Hematological Parameters Versus C-reactive Protein Role in The Diagnosis of Late Onset Neonatal Sepsis in Full Term Neonates at Suez Canal University Hospital

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Abstract

Background: **Late-onset sepsis is a major cause of morbidity and death in neonates. Diagnosis may be complex. Hematological markers and C-reactive protein (CRP) are regularly measured as part of sepsis screening. However, the gold standard for diagnosing sepsis is the isolation of microorganisms in a blood culture.** ***Aim:*** **This study aimed to compare the role of hematological parameters versus CRP as diagnostic markers for late-onset neonatal sepsis (LONS) in full-term neonates.** ***Patients & Methods:*** **This case-control study included 50 neonates with LONS admitted to the Neonatal Intensive Care Unit (NICU) of Suez Canal University Hospital & 50 healthy controls both groups were rigorously investigated, including anthropometric data, clinical symptoms, key biomarkers, including CBC parameters & serum CRP.** ***Results:*** **Among the sepsis group, 43 patients (86%) had positive blood culture results as klebsiella (32%) & mixed organisms had the highest prevalence among positive results, staph (16%) & E.coli (8%). The Sepsis group had a significantly lower mean of Hemoglobin, platelets, & Platelet lymphocytic ratio than the control group with p-value <0.05.The Sepsis group had a significantly higher mean of C-reactive protein, C-reactive protein /Mean platelet volume and C-reactive protein /Albumin than the control group as p- value <0.001. CRP had the best sensitivity (100%), whereas CRP/MPV was more specific (100%).** ***Conclusion:*** **CRP has very good sensitivity for the diagnosis of LONS, while in combination with MPV gave more specificity for the diagnosis of LONS.**

Keywords: C-Reactive Protein, Platelet lymphocytic ratio, C-Reactive Protein to mean platelet volume ratio, Late Onset Neonatal Sepsis

Introduction

Neonatal sepsis is a systemic inflammatory response that occurs in newborns who are < 28 days old & have a confirmed infection. Clinical symptoms may vary from asymptomatic infection to severe local or systemic infection, which may include systemic indications of infection, circulatory shock, & multisystem organ failure(1). In underdeveloped nations, neonatal infections are responsible for around 1.6 million fatalities per year. The majority of these fatalities are caused by sepsis(2).

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It is crucial to have diagnostic tools for neonatal sepsis that are very accurate & capable of detecting the infection early on. This is because the illness may pose a major danger to the newborn, & it is essential to begin treatment as soon as possible. It may take some time to confirm the diagnosis, & diagnostic tests are employed to quickly identify the illness. Both clinical & laboratory testing may be used to determine if a newborn has sepsis[3]. The gold standard for diagnosing newborn infections is the isolation of germs from bodily fluids, such as blood, cerebrospinal fluid, & urine. However, microbiological culture is not accessible until at least 36 to 48 hours[4]. Conventional hematological and microbiological methods, which are routinely used, play an important role in diagnosis of neonatal sepsis. A complete blood count (CBC) obtained 6 to 12 hours after delivery may be helpful in the evaluation of early-onset sepsis. Although both the absolute neutrophil and the ratio of immature to total neutrophil counts (I/T) ratio have been used as markers for neonatal sepsis, they are more useful in identifying neonates who are unlikely to have sepsis than identifying those with sepsis[5]. An elevated I/T ratio has the best sensitivity of the neutrophil indices for predicting neonatal sepsis. In healthy term infants, the 90thpercentile for I/T ratio is 0.27. Exhaustion of the bone marrow reserves will result in low band counts and lead to falsely low ratios; this was illustrated in a cohort study of 3154 neonates who had a blood culture drawn at less than 24 hours and two serial WBC measurements with manual differentials, an abnormal I/T ratio was observed in all neonates with true sepsis[6]. A study found an increase in MPV in the first 24 h in neonates with sepsis compared to without sepsis group. Therefore, elevated MPV values are predictive of EONS. In addition, an increase of mortality and increased CRP values in patients with neonatal sepsis[7]. Another study reported that MPV increases significantly in neonates with sepsis. MPV could be a useful as an early diagnostic marker in neonatal sepsis[8]. CRP levels rise within 6 to 8 hrs of infection and peak at 24 hrs, Inflammation triggers the release of IL-6, which stimulates an increase in CRP concentrations. Individual CRP values of 0.2 to 95 mg/liter (mean, 1.7 mg/liter; median, 10 mg/liter) have a sensitivity range of 41 to 96% and a specificity range of 72 to 100% for neonatal sepsis. A value of 10 mg/liter is the most commonly used cutoff in most published studies (Meem et al., 2015).

This study aimed to compare the role of hematological parameters versus CRP as diagnostic markers for LONS in full- term neonates.

Patients & methods

This analytical case-control study was undertaken on the newborns with LONS hospitalized in (NICU) of Suez Canal University Hospital. it included all newborns who were admitted to the NICU (from March 2023 to March 2024) & met the criteria for inclusion including Temperature instability, Respiratory signs (grunting, intercostal retractions, apnea, tachypnea, cyanosis), Cardiovascular (bradycardia, tachycardia, poor perfusion, hypotension) Neurologic (hypotonia, lethargy, seizures, excessive irritability) Gastrointestinal (feeding intolerance, abdominal distension), Metabolic: either hypoglycemia or metabolic acidosis[6]. Sample size was calculated according to Metwaly study[8] as the difference of mean MPV among sepsis and control group was (0.65±0.5). So this study included 50 cases and 50 controls. The newborns were split into two groups: *Group A*, which consisted of 50 neonates with LONS, & *Group B*, which was the control group (50 neonates). The two groups were similar in terms of age and gender.

The control group involved full-term newborns who were matched for age and gender and who were followed up at Suez Canal University Hospital for non-infectious, unconjugated hyperbilirubinemia. While the case group admitted to the NICU with clinical features of LONS with no illness as metabolic disorders or ischemic hypoxic insult. Sepsis was diagnosed according to the following criteria.

An informed written consent was obtained from each children guardians before taking any data or doing any physical examination. It included an explanation of the study aim, design, and assurance that no harmful maneuvers will be used. All the data was strictly confidential (for research purpose only)

Statistical analysis was performed using SPSS program. Data was presented as tables and graphs when appropriate. Quantitative data was expressed as mean and standard deviation while qualitative data was expressed as numbers and percentages. Comparisons were performed using T- test (for quantitative data) and chi square (for qualitative data). The correlation was done by Pearson's correlation. Significance was considered at p value of < 0.05.

All patients were subjected to maternal history, neonatal assessment & laboratory investigations (C.B.C included Hb, total WBCs, Platelet count, Neutrophils, lymphocytes, N/L ratio. P/L ratio, MPV, C.R.P, CRP/MPV, CRP/albumin ratio & blood culture).

For measurement of CBC, peripheral blood was collected into an EDTA vacutainer tube and analyzed by an automated blood cell counter Serum CRP was measured using a fully automated auto-analyzer Cobas c501.

Results

Demographic and prenatal data

Fifty neonates with LONS admitted to Neonatal Intensive Care Unit (NICU) of Suez Canal University Hospital and 50 healthy control. There were no statistical significant difference between group A patients and group B patients as regard age, weight, gender, gestational age as p value >0.05. Regarding prenatal data, the current study found that sepsis group patients had significantly higher percentage of maternal history of presence of meconium and history of maternal fever than healthy control group with p value 0.05.

In this study, among sepsis group patients 52% with respiratory symptoms, 52% lethargy and depressed activity, 14% GIT symptoms,96% temperature instability, 48% DIC, 4% poor perfusion and hypotension, and 4% neurological symptoms.

Figure 1 revealed that 43 patients (86%) had positive blood culture results with the klebsiella had the highest prevalence among positive results(32%).

Table (1) revealed that group A patients had a significantly lower mean of Hb (9.99±1.94 vs 13.57±1.58), platelets (143.29±144.5 vs 293.66±65.92), N/L ratio (2.44±3.25 vs 3.3±0.77) & P/L ratio (53.88±43.38 vs 87.76±18.48) (p-value<0.001) and a significantly higher mean of MPV (10.94±1.01 vs 9.89±1.04) & CRP/MPV (7.43±6.24 vs 0.17±0.15) than group B patients with (p-value<0.05).

Table (2) revealed that group A patients had a significantly higher mean of CRP (83.78±72.4 vs 1.71±1.44), CRP/MPV (7.43±6.24 vs 0.17±0.15), CRP/Albumin (35.29±33.43 vs 0.48±0.43) (p-value <0.001).

Regarding diagnostic performance in the discrimination of sepsis patients, MPV at cut-off point 10.25 FL had 74% sensitivity and 70% specificity. N/L ratio at cut- off point 2.35 had 92% sensitivity and 62% specificity. The P/L ratio at cut off point 42.1 had 96% sensitivity and 52% specificity. CRP at cut-off point 6 mg/L had 100% sensitivity and 94% specificity . CRP/MPV Ratio at cut-

off point 1.05 had 98% sensitivity and 100% specificity (Table 3).

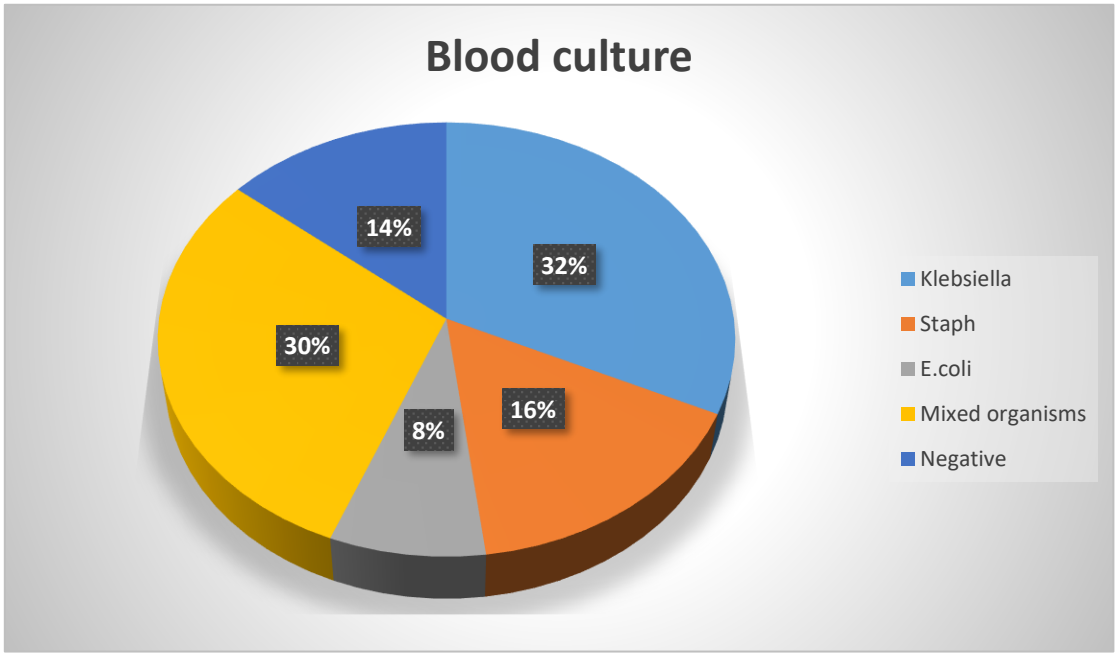

Figure 1: Blood culture of the Study sepsis group.

Table 1: CBC of the studied population.

CBC		Group A (n=50)	Group B (n=50)	*Test value*	*P-value*
Hb	*Mean ± SD*	9.99±1.94	13.57±1.58	*t=9.515*	*<0.001**
	Range	6.5-16.8	9.7-21.3		
	Median	9.5	13.2		
Hb classification *Frequency (%)*	*Normal*	6(12%)	30(60%)	X^2=25.0	*<0.001**
	Anemia	44(88%)	20(40%)		
	Polycythemia	0(0%)	0(0%(		
TLC	*Mean ± SD*	12.89±8.38	10.88±3.36	*MW=0.758*	*0.435*
	Range	0.9-40.6	6.3-26.4		
	Median	11.8	11.55		
TLC classification *Frequency (%)*	*Normal*	17(34%)	22(44%)	X^2=5.085	*0.090*
	Leucocytosis	28(56%)	28(56%)		
	Leukopenia	5(10%)	0(0%)		
Platelets*10^3	*Mean ± SD*	143.29±144.5	293.66±65.92	*MW=305.5*	*<0.001**
	Range	3-709	132-426		

	Median	106	301.5		
Platelets classification *Frequency (%)*	*Normal*	15(30%)	48(96%)	X^2=53.606	<0.001*
	Thrombocytopenia	32(64%)	1(2%)		
	Thrombocytosis	3(6%)	1(2%)		
MPV	*Mean ± SD*	10.94±1.01	9.89±1.04	*t*=96.982	<0.001*
	Range	9.1-14	8.2-12.2		
	Median	10.9	9.8		
N/L ratio	*Mean ± SD*	2.44±3.25	3.3±0.77	*Mw*=568.5	<0.001*
	Range	0.29-23.2	0.38-4.1		
	Median	1.8	3.2		
P/L ratio	*Mean ± SD*	53.88±43.38	87.76±18.48	*MW*=590.5	<0.001*
	Range	1-163	19.6-115.2		
	Median	42	92.35		
CRP/MPV ratio	*Mean ± SD*	7.43±6.24	0.17±0.15	*MW*=6.00	<0.001*
	Range	0.38-26.8	0.01-0.6		
	Median	5.1	0.1		

MW; Man Whitney U test, X2; chi square test; t: student t test. *Statistically significant as p<0.05.
Hb; hemoglobin, TLC; total leucocytic count, MPV; mean platelet volume; CRP; c reactive protein.
MW; Man Whitney U test, X2; chi square test; t: student t test. *Statistically significant as p<0.05.

Table 2 : Comparison of CRP, CRP/MPV ratio & CRP/Albumin ratio between the 2 studied groups.

	Group A (n=50)	Group B (n=50)	Test value	P-value
CRP(mg/L) Mean ± SD	83.78±72.4	1.71±1.44	MW=7.934	<0.001*
Range	4.7-309	0.1-6		
Median	62.5	1.2		
CRP/MPV ratio Mean ± SD	7.43±6.24	0.17±0.15	MW=28.18	<0.001*
Range	0.38-26.8	0.01-0.6		
Median	5.1	0.1		
CRP/Albumin ratio Mean ± SD	35.29±33.43	0.48±0.43	MW=43.015	<0.001*
Range	1.4-133.8	0.02-1.7		
Median	23.2	0.3		

MW; Man Whitney U test. *Statistically significant as p<0.05.

Table 3: Area under the ROC curve of study parameters in discrimination of sepsis among the studied participants

Parameter	Area	S. E.	P-value	Asymptotic 95% Confidence Interval		Cut-off	Sensitivity	Specificity	PPV	NPV
				Lower Bound	Upper Bound					
MPV	0.774	0.047	0.000	0.683	0.865	10.25(FL)	74	70	71.2	72.9
N/L ratio	0.773	0.051	0.000	0.672	0.873	2.35	92	62	70.8	88.6
P/L ratio	0.764	0.054	0.000	0.658	0.869	42.1	96	52	66.7	92.9
CRP	0.999	0.002	0.000	0.966	1.00	6 (mg/l)	100	94	94.3	100
CRP/MPV Ratio	0.998	0.003	0.000	0.992	1.00	1.05	98	100	98	98.03

Discussion

Sepsis is a life-threatening organ failure that occurs when the body's reaction to infection is not functioning properly. It is still one of the most prevalent causes of mortality in critically sick patients. The World Health Assembly & the World Health Organization (WHO) both acknowledged sepsis as a global health priority, & it has since become a public concern[9].

Blood culture is still the best method for diagnosing sepsis, as it can identify pathogens & perform antibiotic sensitivity tests to guide the treatment of bacterial infections. However, it is a time-consuming process & has a high false negative rate, especially after antibiotics have been used[10].

Sick newborns who are hospitalized need laboratory testing & several blood samples, that can be used to diagnose late-onset neonatal sepsis in its early stages[11]. The study revealed that klebsiella was the most common causative agent of LOS (32%) followed by staph (16%).

This In concordance with many studies 10,11,12 that revealed that Klebsiella was the most prevalent cultivated gram-negative organism, Coagulase negative staphylococcus aureus was the most prevalent gram positive cultured bacteria in sepsis.

In the current research, the sepsis group patients had a substantially lower average of Hb, platelets and P/L ratio than the healthy control group. This is consistent with another case-control research that was done on babies who were hospitalized for sepsis. That study indicated that newborns who did not have sepsis had higher levels of neonatal hemoglobin (13.20 g/dL) than those who did have sepsis (11.90 g/dL)[12].

This is similar to the research by Omran et al.[11], which found that septic neonates had a substantially lower platelet count than the control group (P = 0.012).

At a cutoff point of 2.35, the N/L ratio revealed a sensitivity of 92% and specificity of 62% when distinguishing sepsis patients. At the cutoff point, the P/L ratio was 2.35, which had a sensitivity of 96% & a specificity of 52% when distinguishing between sepsis patients.

In concordance with previous research, it was discovered that the sepsis group had considerably greater neutrophil counts, NLRs, PLRs, & C-reactive proteins than the healthy control group. In the LOS group, there was a positive correlation between

neutrophil counts, NLR, & PLR. The predicted cutoff value of neonate LOS was discovered to be an NLR of 6.76 (sensitivity 97.4%; specificity 100%; area under the receiver-operating characteristic curve 0.99; P=0.001). The predicted cutoff value of neonate LOS was found to be a PLR of 94.05, with a sensitivity of 97.4, a specificity of 100%[13].

According to this research, patients in the sepsis group had a mean CRP of 83.78±72.4mg/L, which was substantially higher than the mean CRP of 1.71±1.44mg/L in the healthy control group .values were less than 0.001. When the cutoff threshold was 6 mg/L, CRP had a sensitivity of 100% and a specificity of 94% in distinguishing individuals with sepsis.

These results are in agreement with prior research that shows the importance of serum CRP in diagnosing respiratory infections. Kumar et al. discovered that when utilizing a CRP cut-off value of 5 mg/L, the total serum CRP accuracy for diagnosing LONS was higher, ranging from 96.5% in proved sepsis to 99.1% in suspected sepsis, with a specificity of 85.3%. This indicates that CRP is quite accurate in diagnosing infants who are at risk of sepsis[14].

Furthermore, Omran et al. discovered that newborns with LOS had a mean serum CRP of 29.4 ± 13 mg/L, which is a considerable increase[15].

These values vary greatly, ranging from 1.5 to 20 mg/L, & are linked to different levels of sensitivity & specificity. For example, sensitivity levels may be anywhere from 74% to 98%, & specificity values can be anywhere from 71% to 94%, regardless of whether a single measurement is used at least 12 hours following the commencement of symptoms or serial CRP measurements[16–20].

In the current research, the patients in the sepsis group had a mean MPV & CRP/MPV that were substantially greater than those of the control healthy group, with p<0.05. At the cutoff point of 10.25FL, the MPV revealed a sensitivity of 74% & a specificity of 70% when it came to distinguishing sepsis patients. The CRP/MPV ratio at the cutoff point of 1.05 revealed a sensitivity of 98% & a specificity of 100% when it came to distinguishing individuals with sepsis.

Another research found that the CRP/MPV ratio was very effective in identifying sick newborns, with a sensitivity of 97.14% & a specificity of 85.71% when the cutoff value was greater than 0.88. In children, the CRP/MPV ratio may be useful for distinguishing between bacterial & viral pneumonia & for predicting sequelae[21].

In the current research, patients in the sepsis group had a mean CRP/Albumin Ratio of 35.29±33.43, which was substantially higher than the control group's mean of 0.48±0.43, with a p-value of less than 0.001.

This is consistent with a recent research that revealed that the CRP/Albumin ratio may be utilized as a possible predictor for gram-negative bacteremia in LONS[22].

Conclusion

CRP has very good sensitivity for the diagnosis of LONS, while in combination with MPV gave more specificity for the diagnosis of LONS.

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