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RESEARCH ARTICLE

Evaluation of platelet count, platelet indices and C-reactive protein in culture proven neonatal sepsis in a tertiary care centre

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ABSTRACT

Background: Neonatal sepsis is the most prevalent and significant cause of neonatal morbidity and mortality, requiring prompt diagnosis and treatment. Positive blood culture is the gold standard for diagnosis; the results of which are delayed by 48 hours with only 30-75% positivity rate and a well-equipped laboratory setting is necessary. Emerging studies suggests platelet count and its indices such as plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) as reliable and affordable indicators obtainable during regular complete blood counts. **Aims and objectives:** To determine the platelet count and platelet indices in culture positive neonatal sepsis and its correlation with C-reactive protein in a tertiary care centre. **Method:** It was a hospital based prospective study carried out in NICU, Gauhati Medical College and Hospital, Guwahati, Assam. The study included 94 culture positive neonatal sepsis admitted to the above centre from 1st June, 2021 to 31st May, 2022. **Result:** Out of 1120 cases, 94 culture positive cases were taken in the study of which 87% had high CRP, 75.5% neonates had thrombocytopenia, 65.9% had high mean platelet volume and 40.4% with high platelet distribution width.CRP had significant correlation with MPV and PDW with p-values of 0.023822 and 0.011713 respectively but not with plateletcrit. A high MPV of 10.6% in gram positive sepsis cases, 30.8 % gram negative and 24.5% in fungal sepsis cases. **Conclusion:** Platelets and its indices can be considered as a diagnostic tool for neonatal sepsis as it is cheap, rapid, and easily available and does not require additional equipment.

Keywords: Blood culture, platelet distribution width, plateletcrit, mean platelet volume.

A bloodstream infection in newborn babies younger than 28 days old is referred to as neonatal sepsis. Early-onset sepsis (EOS) (<72 hours of life) and late-onset sepsis (LOS) (at or after72 hours of life) are two categories of neonatal sepsis based on when symptoms first appear after birth.

In underdeveloped nations like India, neonatal sepsis is the most prevalent and significant cause of neonatal morbidity and mortality. Neonatal sepsis has modest, non-specific signs and symptoms, making a clinical diagnosis challenging. According to the National Neonatal Perinatal Database, infections account for 18.6% of intramural neonatal fatalities and 39.7% of extramural neonatal mortality [1]. In 2013, the global analysis revealed that the major contributing factors for mortality in neonates were infections and preterm with associated complications in developing countries [2].

Fortunately, if discovered quickly and treated with the right medications, neonatal sepsis is curable. However, early sepsis diagnosis is still difficult since the early symptoms of neonatal sepsis can be mistaken for those of non-infectious causes. Additionally, starting antibiotics before a sepsis diagnosis may lead to an unneeded and protracted antibiotic exposure. This raises the possibility of adverse antibiotic reactions as well as the development of drug-resistant

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bacteria. Neonatal sepsis can develop quickly or slowly. Respiratory distress and pneumonia are the typical first symptoms of early-onset sepsis, which most likely has perinatal risk factors as its cause. After 72 hours of age, septicaemia and pneumonia are typically the first symptoms of late-onset sepsis, which is linked to hospital acquired infections.

Positive blood culture results are a definitive diagnosis; but the procedure is time consuming [3]. Only 30-75 percent of cases have a reported culture positivity rate, and a well-equipped laboratory setting is necessary [4].

Among inflammatory markers, CRP is a preferable screening tool in initial assessment of neonatal sepsis. Moreover, CRP has better screening validity in neonatal sepsis and considered as preferable method to screen a new-born for possible neonatal sepsis[5]. Other tests such as procalcitonin and IL-6 are very expensive and not easily affordable by most of the patients in developing countries.

Sepsis is a non-specific inflammatory defence mechanism and is considered a generalised process where every organ and system can be involved. The haemostatic system is frequently disturbed during sepsis.

One of the most common and independent risk factors for sepsis-associated death in newborn sepsis is thrombocytopenia [6]. An increased destruction and inadequate production of platelets during sepsis induced thrombocytopenia led to release of young platelets in circulation. Young platelets are larger than old platelets and platelet activation changes its shape to discoid with pseudopodia[7].Mean platelet volume (MPV) and platelet distribution width (PDW) can help to differentiate consumptive from hypoplastic thrombocytopenia. MPV is a measurement of average size of platelets found in blood. This value normally has inverse relationship to platelet count and increases as more young platelets are present in the circulation due to increased destruction of platelets. PDW is an indicator of variation in platelet size. Platelet count (PC), mean platelet volume (MVP) and platelet distribution width (PDW), easily available in coulter alongwith complete blood count, are widely and routinely used in clinical practice across the globe. Easily accessible, inexpensive, and widely used laboratory tests that show the severity of sepsis are important. The role of platelet indices in sepsis has been reported in adult studies. Therefore, the aim of the present study is to evaluate the platelet count and platelet indices in neonatal sepsis and to determine its correlation with C-reactive protein.

Method

The study was conducted at Gauhati Medical College and Hospital, Guwahati in NICU, department of Paediatrics, from1st June 2021 to 31st May 2022.It was a hospital based prospective study. It is a one-year study involving all the neonates admitted with clinical features or risk factors suggestive of sepsis, admitted to the neonatal intensive care unit (NICU), Gauhati Medical College and Hospital, Guwahati, Assam.

Inclusion criteria: All neonates (< 28 days) presenting with symptoms and signs of sepsis like poor feeding, lethargy, tachypnoea, hypothermia , convulsion , etc were included in the study.

Exclusion criteria: 1) All newborns with neonatal hyperbilirubinemia due to causes other than sepsis like physiological jaundice, Rh, ABO incompatibility, TTNB, MAS without clinical or laboratory suspicion of sepsis were excluded from the study. 2) Mother and neonate with haematological disorder. 3) Neonate with major congenital anomalies.

Definitions and nomenclature of neonatal sepsis:

1. Probable sepsis: When clinical and laboratory findings consistent with bacterial infection without a positive culture.

2. Clinical sepsis: When the screen and blood/ cerebrospinal fluid (CSF) culture is negative, but there is a suggestive history with a high clinical suspicion.

3. Culture proven or definitive sepsis (Culture positive sepsis): In an infant who has clinical features suggestive of septicaemia, pneumonia or meningitis and there is isolation of organisms from blood, CSF, urine, or abscess cavity.

4. Neonatal sepsis: Best defined as presence of systemic features associated with pure growth of bacteria from one or more sites.

5. At risk (ruling out sepsis): Often we “suspect” sepsis based on risk factors and clinical features, but the clinical course (rapid recovery within few hours) and “screening tests” are not suggestive; we should not label these as “suspected sepsis”. They are more like “rule out sepsis”.

Definition of intra-amniotic infection or inflammation or both (Triple I)

• Maternal fever: Maternal oral temperature ≥39ºC on any one occasion. If oral temperature is 38ºC (102ºF), repeat after 30 minutes, if repeat value remains at least 38ºC (100.4ºF), it is defined as fever.

• Suspected triple I: Fever without a clear source plus any of: ▪ Baseline fetal tachycardia (>160/min for > 10 minutes),

▪ Maternal TLC >15000/cmm in absence of corticosteroids ▪ Definite purulent fluid from cervical os.

• Confirmed triple I: All the above plus laboratory findings such as – ▪ Positive amniotic fluid gram stain/culture, ▪ Histopathological evidence of infection or inflammation or both in placenta.

Table 1: The parameters of sepsis screen

Parameter	Abnormal value
➢ Total leukocyte count	20,000/mm³ Low
➢ Absolute neutrophil count	Counts as per Monroe chart for term infants and Mouzinho chart for VLBW babies.
➢ Immature or band cells to total neutrophil ratio.	>0.2
➢ Micro -ESR	>15 mm 1st hour
➢ C-Reactive protein	>1mg/dl

The sepsis screen: Any 2 parameters positive is considered positive sepsis screen

All the neonates were admitted in NICU, Gauhati Medical College and Hospital, Guwahati with clinical suspicion of sepsis. The clinical features and risk factors of the neonates fulfilled the National Neonatology Forum, India guidelines. Ethical clearance was taken from the institutional ethical committee of Gauhati Medical College and Hospital before the start of study.

In the selected neonates, detailed birth history (birth weight, gestation age, mode of delivery etc.) and maternal history along with clinical features (respiratory rate, refusal to feed etc.) and investigation results (WBC count, neutrophil count, platelet count and indices, blood culture report, serum CRP etc.) were recorded in a predesigned proforma.

Sepsis screen was sent along with platelet count and its indices-MPV, PDW and PCT, C-reactive protein (CRP) and blood culture for all the patients. SYSMEX-XN 1000 (BECKMAN COULTER principle) analyzer, Vitros 5600 were used for complete hemogram and platelet indices and CRP respectively and as when required X-ray chest, urine C/S, and CSF culture was done.

Statistical analysis: Data were collected from the patient records and compiled in MS Excel. Data were analysed using arithmetic mean, p-value and also expressed in percentages. Percentage is used to compare one quantity against another.

Results

There are a total of 48 preterm and 46 term culture positive neonates in the present study. A slightly higher number of preterm neonates (51%) are present in this study (table 2).

In the present study, out of 94 culture positive neonates, 34 cases are with normal birth weight (>2.5kg) and 60 cases are with birth weight (table 3).

Table 2: Distribution of cases according to age of gestation

Gestational age	No. of neonates	Total
Preterm	48(51%)	94
Term	46(48.9%)	

Table 3 : Distribution according to birth weight of neonates

Birth Weight(kg)	No. of neonates		Total
Normal (>2.5kg)	34(36.1%)		
LBW (1.5-2.5kg)	27	60(63.8%)	94
VLBW(1-1.5kg)	24		
ELBW(<1kg)	7		

Table 4: Thrombocytopenia in neonates

Platelet count	Number	Percentage(%)
Normal	23	24.4
Thrombocytopenia	71	75.5

Table 5: Grading thrombocytopenia in total cases

Thrombocytopenia (Cumm)	Number	Percentage
Mild (100-150 x 10^3)	28	39.4
Moderate (50-100 x 10^3)	32	45
Severe (<50 $X10^3$)	11	15.4
Total	71	100

Table 6: Distribution of cases according to raised reactive protein (CRP)

CRP	No. of neonates	Percentage(%)
Normal	12	13
Increased	82	87
Total	94	100

Table 7: Correlation of c-reactive protein(CRP) with thrombocytopenia and platelet indices(PDW, MPV, Pct)

Platelet indices	Mean value	Mean value of CRP	P-value
Thrombocytopenia	115.5±55.37	26.99±16.31	0.000133
Mean platelet volume (MPV)	13.94±3.24		0.023822
Platelet distribution width(PDW)	3.4±3.22		0.011713
Plateletcrit (PCT)	0.21±0.14		0.5092

There were 71 cases of culture proven sepsis with platelet count below 150 $x10^3$cumm and 23 cases with normal platelet count (table 4). Out of total 94 culture positive cases, 71 (75.3%) cases have thrombocytopenia. 28 (39.4%) cases have mild thrombocytopenia, 32(45%) moderate and 11(15.4%) with severe thrombocytopenia out of 71 total thrombocytopenic neonates (table 5).Out of total 94 culture proven sepsis cases; CRP is raised in 82 cases (table 6). From the study, the mean value of CRP is calculated to be 26.99±16.31. A significant correlation between CRP with thrombocytopenia and platelet indices- MPV and PDW however PCT is not significantly correlated (table 7). In cases of gram-negative sepsis, 29 cases have thrombocytopenia, 17 cases have high PDW, 29 cases have raised MPV and 9 have raised PCT. In cases of gram-

positive sepsis, 4 cases have thrombocytopenia, 3 cases have raised PDW, and 10 cases have raised MPV. In cases of fungal sepsis, 22 cases have thrombocytopenia, 11 have raised PDW, 23 have raised MPV and 14 cases have raised plateletcrit.

Discussion

Neonatal sepsis is a clinical phenomenon characterised by infection related signs and symptoms in the first month of life, either with or without bacteraemia. Neonatal sepsis with its high mortality and morbidity remains a diagnostic and treatment challenge to the health care providers despite the use of higher antibiotics and advanced supportive care. The institution of therapy at the earliest is aided by an early and prompt diagnosis and thus also prevents the unnecessary use of antibiotics thereby keeping the emergence of drug resistance in check.

Blood culture provides the conclusive diagnosis of sepsis, which requires at least 48 to 72 hours, with a 30 to 40% success rate of circumstances. Thrombocytopenia is a frequent complication of various diseases that affect critically sick individuals and is linked to a higher death rate. It is also a frequent symptom of neonatal sepsis. A study by Mannan et al showed that 50% cases of neonatal sepsis had thrombocytopenia [8]. In the present study, 75.5% of cases of culture proven sepsis have thrombocytopenia with 39.4% neonates with mild, 45% moderate and 15.5% severe thrombocytopenia. In the present study, gram negative (52.7%) and fungal sepsis (22%) had higher incidence of thrombocytopenia which was comparable to studies by Guida et al[9] and Ree et al[5].

The gestational age distribution in the present study constitutes more preterm cases (51%) than term which is comparable to studies done by Khatua et al[10] and Joshi et al[11] where most cases were preterm with 63% and 52.6% respectively. Preterm neonates are at higher risk for sepsis or infection than term neonates. The increased susceptibility for infection is mainly due to: 1) Immune system deficit, mostly caused by reduced IgG antibodies and ineffective opsonization and complement activation; 2) Poor mucosal defences and low storage reserve of neutrophil; 3) The increased need for invasive devices (vascular access, endotracheal tube, feeding tubes, exchange blood transfusion and urinary tract catheters) due to associated severe illness. While in term babies, sepsis can be due to presence of predisposing risk factors like intra uterine growth retardation, birth asphyxia, prolonged rupture of membranes etc.In the present study, higher incidence of culture proven cases in low birth weight i.e., weight <2.5kg which is comparable to studies done by Joshi et al[11] (50.4%), I Roy et al[3] (63.8%) and Bangi et al[12] (60.83%).

Madani S et al [13] and Priti Singh et al[14] showed that increase in platelet indices in septic neonates with thrombocytopenia. In the present study out 94 culture positive cases, 65.9 % have high MPV, 33 % with increased PDW and 30.8% with high PCT. Out of 71 thrombocytopenic neonates with culture proven sepsis,67.6 % (p value 0.000023) have high MPV, 41 % (p - value 0.00003) with high PDW and 35 % (p -value 0.982447) with high PCT. It is similar to the study by Bhat et al[15], Sartaj Naik et al[16].

MPV reflects the size of platelets. Young platelets are larger than old platelets. An increased number of young platelets indicate increased platelet production due to overconsumption increased by inflammation. The MPV of thrombocytopenic neonates was significantly higher than that of non-thrombocytopenic neonates ($p < 0.01$) however other indices are less studied. Bhat et al[15] had reported higher MPV in gram negative and fungal sepsis than gram positive sepsis. In the present study, number of culture positive neonates with high MPV is 10.6% in gram positive sepsis cases, 30.8 % gram negative and 24.5% in fungal sepsis cases.

C- reactive protein acts as a scavenger causing opsonisation of bacteria and activation of the complement system thereby facilitating phagocytosis during inflammatory response. The aberrant - globulin CRP is a quickly responding acute phase reactant that the liver produces within 4-6 hours of an inflammatory stimulus, peaks at 24-48 hours, and then decreases over time as the inflammation subsides. In the present study, 64 (78 %) neonates with high CRP had thrombocytopenia (p value 0.000133) is similar to the study done by Rabindran et al[17]. In their study, it was also found that thrombocytopenia and increased CRP were more with fungal sepsis. In this study, increased CRP was more with gram negative sepsis (41.7%) followed by fungal (30.3%) and then gram-positive sepsis (27.8%). Catal et al[6] found a statistical correlation of MPV and CRP in neonatal sepsis. In the present study, 55 culture positive neonates have high MPV with high CRP (p-value 0.023822). Further, in this study, 29 (p-value 0.011713) neonates with high CRP had high PDW and 25 (p-value 0.50592) with high PCT.

Limitations in clinical utility of platelet indices - Concerns are raised about the recommended anticoagulant

for platelet counting, K2 or K3 EDTA, because it affects MPV. ACD/Na2EDTA has been suggested as a perfect anticoagulant for the study of MPV because it inhibits platelet activation while maintaining the platelets in their normal discoid shape. An overestimation of MPV, a better PDW and a rise in fraction of large cells may occur if red blood cells are misclassified as platelets. In severe thrombocytopenia, difficulties in obtaining a sufficient platelet distribution curve may limit the calculation of other platelet indices.

Conclusion

Blood culture is the gold standard for diagnosis of neonatal sepsis but in neonates with signs and symptoms of sepsis along with risk factors, whose culture reports are awaited, can be started on antibiotics if both platelet indices and serum CRP levels are high. This is to avoid delay in instituting therapy, thus reducing the mortality and morbidity rates. This is to avoid delay in instituting therapy, thus reducing the mortality and morbidity rates.

Conflict of interest: None. **Disclaimer:** Nil.

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