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Original Article

Complete blood counts parameters and neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis is one of the common causes of neonatal mortality. Blood culture is the gold standard procedure to confirm sepsis. However, the rapid rate of disease progression makes its timely diagnosis and prompt management very crucial before the availability of culture. The study aimed to determine the significance of complete blood count parameters including platelet indices in diagnosing neonatal sepsis.

Materials and Methods: This study included 129 suspected cases of neonatal sepsis admitted to KIST Medical College Teaching Hospital from 15th April 2018 to 14th April 2019. Complete blood count, platelet indices, micro erythrocyte sedimentation rate, C-reactive protein, and blood culture were performed and analyzed.

Results: Of 129 suspected neonatal sepsis, abnormal leukocyte count was encountered in 31 (24.0%) patients. Culture-proven sepsis was seen in 25 (19.1%) patients. The sensitivity and specificity of Neonatal Sepsis Screening Criteria were 10.0% and 80.0% respectively.

Among platelet-derived indices, PDW (full form) had a sensitivity of 80% and specificity of 56% with a cut of 17.02 CV. MPV (full form), with a cut-off value of 9.78 fL had a sensitivity of 88 % with a specificity of 59%. Both MPV and PDW had low positive predictive values (33.3%) but a high negative predictive value (95.2%).

Conclusions: Along with total leukocyte count, platelet indices have shown their significance in determining neonatal sepsis with significant negative predictive value. The addition of MPV and PDW to the Neonatal Sepsis Screening Criteria might help the treating physician to rule in or rule out neonatal sepsis and manage it accordingly.

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INTRODUCTION

Neonatal sepsis (NNS) is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first four weeks of life. NNS is confirmed with positive blood and/or cerebrospinal fluid culture. But since often the culture comes inconclusive, NNS is also defined clinically.[1] According to the onset of the disease, neonatal sepsis can be classified as Early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS is seen in the first 3 days of life and LOS from 4 to 28 days of life. Clinical features of NNS are vague and ill-defined. The rapid rate of disease progression makes its timely diagnosis

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and prompt management very crucial. Though blood culture is considered a gold standard for the diagnosis of neonatal sepsis, its use has significant limitations which include false negativity secondary to maternal antibiotic use or low microorganism concentration, delayed reporting, and false positivity secondary to contamination.[2]

Neonatal sepsis screening is a commonly used biomarker that includes total leukocyte count (TLC), immature to the total neutrophil ratio (I/T ratio), Absolute neutrophil count (ANC), micro-erythrocyte sedimentation rate (m-ESR), and C reactive protein (CRP).[3] Routine complete blood count varies significantly with the day of life and gestational age, making it difficult to interpret. While low white blood cell count, low values of ANC, and high I/T ratio are associated with early-onset sepsis, high WBC count and high ANC count is not informative in this case. Similarly, high WBC count, high ANC count, low I/T ratio, and low platelet count are associated with late-onset sepsis. Despite their association with infection, the above-mentioned findings have low specificity.[4] Similarly, the physiological rise of CRP is seen after 3 days of birth and is lower in premature infants, so a single value for all newborns might be suboptimal and is also seen to have very low sensitivity.[4] Due to these limitations, there is an ongoing search for new parameters that aid in the early diagnosis of NNS. Inflammatory markers such are procalcitonin, and IL-6 are currently in use for that purpose, however, these tests are not cost-effective and are not easily available.

Platelet count is markedly decreased in case of sepsis which can be due to increased platelet destruction following disseminated intravascular coagulation or when bacteria and its product damage the endothelium leading to platelet aggregation or when they bind directly to platelet causing its aggregation and elimination from circulation. Among other platelet indices, indices related to morphology and platelet kinetics such as mean platelet volume (MPV), platelet volume distribution width (PDW), and plateletcrit (Pct) are studied in sepsis.[5] MPV refers to the average size of platelets and is considered a marker of production rate and platelet activation. Its level is increased in destructive thrombocytopenia and decreased in thrombocytopenia resulting from decreased production. PDW is an indicator of variation in platelet size and increases in platelet consumption when turnover is increased.[6] Platelet lymphocyte ratio which is an indicator of the balance between inflammation and thrombosis can also be used to study sepsis. It is confirmed that it has a higher specificity and positive predictive value in comparison with other biomarkers used in the diagnosis of early-onset sepsis.[2] Although above mentioned hematological parameters are readily available when CBC is done by automated coulter counter, they are studied less.

In this study, we aimed to investigate the diagnostic value of plateletcrit, MPV, and PDW, platelet/lymphocyte ratio in neonatal sepsis; compare them with other neonatal sepsis screening laboratory criteria[3] and also correlate these parameters with blood culture/CRP levels.

MATERIALS AND METHODS

This was a cross-sectional study conducted at KIST Medical College, Teaching Hospital from April 15th, 2018 to 14th April 2019. Permission was obtained from the Institutional Review Board prior to the study. Parents of the newborn suspected of NNS were explained regarding the study and subsequently enrolled in the study. The relevant data were collected in the predesigned proforma. CBC was performed and laboratory findings like TLC, differential count, hemoglobin (Hb), Neutrophil/Lymphocyte (N/L) ratio, Platelet count, Platelet/Lymphocyte (P/L) ratio, Plateletcrit, MPV, and PDW were noted as well. Along with these tests, micro-ESR, CRP, and blood culture were performed. Lumbar puncture with CSF analysis, chest X-ray, and urine R/E was done whenever indicated. The manual differential count was performed only when felt necessary by the reporting pathologist. Leukocyte count and lymphocyte count were done by the flow cytometry method using a laser light scattering technique. Platelet count was performed using the impedance method with hydrodynamic focusing. MPV value was generated in the coulter counter. The platelet volume distribution curve was produced by utilizing a lower threshold (2-6 fL) and an upper threshold (12-30fL). The MPV was then obtained by dividing Pct by platelet number and PDW was the width of the size distribution curve at 20% of the peak. CRP andmicroESRwere also performed. TLC > 25,000 mm^3 was considered leukocytosis and less than 5,000 mm^3 was considered leukopenia. Likewise, ANC <1800/mm^3 was considered neutropenia. Micro-ESR more than the day of life plus 3 mm/hr. to a maximum of 15 mm/hr.was considered as raised. CRP of more than 1.0 mg/L was considered positive. Positive culture or positive CRP level was considered as Sepsis. All these findings were recorded in the proforma.

Statistical Analysis

The data were entered into Microsoft Excel. Analysis of the data was done by using SPSS (the statistical program for social science version 16). Variables were given as mean ± SD and percentage. Pearson correlation coefficient test (r test) was used to test the correlation between two quantitative variables. Linear regression test was used to find the relation between platelet indices and other parameters. Receiver Operator Characteristic (ROC) curve was used to find the cut-off value, sensitivity, and specificity of the MPV, and PDW. A p-value of <0.001 is considered highly significant. If p is <0.05, it is considered as significant.

RESULTS

A total of 129 suspected neonatal sepsis neonates were included in the study. Among them, 73 (56.6%) were male and 56 (43.4%) were female. The mean gestational age at delivery was 38 (SD±2.09) weeks ranging from 30 weeks to 42 weeks with 19 (14.7%) preterm deliveries. Mean

birth weight was 2.87 (SD±0.49) kg (Table 1) in which two neonates (1.6%) were of very low birth weight, 15 neonates (11.6%) were of low birth weight, and the remaining (n=112; 86.8%) of normal birth weight. The mean weight among preterm deliveries was 2.24 kg (SD±0.45) with a minimum being 1.18 kg. Term deliveries were 110 (85.3%) with a mean weight of 2.9 (SD±.042) kg (Table 2) Fifty-three patients (41.08%) had at least one of the findings listed in Table 1. The most common associated finding was a premature rupture of membrane (PROM) which was seen in 35 (27.1%) patients followed by chorioamnionitis (n=8; 6.2%). The birth weight of both male and female newborns was compared among preterm and normal deliveries as depicted in table 2. Overall, the mean gestational age was 38.0 weeks(SD±2.09) and birth weight was 2.87 kg (SD±0.49)

Table 1: Perinatal data of the studied neonates (n=129)

Variables		Number (%)
Gender	**Male**	73 (56.6%)
	Female	56 (43.4%)
Gestational age	**Preterm**	19 (14.7%)
	Term	110 (85.3%)
	Birth weight	2.87 kg
	Premature Rupture of Membrane	35 (27.1%)
	Chorioamnionitis	8 (6.2%)
	Prolonged 2nd stage of labour	1 (0.8%)
	Maternal Urinary tract infection	6 (4.7%)
Hematological findings	**TLC**	17, 245 / mm3(SD±6507)
	Neutrophils	61.29% (SD±15.04)
	Band forms	3.48% (SD±3.1)
	Lymphocytes	36.41% (SD±14.5)
	Platelet	244,567 / mm3(SD±77849)

Table 2: Comparing mean birth weight among Preterm and Term deliveries (n=129)

Parameters		Gestational age (mean)	Birth weight
Overall		38.0 (SD±2.09)	2.87 kg (SD±0.49)
Preterm	**Male**	34.13(SD±1.9)	2.29 (SD±0.47)
	Female	34.01(SD±2.1)	2.17 (SD±.45)
Term	**Male**	38.5(SD±1.2)	2.99(SD±0.34)
	Female	38.8(SD±1.1)	2.96 (SD±0.50)

Relevant CBC findings are shown in table 1. The mean total leukocyte count was 17,245 mm^3 (SD±6,507) with a maximum of 46,000/mm^3 and a minimum of 4,700/ mm^3. Leukopenia was observed in 15 (11.6%), whereas, 16 patients (12.4%) had leukocytosis, and 98 (76%) patients had normal leukocyte count. Similarly, the mean value of an absolute count was 11,220/mm^3 with a minimum of 799/ mm^3. Neutropenia was observed only in 3 patients (2.4 %; ref range <1800/cumm). Furthermore, micro-ESR was elevated in 11 (8.5%) cases and the I/T ratio was elevated in 3 (2.3%) cases.

Based upon Neonatal Sepsis Screening criteria, 30 (23.3%) patients were of probable (clinical) sepsis. Thirty-eight (29.5%) patients had a positive CRP level. Twenty-five (19.4%) patients were culture-positive cases of neonatal sepsis. 49/129 (38%) patients were either culture or CRP-positive. In this study, a significant correlation was observed between the neonatal sepsis screening criteria and sepsis cases ($p<0.001$).

The ROC curve was plotted to analyze the importance of platelet-derived indices among culture-proven cases of NNS. Among platelet-derived indices that were analyzed, PDW had a sensitivity of 80.0% and specificity of 55.8% at the cut-off value of 17.02 CV. (Table 3). Area of Platelet count, Plateletcrit and Platelet lymphocyte ratio were less than 0.5. (fig. 1) MPV with a cut-off value of 9.78 fL has a sensitivity of 88.0 % with a specificity of 59.0%. (Table 3)

Table 3: Determination of sensitivity, specificity, and p-value of platelet indices among the studied neonates

Variable(s)	Area	95% Confidence Interval	Cut-off value	Sensitivity of the given cut-off	Specificity of the given cut-off	p-Value
MPV	0.809	0.809-0.932	9.79	76.3 %	91.8 %	<0.001
PDW	0.802	0.802-0.927	16.81	70.0%	95.9%	<0.001

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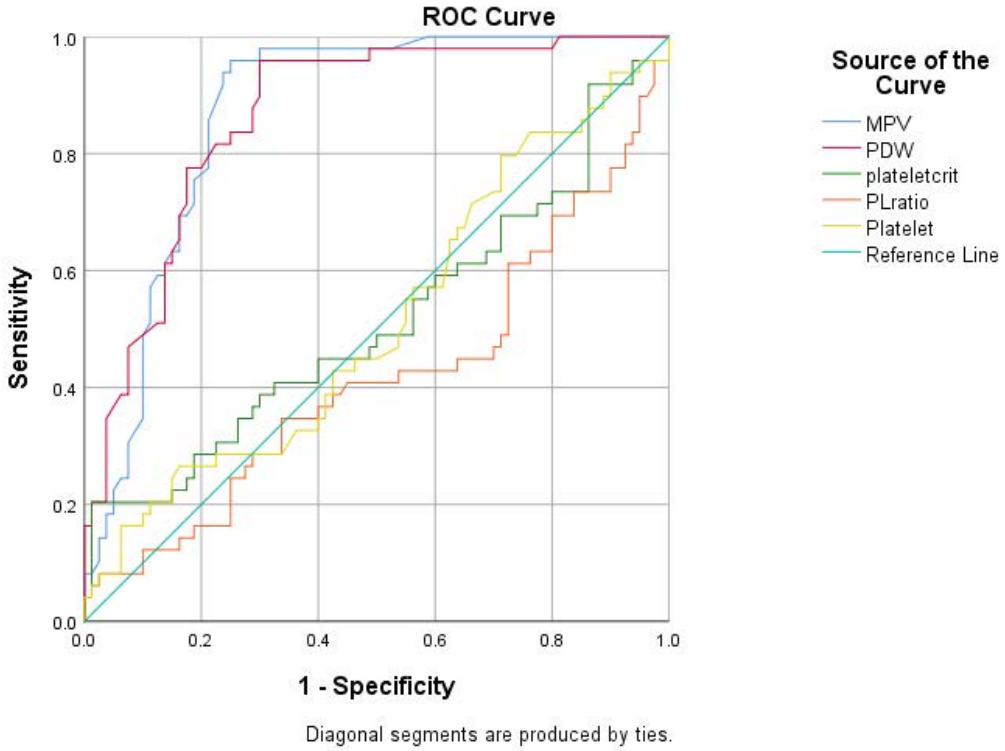

Figure 1: ROC curve of different platelet indices

When PDW, MPV, Plateletcrit, and Platelet/Lymphocytes ratio were included, no significant changes were observed (p-value> 0.05). However, PDW and MPV when were analyzed separately, a significant correlation was observed with a p-value of <0.001 (Table 3). The positive predictive value of both PDW and MPV was 96.6% and 93.8% respectively. Similarly, the negative predictive value of PDW was 66.2% and it was 70.3% for MPV.

DISCUSSION

Newborns, especially preterm babies are more susceptible to infection than children of any other age. Impaired cytokine production, decreased expression of adhesion molecules in neutrophils, and reduced response to chemotactic factors affect their innate immunity. Furthermore, transplacental passage of antibodies which started in the 2nd-trimester peaks in the 3rd trimester thus reducing the humoral response in preterms and making them susceptible to infections.[4]

Newborns rarely present with specific clinical features of NNS, laboratory findings remain the only possible tool for its detection. Among 129 suspected cases of NNS, 19 (14.7%) were preterm deliveries. The rate of preterm deliveries coincides with the other study done by KC et al in 2015[7] where the author found 14% of preterm deliveries.

Premature rupture of the membrane was the most common cause for suspicion of NNS (35; 27.1%). In a study done by Poudyal et al[8] in Dhulikhel, Nepal, PROM was observed in 28.29% which is in concordance with our study. Similarly, chorioamnionitis was the reason for suspicion of NNS in 6.2% of newborns. Chorioamnionitis was encountered among 12% of newborns in Mulagao hospital, Kampala, Uganda.[9] In this study, 4.7% of the newborn were suspected of NNS because of UTI. The incidence of UTI was lower than in other study done in Dhulikhel, Nepal by Poudyal et al.[10]

The positive neonatal septic screen should have at least two of the following 5 criteria[3]: a) Leukopenia (TLC<5,000 mm^3; B) CRP ≥1.0 mg/dL; C)I/T ratio: ≥ 0.2; D)Micro ESR (> 15mm 1st hour) and E)Absolute Neutrophil count: <1800 mm^3. Various laboratory investigations were performed. Among all the patients with suspected NNS, 31 (24.0%) patients had leukopenia or leucocytosis. Based upon septic screening criteria, among the suspected NNS newborns, 30 (23.3%) patients were of neonatal sepsis. Leukopenia or leucocytosis is associated with infections and in the case of NNS, leukopenia is usually associated with early-onset of NNS whereas, leucocytosis is associated with late-onset NNS.[4] According to the septic screening criteria, Leukopenia is one of the five criteria to diagnose NNS. In this study, even leukopenia was observed in 16 (12.4%) patients of which, 7 (5.4%) patients had proven NNS. However, the remaining 9 (7.0%) patients with leukopenia were not proven NNS. Similarly among 15 (11.6%) patients with leucocytosis, only 2 (1.5%) patients are proven NNS. 16 patients (12.4%) with normal total leukocyte count had proven sepsis. Culture is

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the gold standard to diagnose neonatal sepsis. However, up to 68% of the patients with NNS had a low level of bacteremia (<10 colony-forming unit/ml) and 42% had <1 colony-forming unit, which would be reported as a negative culture report.[11]

C-reactive protein is synthesized in the liver and is a marker of inflammation. It is one of the acute phase proteins. Though it is commonly seen in acute inflammation, it is also elevated in the chronic inflammatory process. Hence it is not specific to sepsis. However, it is one of the widely used inflammatory markers along with IL-6, IL-9, and procalcitonin. CRP was positive in 38 (29.5%) of patients suspected of NNS. Contrary to this study, a study done by Hisamuddin et al found an accuracy of 70.07% for CRP in 72 hours of life.[12] Benitz et al reported a 40% sensitivity of CRP tests.[13] The low percentage of sensitivity for CRP tests might be related to the premature immune system of the newborn. As the newborn grows, the CRP tests become more sensitive and specific.[10,14] The low positive CRP value might be because of the collection of blood samples within a few hours of birth for sepsis screening. On the other hand, another study has observed satisfactory sensitivity and specificity.[15]

Platelet plays a vital role during the acute inflammatory process. It regulates leukocyte function during inflammation. Platelet either directly interacts with a cellular component of natural immunity, namely neutrophil, and macrophages, or releases chemokines and cytokines which further attract inflammatory cells.[16,17] Platelets promote endothelial adhesion and extravasation of leukocytes at sites of inflammation while securing vascular integrity at the site of transmigration.[18] Thrombocytopenia has been associated with adult sepsis and has been included in the SOFA score. The platelet count has been inversely correlated with sepsis severity.[19] In this study, 10/129 patients had thrombocytopenia. Among 25 culture-proven NNS, 2 had severe thrombocytopenia, 1 had moderate thrombocytopenia and 2 had mild thrombocytopenia. Platelet parameters were analyzed and MPV with a cut-off value of 9.79 fL has a sensitivity of 76.3 % with a specificity of 91.8 %. The baseline MPV value was increased in the 64/129 suspected NNS patients. Twenty-one (84.0%) culture-proven patients had elevated baseline MPV values. Guida et al, in their study among 154 blood culture-proven NNS patients also observed elevated baseline MPV values.[20] MPV is the average size of the platelet. As there is the consumption of platelet during sepsis, larger, newly formed platelets are released in the peripheral blood, which increases the MPV. Similarly, Catal et al studied 91 preterm NNS patients and reported a positive correlation between MPV, IL-6, and CRP.[21] PDW with a cut-off value of 16.81 has a sensitivity of 70.0 % with a specificity of 95.9 %. Among culture-proven NNS, 20 (80.0%) had elevated baseline MPV values. Overall, 66/129 studied suspected population PDW was elevated. PDW was elevated in a study done by Akarsu et al. In 72.1% of neonatal sepsis, the PDW was elevated.[22] A similar finding was reported by Celeste et al. where among 156 NNS patients the MPV and PDW showed high specificity to detect bacteremia (95% and 79% respectively) and had appreciable negative predictive value.[23]

MPV had a high positive predictive value (96.6%) and negative predictive value (93.8%) and PDW had a positive predictive value (66.2%) and negative predictive value (70.3%).

Limitations: The study was done in a single center with a small sample size (129) of which only 49 cases were culture/CRP positive. Hence, further study with larger sample size and multiple samples in different timeframes may help establish its prognostic value in neonatal sepsis.

CONCLUSIONS

CBC and its parameters (especially platelet related) are readily available and not well studied in cases with suspected NNS. In addition, in Neonatal Sepsis Screening Criteria only TLC, I/T ratio, and ANC are being utilized. Our study revealed that platelet indices had shown their significance in determining NNS with significant positive predictive value. Hence, the addition of MPV and PDW to the Neonatal Sepsis screening Criteria might help the physician to rule in or rule out neonatal sepsis and manage it accordingly.

Conflict of interest: None

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