Control group N=118	Test value	P-value
CRP (mg/l)	Negative Positive	12 (11.2%) 95 (88.8%)	118 (100.0%) 0 (0.0%)	181.326**	0.000
Hb (g/dl)	Mean±SD Range	12.02 ± 3.04 4.5 – 21.7	13.37 ± 2.71 8.2 – 18.3	-3.541**	0.000
TLC ($x10^3$/cmm)	Median(IQR) Range	11.5 (7.7 – 14.9) 1.8 – 46.2	10.7 (83 – 14.4) 5.1 – 34	-0.407	0.684
Plts ($x10^3$/cmm)	Median(IQR) Range	129 (75 – 233) 12 – 1316	279.5 (209 – 360) 104 – 732	-7.479**	0.000
tPMN ($x10^3$/cmm)	Median(IQR) Range	5.7 (3 – 8.8) 0.2 – 31.2	4.3 (2.8 – 6) 1.4 – 412	-2.296**	0.022
Imm cont (/cmm)	Median(IQR) Range	990 (658 – 1560) 20 – 7520	268 (190 – 406) 75 – 2745	-9.858**	0.000
I/T	Mean±SD Range	0.19 ± 0.08 0.02 – 0.5	0.07 ± 0.04 0.02 – 0.34	14.581**	0.000
I/M	Mean±SD Range	0.25 ± 0.15 0.03 – 1	0.08 ± 0.05 0.02 – 0.5	11.825**	0.000
Degenerative changes	No Cytoplasmic vacules Toxic granules	55 (51.4%) 25 (23.4%) 27 (25.2%)	116 (98.3%) 2 (1.7%) 0 (0.0%)	67.978**	0.000
Rodwell score	Median (IQR) Range	4 (3-5) 1 - 7	0 (0 – 1) 0 - 4	-12.237**	0.000
Monocytes CD64	Median(IQR) Range	8.44 (5.79 – 10.9) 2.26 – 28.2	5.58 (3.19 – 8.01) 1.83 – 18.1	-4.365**	0.000
Monocytes CD11b	Median(IQR) Range	2.55 (1.61 – 3.86) 1.01 – 17.2	2.05 (1.45 – 3.58) 0.96 – 56.3	-1.277	0.202
Neutrophils CD64	Median(IQR) Range	5.02 (3.68 – 6.5) 1.8 – 10.7	2.49 (2.05 – 3.09) 1.33 – 7.98	-7.354**	0.000

Neutrophils CD11b	Median(IQR)	3.61 (2.18 – 5.8)	3.43 (1.6 – 6.42)	-0.800	0.424
	Range	1.33 – 18	1.14 – 88.4		

***P-value <0.05 Significant (S); **P-value< 0.01**

107 cases and 118 control patients were enrolled in the current study , the CRP ranged from negative (<6mg/l) to 192 mg/l in the patient group with a median of 24 mg/l. CRP was positive in most cases of culture proven sepsis, while all subjects enrolled in the control group were CRP negative.

Regarding the blood picture parameters, Hemoglobin level was significantly lower in the sepsis group compared to the control group (P=0.000). Platelet count was significantly decreased in the patient group in comparison to the control group (P=0.000). Whereas the ANC, immature neutrophil count, I/T ratio and I/M ratio were all significantlyhigher in the patient group compared to the control group (P= 0.000).

Hence, the Rodwell score was significantly higher in patients in comparison to the controls (P=0.000).

Regarding the CD64 expression patterns, CD64 expression was significantly increased on both monocytes and neutrophils among patients compared to controls (P= 0.000).

There was a significant increase in CD64 on neutrophils in EOS compared to LOS (P=0.010).

No correlation was found between Rodwell score and CD64 & CD11b expression levels on neutrophils or monocytes, while there was a significant positive correlation between Rodwell score and CRP levels (P= 0.000), where Rodwell score was highly significantly increased with the increase in CRP levels (P=0.000) , while Rodwell score was insignificantly changed with the prognosis (P=0.328) .

No relation was found between the CRP levels and CD64 & CD11b expression levels on the surface of monocytes nor with the CD64 expression on the neutrophils , however CD11b expression levels on neutrophils were found to be significantly increased with the increase in CRP levels (P=0.034).

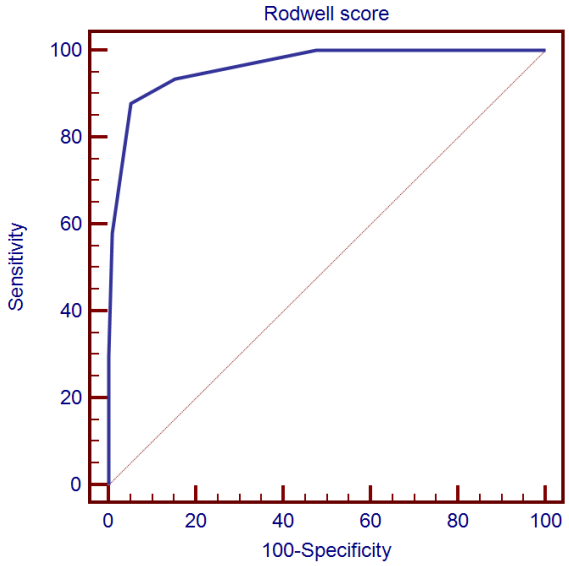
Table 4. Correlation between Rodwell score and sex, CRP, admission, and prognosis

Variable		Rodwell score		Test value	P-value
		Median (IQR)	Range		
Sex	Female	4 (3 – 4)	1 – 7	-1.055	0.291
	Male	4 (3 – 5)	1 – 7		
CRP	Negative	1.5 (1 – 3)	1 – 4	-4.298**	0.000
	Positive	4 (3 – 5)	1 – 7		
NICU admission	No	–	–	-	-
	Yes	4 (3 – 5)	1 – 7		
Prognosis	Died	4 (3 – 5)	3 – 7	-0.977	0.328
	Improved	4 (3 – 5)	1 – 7		

***P-value <0.05 Significant (S); **P-value< 0.01**

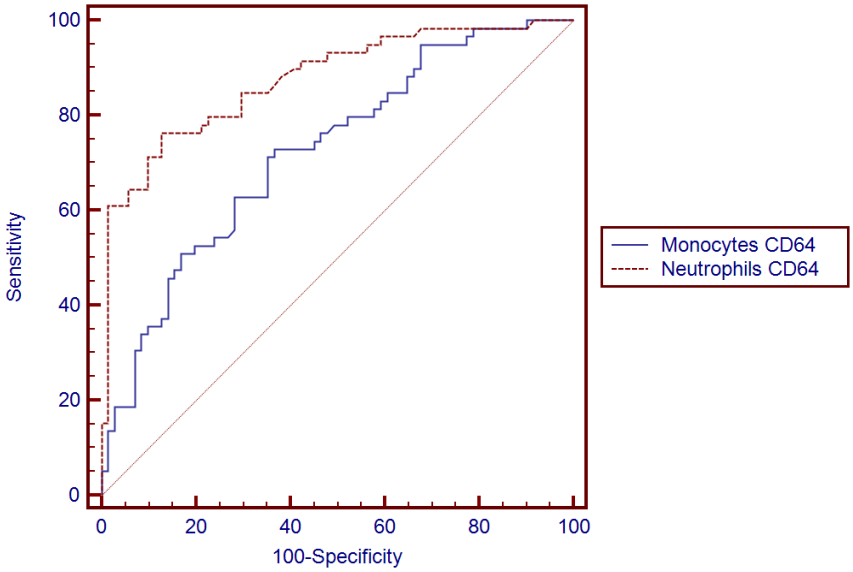
This table shows that Rodwell score is highly significantly increased with CRP, while it was insignificantly changed with the prognosis.

ROC curve for Rodwell Score only



AUC= 0.964 , cut of point >2, sensitivity 87.85, specificity 94.92, PPV 94 and NPV 89.6

ROC curve for monocyte CD64 and neutrophil CD64 in one ROC



Monocytes CD64:

AUC= 0.832 , cut of point >3.95, sensitivity 94.92, specificity 59.32, PPV 53.8 and NPV 95.9

Neutrophils CD64:

AUC= 0.875 , cut of point >3.62, sensitivity 76.27, specificity 87.32, PPV 83.3 and NPV 81.6

Discussion:

The current study is a prospective statistical analysis including 107 of neonates as group A with culture proven sepsis and 118 neonates as group B with no evidence of clinical sepsis and

negative cultures. Samples were collected from NICU of Cairo university and Almaza district NICU center from July 2017 to July 2018.

Group A was divided into 47 babies with early onset sepsis where 18 of them were females and 29 of them were males, and 60 babies with late onset sepsis where 31 were females and 29 were males.

In our study we found no significant difference between group A and B regarding gender with (P=0.614).

There was also highly significant difference between EOS and LOS groups regarding gestational age where EOS group has mean gestational age of 34.6 weeks while in LOS was 32.9 weeks with (p=0.005).

Also in our study, CRP was highly significant in sepsis group than in control group, where 88.8% were having positive CRP in sepsis group.

In our work, E coli was found to be the most common causative organism of sepsis where it was detected in 56% of patients in group A followed by staphylococcus aureus and Klebsiella pneumonia was detected in 17% and 15% of patients in group A respectively. There was no significant difference between different causative organisms regarding EOS and LOS.

In another study in Egypt by **Yousif et al, 2018**, the most commonly isolated organism was also E.coli, followed by Klebsiella pneumoniae and Staphylococci.

In another Egyptian study conducted by **Mohamed et al., 2012,** they reported that the organisms isolated from their cultures were mostly Gram-positive organisms (69.2%), among them Staphylococcus 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 Escherichia coli isolated in 7.7% of cases**.**

Shehab El- Din et al.,2015 reported that Gram-positive bacteria were responsible for most cases of neonatal sepsis in their study. Coagulase negative staphylococci (CoNS) were the most frequent isolated pathogens in EOS and LOS, followed by Klebsiella pneumoniae and Serratia marcescens**.**

Shobowale et al.,2015 reported that the most common organisms isolated in their study in Lagos university teaching hospital were Klebsiella pneumoniae (36.5%), followed by Staphylococcus aureus (18.8%)**.**

Bulkowstein et al.,2016 reported that in southern-Israel population, Streptococcus pneumoniae infections were less common in EOS than in LOS while Streptococcus agalactiae infections were more common in EOS. In both groups, Gram-negative bacteria predominated**.**

Shehab El-Din et al., 2015 reported positive CRP in about 85% of cases with culture proven sepsis**.**

Usha et al., 2015 reported that C-reactive protein increase in inflammations due to infection or tissue damage, with a sensitivity of 47-100% for detection of bacterial sepsis.

In our study hemoglobin in group A was significantly lower than that in group B with (P<0.001).

Also platelets count was significantly decreased in group A than that among group B with (P<0.001).

Yousif et al., reported that there was significant decrease in hemoglobin and platelets in their sepsis group compared to the controls **(Yousif et al., 2018).**

Ree et al., reported that thrombocytopenia occurred in 49% of their septic neonates **(Ree et al., 2017).**

Also Ognean et al explain thrombocytopenia in their study was either due to increased destruction, sequestration secondary to infection, failed platelet production secondary to reduced megakaryocytes, or damaging effect of endotoxins is frequently associated with neonatal sepsis but is, usually, a late sign of infection, indicating a poor prognosis (**Ognean et al., 2017).**

Regarding ANC, immature neutrophils count, I/T ratio and I/M ratio we found that they were significantly increased with group A with (P=0.022, <0.001, <0.001) respectively.

And also we have found that ANC, and immature count were significantly lower in EOS group than that in LOS group with (P= 0.037, 0.038) respectively.

While the total leucocytes count was insignificantly different between sepsis and control groups.

Misra et al reported that leukopenia was found to be a better predictor for neonatal sepsis compared to leukocytosis (sensitivity of 87.5% versus 25%) and is more useful in infections with gram negative germs **(Misra et al., 2013).**

Others have demonstrated that TLC increases in severe neonatal infections (both mature and immature cells) possible secondary to growth factors and cytokine release that stimulate the bone marrow production **(Borna H and Borna S, 2005).**

Shahab El-Din et al found that the abnormalities in the CBC were found in (66.8%) the septic group of neonates with (6.9%) having leucopenia (WBC < 5,000/mm3), (22.3%) leukocytosis (WBC > 20,000/mm3), (23.2%) neutropenia, and (45.5%) thrombocytopenia (platelets < 140,000/mm3) **(Shehab El-Din et al., 2015).**

In our study Rodwell score (HSS) was significantly higher in cases (group A) than the control group (group B) with cut off value of >2 (P<0.001), but it showed no different between EOS and LOS groups.

Rodwell et al., stated that the hematologic scoring system should improve the diagnostic accuracy of the complete blood cell count as a screening test for sepsis and could simplify and standardize the interpretation of this global test **(Rodwell et al., 1988).**

Also Orgnean et al state that since sensitiveity and specificity of the hematological indices are increasing when more parameters are evaluated, and said that hematological scoring systems (HSS) were proposed for neonatal sepsis detection. Such scores are available, relatively rapid, with low cost, and practically accessible in most laboratories but they still need simplification and standardization **(Ognean et al., 2017).**

Narasimha and Kumar also reported that the higher the score, the greater was the likelihood of sepsis. Where 100% of cases have a score of >5, 42.30% infants with history of probable infection had scores >5 and 30.76% had scores ≥3–4 suggesting the possibility of sepsis. 16.66% of normal infants had score >5 suggesting the presence of sepsis and 41.66% had scores >3 or 4 suggesting the possibility of sepsis in these cases. 41.66% of normal infants and 27% infants with probable infection had score ≤2, which implies sepsis was unlikely in these cases.

Also Bhalodia et al found comparable results regarding HSS where all culture-positive cases showed hematological score ≥5 with (P < 0.001)**. (Bhalodia et al., 2017).**

Makkar et al. also found that among cases with culture proven sepsis, (83.33%) infants had score ≥5, and (16.67%) had scores 3-4, (54.55%) among cases with probable infection had scores 3-4;and (27.27%) had score ≥5.

They found in this score that immature PMN count was highly sensitive (96.87%), followed by immature: Total (I:T) ratio (93.75%) and total PMN count (90.62%). Immature: Mature (I:M) PMN ratio (97.22%), followed by I:T PMN ratio (94.44%), degenerative changes

(94.44%) and platelet count (94.44%) were highly specific tests for diagnosing sepsis. They said that combination between increased I:T ratio and immature neutrophil count had the best sensitivity for EOS and probable EOS prediction, while increased I:T and I:M ratios were the most specific; a higher score was associated with a higher accuracy and probability of EOS, allowing a satisfactory identification of EOS and decision to treat (**Makkar et al., 2013).**

However, some papers criticize certain components of HSS that appear repetitive due to same pathogenesis mechanism. For example, raise in immature neutrophil count invariably increase the score by 3 in the current form of HSS. Apart from that, neutropenia which is very common and more sensitive in diagnosis of sepsis has a similar score as leukocytosis which is frequently seen in neonates due to non-specific reasons **(Hornik CP, 2012) and (Mikhael M, 2014).**

It showed high sensitivity (73.42%) and PPV (89.17%) but low specificity(32.76%) and NPV (59.09%) with p-value -0.742. This was comparable to some of the other studies [24-29].

Regarding CD64 we found that on both monocytes and neutrophils it was significantly higher among group A than that among group B with (P<0.001) for both, while there was no significant different for CD11b on both monocytes and neutrophils between groups A and B.

Yousif et al, showed in their study that the mean level of CD64 in sepsis group was much higher (95.54±9.44) than that of control group (14.51 ±7.51) and this difference was highly significant (p-value <0.001). The mean CD11b level was also higher among sepsis group (86.04± 11.58) than that in healthy group (15.43±10.9) and this difference was highly significant (p-value <0.001) **(Yousif et al., 2018).**

Jamsa et al found that the best marker for diagnosing severe sepsis in the ICU was neutrophil CD64 (P < 0.05), but also neutrophil and monocyte CD11b may be applicable (**Jamsa et al., 2015).**

Jamsa et al also reported that both monocyte and neutrophil CD11b and CD64 expression was highest in the start of infection and decreased over time. CD64 levels were reported previously to remain increased for at least 14 days. Also It they assumed that antibiotic treatment may lower CD64 expression in septic patients **(Jamsa et al., 2015).**

It is known from previous studies that CD11b expression is highly affected by pre-analytical factors and analytical settings and a lot of factors have been described influencing the behavior of PMN and the corresponding cell-surface expression. Artifactual CD11b variations have been linked to different technical features and the type of anticoagulant used to collect the blood **(Latger-Cannard et al., 2004).**

Also, other studies reported that monocyte CD11b increased in sepsis compared with non- septic infants **(Russwurm et al., 2002) & (Brunialti et al., 2006)**. And, Nuutila et al., reported that neutrophil CD11b in sepsis are controversial, possibly because of pre-analytical and analytical factors **(Nuutila et al., 2009).**

Moreover, Jamsa et al, had shown that also monocyte CD64 expression may be affected by the pre-analytical procedures **(Jamsa et al., 2011).**

In contrast to our study, other studies in emergency department and ICU population, neutrophil CD64 was criticized for low sensitivity for bacterial infection. Different from our study with majority of gram-negative blood cultures, there was majority of gram positive infections which may partly explain the low sensitivity **(Gamez-Diaz et al., 2011) & (Gros et al., 2012).**

Back to our study, we found no significant different between EOS and LOS groups regarding CD64 on monocytes or CD11b on monocytes and neutrophils, While there was significant increase in EOS than LOS groups regarding CD64 on neutrophils with (P=0.010).

Similarly El-Badawyet al., reported that CD64 expression on neutrophils increases significantly in neonates with EOS (p value <0.001), and can be considered a useful diagnostic marker for the early diagnosis of neonatal infection. However, its combination with CRP further increases its diagnostic value. While CD64 expression on monocytes was increased in neonates with EOS but with no statistical difference from non-infected and control groups **(El-Badawy et al, 2012).**

Motta et al., also reported that the increased expression of CD64 was significantly associated with subsequent infections (relative risk 1.54; 95% confidence interval 1.02–2.33), so that CD64 could be used as a reliable marker of EOS in VLBW neonates and it is an independent risk factor for late-onset infections, **(Motta et al., 2014).**

In our study we found no correlation between Rodwell score and CD64 or CD11b on both neutrophils and monocytes, nor between CD64 and CD11b with CRP, while there was positive correlation between CD11b on neutrophils and monocytes (r=0.839, P<0.001), also between CD64 on neutrophils and monocytes (r=0.433, P=0.001).

And also there was positive correlation between CRP and Rodwell score (r=0.273, P=0.007).

El-Badawy et al, on correlating the results of CD64 expression with other diagnostic markers of neonatal sepsis they found a significant correlation between neutrophil CD64 and CRP values (r = 0.3, p value 0.006) and between the monocytes expressing CD64 and thrombocytopenia (r = 0.3, p value 0.01) **(El-Badawy et al, 2012).**

Mohamed et al., found a significant positive correlation between CD64 expression and HSS (r=0.6581, P<0.001) and CRP (r=0.7531, P<0.001). with accuracy 86.1%, specificity and a positive predictive value (PPV) of 82.6 and 75%, respectively; HSS and CRP sensitivities were 92.3 and 100%, respectively, but with lower specificities (73.9 and 52.2%, respectively) and PPVs (66.7 and 54.2%, respectively) **(Mohamed et al., 2012).**

In our study we found that the Roc curve for Rodwell score showed sensitivity of 87.85% and specificity of 94%, positive predictive value (PPV) of 94% and negative predictive value (NPV) of 89.6%.

Sonawane et al. found that CBC has a sensibility of 37.5%, specificity of 75%, positive predictive value (PPV) of 69.2% and negative predictive value (NPV) of 44.4% for neonatal sepsis detection and underlined that CBC has a higher specificity compared to CRP (**Sonawane et al., 2014).**

An extensive review performed by da Silva et al. that found a large range of sensibilities - 17-90% - and specificities - 31-100% - for leukocytes and leukocyte indices especially in EOS screening, explained the results by laboratory experience variances, different postnatal age of the patients, and influence of noninfectious factors affecting neutrophils parameters and concluded that serial measurements of the leukocyte indices may increase the accuracy of EOS diagnosis and allow monitoring of the treatment **(Silva et al., 1995) and (Newman et al., 2010).**

A more recent review, performed by Bhat and Rao , based on 17 studies, showed that a leukocyte count < 5000 cells/mm3 or > 20.000 cells/mm3, a platelet count < 150.000/mm3, an I:T ratio ≥ 0.2, and the presence of vacuoles in the cytoplasm or toxic granulation on the peripheral smear suggest a risk of sepsis with sensitivity that range between 15.6 and 81% (**Bhat , Rao, 2010).**

Regarding Roc curve for CD64 on monocytes and neutrophils in our study it was showen that the sensitivity of 94.9% and 76.2%, and specificity of 59% and 83%, PPV of 53.8% and 83.3% , NPV of 59.9% and 81.6% , and a cut of point of >3.95 and >3.62 respectively.

Jamsa et al., reported that neutrophil CD64 having the highest AUC of 0.990 (95% confidence interval 0.968–1.000) with a cutoff value of 9172 (MESF) with sensitivity of 100% and specificity of 93%. Inspite they reported that monocyte CD64 expression was significantly lower in severe sepsis than the peak values in control group **(Jamsa et al., 2015).**

Yousif, et al. found that ROC analysis for their study showed that both CD64 and CD11b could be used for early detection of sepsis with sensitivity and specificity of more than 97%. Also, ROC analysis showed that both CD64 and Cd11b could be used for prediction of positive blood culture patients with sensitivities of more than 97% and specificities of 93% and 98%; respectively **(Yousif, et al., 2018).**

Finally, in view of our results, we conclude that Rodwell score is a simple, quick, cost effective tool which can be used as a screening test for early diagnosis of neonatal sepsis which may aid the clinicians in identifying sepsis and to institute proper anti-biotic therapy. Unnecessary exposure of infants to antibiotic therapy can thus be avoided.

Also CD64 on monocytes and neutrophils has high sensitivity and specificity for sepsis detection which may significantly help in limitation of un-useful use of antibiotics.

References

1. Hussein Attia Hussein Mahmoud, Ritika Parekh, Sarvani Dhandibhotla, Tarun Sai, Aayush Pradhan, Shiny Alugula, Martin Cevallos-Cueva, Barbara K . Insight Into Neonatal Sepsis: Cureus. 2023 Sep; 15(9): e45530.
2. Akinrinmade OA, Chetty S, Daramola AK, et al. CD64: An Attractive Immunotherapeutic Target for M1-type Macrophage Mediated Chronic Inflammatory Diseases. Biomedicines 2017 Sep; 5(3): 56.
3. Bhalodia MJ, Hippargi SB, Patil MM. Role of hematological scoring system in diagnosis of neonatal sepsis. Clinic Neonatology J. 2017; 6 (3): 144-147.
4. Bhat R, Rao A. The Performance Of Haematological Screening Parameters And CRP In Early Onset Neonatal Infections. Journal of Clinical and Diagnostic Research. 2010; (4):3331-36.
5. Borna H, Borna S. Value of Laboratory tests and C-Reactive Protein in the detection of Neonatal Sepsis. Internet J Pediatr Neonatol. 2005; 5:60-68.
6. Brunialti MK, Martins PS, Barbosa de Carvalho H, et al. TLR2, TLR4, CD14, CD11B, and CD11C expressions on monocytes surface and cytokine production in patients with sepsis, severe sepsis, and septic shock. Shock 2006; 25: 351–57.
7. Bulkowstein S, Ben-Shimol S, Givon-Lavi N, et al. Comparison of early onset sepsis and community-acquired late onset sepsis in infants less than 3 months of age. BMC Pediatr 2016; 16: 82.
8. Chan YH. Biostatistics 102: Qualitative Data – Parametric & Non- Parametric tests. Singapore Med J 2003; 44(8): 391-396.
9. Chan YH. Biostatistics 103: Qualitative Data – Tests of independence. Singapore Med J 2003; 44(10): 498-503.
10. El-Badawy AA, Abdellatif DAK, Mokhtar DA, Faragallah HHA. The Use of Neutrophil CD64 as a Diagnostic Marker of Early Onset Neonatal Sepsis, Thesis Submitted for

fulfillment of master degree in pediatrics 2012;erepository.cu.edu.eg/index.php/cutheses/article/view/3385/3340.

11. Gamez-Diaz LY, Enriquez LE, Matute JD, et al. Diagnostic accuracy of HMGB-1, sTREM-1, and CD64 as markers of sepsis in patients recently admitted to the emergency department. Acad Emerg Med 2011; 18: 807–15.
12. Ghosh S, Mittal M, Jaganathan G. Early diagnosis of neonatal sepsis using a hematological scoring system. Indian J Med Sci 2001; 55(9):495–500.
13. Gros A, Roussel M, Sauvadet E, et al. The sensitivity of neutrophil CD64 expression as a biomarker of bacterial infection is low in critically ill patients. Intensive Care Med 2012; 38: 445–52.
14. Hornik CP, Benjamin DK, Becker KC, Benjamin DK, Li J, Clark RH, et al. Use of the complete blood cell count in late-onset neonatal sepsis. Pediatr Infect Dis J. 2012;31(8):803-7.
15. Ince Z. Diagnosis of neonatal sepsis: What the clinician expects, what the laboratory tells. Clinical Biochemistry 2014; 47: 754–755.
16. Jamsa J, Huotari V, Savolainen ER, et al. Kinetics of leukocyte CD11b and CD64 expression in severe sepsis and non-infectious critical care patients. Acta Anaesthesiologica Scandinavica, 2015; 59: 881–891.
17. Kawai K, Tsuno NH, Matsuhashi M, et al. CD11b-mediated migratory property of peripheral blood B cells. J Allergy Clin Immunol. 2005 Jul; 116(1):192-7.
18. Khair BK and Rahman AS, Sultana T et al., Role of Hematologic Scoring System in Early Diagnosis of Neonatal Septicemia, BSMMU J 2010; 3(2): 62-67.
19. Latger-Cannard V, Besson I, Doco-Lecompte T, Lecompte T. A standardized procedure for quantitation of CD11b on polymorphonuclear neutrophil by flow cytometry: potential application in infectious diseases. Clin Lab Haematol 2004; 26: 177–186.
20. Majumdar A, Jana A, Biswas S, Bhattacharyya S. Hematologic scoring system (HSS): A guide to decide judicious use of antibiotics in neonatal septicemia in developing countries. J of applied Hematology 2013; 4(3):110-113.
21. Makkar M, Gupta C, Pathak R, et al. Performance Evaluation of Hematologic Scoring System in Early Diagnosis of Neonatal Sepsis. J Clin Neonatol. 2013; 2(1): 25-9.
22. Mikhael M, Brown LS, Rosenfeld CR. Serial neutrophil values facilitate predicting the absence of neonatal early-onset sepsis. J Pediatr. 2014;164(3):2013-4.
23. Misra RN, Jadhav SV, Ghosh P, et al. Role of sepsis screen in the diagnosis ofneonatal sepsis. Med J DY Patil Univ. 2013; 6(3):254- 7.
24. Mohamed HE, Akl HK, El Banna EA. CD64 (diagnostic accuracy compared with the hematologic scoring system and CRP in neonatal sepsis). Eygptional hematology J 2012; 37(4): 193-199.
25. Motta M, Zini A, Regazzoli A, et al., Diagnostic accuracy and prognostic value of the CD64 index in very low birth weight neonates as a marker of early-onset sepsis. Scandinavian Journal of Infectious Diseases 2014; 46 (6): 433-439.
26. Newman TB, Puopolo KM, Soora W, et al. Interpreting Complete Blood Counts Soon After Birth in Newborns at Risk for Sepsis. Pediatrics. 2010; 126(5):903-9.
27. Nuutila J, Jalava-Karvinen P, Hohenthal U, et al. CRP/CD11b ratio: a novel parameter for detecting gram-positive sepsis. Hum Immunol 2009; 70: 237–43.
28. Ognean ML , Boicean A, Sular F, Cucerea M. Complete blood count and differential in diagnosis of early onset neonatal sepsis. Ianuarie, 2017;25; 101-108.

29. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 1988; 112:761–767.
30. Russwurm S, Vickers J, Meier-Hellmann A, et al. Platelet and leukocyte activation correlate with the severity of septic organ dysfunction. Shock 2002; 17: 263–68.
31. Salim M.S, Abdelmoktader A.M, ElHamid A. Correlation between Neonatal Sepsis and Red Blood Cell Distribution Width (RDW). FUMJ. 2019; 2(1):71-78.
32. Shehab el-Din EM, El-SokkaryMM, Bassiouny MR and HassanR. Epidemiology of neonatal sepsis and implicated pathogens: A study from Egypt. Biomed Res Int 2015; 11: 509484.
33. Shobowale EO, Ogunsola FT, Oduyebo OO, Ezeaka VI. A study on the outcome of neonates with sepsis at the Lagos University Teaching Hospital. International Journal of Medicine and Biomedical Research 2015; 4(1):41-49.
34. Sonawane VB, Gaikwad SU, Kadam NN, Gavhane J. Comparative Study of Diagnostic Markers in Neonatal Sepsis. J Nepal Paediatr Soc. 2014; 34(2):111-4.
35. Stylianou-Riga p, Boutsikou T, Kouis P, Michailidou K, Epidemiology, risk factors, clinical presentation and complications of late-onset neonatal sepsis among preterm neonates in Cyprus: a prospective case-control study. BMC Pediatrics . 2024 volume 24, Article number: 50.
36. Supreeth MS, Sathyavathi RA, Shivendra VS, and Kariappa TM. Evaluation of neonatal septicaemia using hematological parantelers. International Journal of Recent Scientific Research Research, 2015; 6(2):2775-2778.
37. Turunen R, Andersson S, Nupponen I, et al. Increased CD11b-density on circulating phagocytes as an early sign of late-onset sepsis in extremely lowbirth weight infants. Pediatr Res 2005; 57:270–275.
38. Usha P, Reddy S, Uma P, Lakshmi AB. Clinical correlation of neonatal and maternal hematological parameters as predictors of neonatal sepsis. IJRRMS. 2015; 5(1):1-8.
39. Yousif LM , MohameD AA, and EL-Masry HMA. CD64 and CD11b vs. Conventional Bacteriological Methods in Early Detection of Bacterial Neonatal Sepsis. Med. J. Cairo Univ. 2018; 86(7): 3579-3587.