Original Article *Iran J Ped Hematol Oncol. 2022, Vol 12, No 3, 174-181*

Volume, Conductivity and Scatter parameters of Neutrophils in Neonatal Sepsis – Is it a Cost-Effective Tool?

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Received: 04 July 2021 **Accepted:** 17 June 2022

Abstract

Background: Early diagnosis of neonatal sepsis is quite challenging. I/T ratio (immature to mature neutrophil ratio) is a highly sensitive marker of sepsis but is time-consuming and subjective. Off late, volume conductivity and scatter (VCS) of neutrophils are among the newer parameters available for screening a septic neonate. This study aimed to determine the correlation between the I/T ratio and VCS parameters in neonatal sepsis and estimate a cut-off value of VCS parameters to diagnose neonatal sepsis using receiver operating curve analysis.

Materials and Methods: This prospective observational study was conducted by the Department of Pathology and Neonatology from April 2019 to March 2020 in a tertiary care center. A total of 110 newborns were included in this study and were divided into two groups (probable sepsis and sepsis). Data were collected from the hospital database, and analysis was done using SPSS software. Correlation between the I/T ratio and VCS parameters was done using Spearman's correlation. Results were expressed as mean ± standard deviation (SD). A P-value of <0.05 was considered statistically significant.

Results: Correlation between the I /T ratio and VCS parameters showed negative correlation values of -0.22, -0.23, and +0.39 (P-values of 0.0198, 0.0153, and <0.0001) for mean neutrophil conductivity (MNC), mean neutrophil scatter (MNS) and mean neutrophil volume (MNV) respectively. MNV with a cut-off of > 156.4 had sensitivity and a negative predictive value of 100 % in the diagnosis of sepsis and was found to be higher in the sepsis group when compared to the probable sepsis group.

Conclusion: Based on the significant difference in VCS parameters of neutrophils in the sepsis group, this aids as an additional marker for the early diagnosis of neonatal sepsis.

Keywords: Neonate, Neutrophils, Sepsis

Introduction

Neonatal sepsis is defined as "A clinical syndrome characterized by signs and symptoms of infection with accompanying bacteremia in the first month of life." It is classified into Early Onset Sepsis (EOS) or Late-Onset Sepsis (LOS) based on the day of onset of symptoms. It includes neonatal septicemia, meningitis, pneumonia, osteomyelitis, and urinary tract infections (1). According to World Health Organization, of the 4 million neonatal deaths worldwide, severe sepsis/pneumonia accounts for about one-third. Though there are continuous advances in neonatal care, early-onset neonatal sepsis remains a life-threatening disease (2). The incidence of neonatal sepsis in India is quite high and accounts for 17,000 / 1,00,000 live births (3), and in Asia, it accounts for about 7.1 to 38 per 1000 live births (4). Diagnosis of neonatal sepsis can be made clinically and/or microbiologically by positive blood / cerebrospinal fluid (CSF) cultures. Apart from imaging modalities, the laboratory investigations done in suspected cases of neonatal sepsis screening are complete blood count, blood culture, urine culture, and CSF analysis (5). Non-specific clinical presentation makes diagnosis difficult at times. The key factors in a case of suspected neonatal sepsis are early diagnosis, and prompt treatment, a delay in diagnosis leads to a fatal outcome, and

[DOI: 10.18502/ijpho.v12i3.10060]

inappropriate use of antibiotics. Immature to mature neutrophil ratio (I/T ratio) is calculated from the peripheral smear examination of neutrophils and their precursors and is a highly sensitive indicator of sepsis. Despite its advantage, its utility is limited by Intra / interobserver variations and constraints of time (6). Beckman Coulter hematology analyzers (such as LH series) have specialized parameters based on Volume, Conductivity, and Scatter (VCS). These parameters are helpful in the diagnosis of sepsis in adults. However, literature on the utility of these parameters in neonatal sepsis is limited (7). As a protocol, the neonatologists of our hospital order for Complete Blood Count (CBC), peripheral smear (PS), and I/T ratio in all neonates suspected to have sepsis. VCS data can be obtained from a regular CBC run itself.

Materials and Methods

The present study tried to find a correlation between the I/T ratio and VCS parameters in neonatal sepsis and a cut-off value of VCS parameters of neutrophils to diagnose Neonatal sepsis using the Receiver operating curve (ROC) analysis.

This prospective observational study was conducted by the Department of Pathology and Neonatology from April 2019 to March 2020 in a tertiary care center in South India. Neonates with significant congenital anomalies, a recent history of blood transfusion, and suspected congenital immunodeficiency were excluded from the study. As this study utilizes the sample which was sent as part of routine investigations, informed consent was not obtained. All samples were taken at the time of admission. The newborns were divided into two groups based on their clinical history and culture reports; newborns with a positive culture and clinical sepsis with a negative culture report (probable sepsis). The following data was collected from the hospital information system: Baseline data (Sex, Birth weight, and Gestational age), A Pathologist calculates I/T Ratio, Level of C - reactive protein (CRP) - by Immunoturbidometric method, Blood culture report (BACTEC method). Complete blood count and VCS parameters (Coulter LH780 Hematology Analyzer). Total leukocyte count (TLC) and absolute neutrophil count (ANC) were obtained during cell passage through the aperture and measured by a coulter LH780 analyzer. The VCS technology of the Coulter cell analysis system (9) can obtain data using direct current impedance to measure cell volume (V), radiofrequency opacity to characterize Conductivity (C) for the internal composition of each cell, and a laser beam to measure light Scatter (S) for cytoplasmic granularity and nuclear structure. VCS parameters of neutrophils were obtained. Such parameters reflected the Mean Neutrophil Volume (MNV), Mean Neutrophil Conductivity (MNC), and Mean Neutrophil Scatter (MNS).

Sample size calculation

The formula is n = (p (100-p) Z2) / E2; where n is the sample size required, p is the percentage occurrence (prevalence), E is the percentage maximum error required, and Z is the value corresponding to the level of confidence required. Assuming the prevalence of neonatal sepsis in India (8) to be 19%, at 95% confidence level and 10% maximum error, the sample size is given by, n = (19 x (100-19) x 1.962) /102 = 59. Hence, the minimum sample size required is 59. As the sample size increases, the accuracy of results also increases. Thus, the sample size is taken to be 110, and these are the cases with suspicion of clinical sepsis.

Ethical Consideration

After obtaining Institutional Ethics committee approval (PSG/ IHEC / 2020 / appr/exp/039), newborns admitted to the Neonatal unit in this study period with suspected sepsis were enrolled.

[DOI: 10.18502/ijpho.v12i3.10060]

Statistical analysis

Data were entered in a Microsoft Excel spreadsheet, and analysis was done using SPSS software by Microsoft V.22.0. Correlation between the I/T ratio and VCS parameters was done using Spearman's rank correlation (non-parametric test). The parameters were compared between the different groups using analysis of variance (ANOVA). Results were expressed as mean ± standard deviation. A P-value of <0.05 was considered statistically significant. Receiver operator characteristic (ROC) curves tested the optimum cut-off value for each variable. The sensitivity, specificity, and area under the ROC curve were then assessed between the sepsis and probable sepsis group. The area under the curve (AUC) measures the overall performance of the test. The test with an AUC value closer to 1 is accurate, and the practical lower limit in the diagnostic test is 0.5

Results

A total of 110 neonates were included in the study. Out of which 100 were in culture-negative (probable sepsis), and ten were in the culture-positive sepsis group. The most common pathogen isolated was Klebsiella pneumonia. Data on gestational age and birth weight distribution; Sepsis screen parameters such as TLC, ANC, and I/T ratio were compared between sepsis and probable sepsis groups and are shown in Table I. I/T ratio value of more than 20 % were considered significant. CRP value of more than 0.2 mg/dl was considered significant (10). The TLC and I/T ratio parameters showed a statistically significant difference between the two groups ($p<0.05$). Among the VCS parameters of neutrophils, MNV is highest in the positive culture group compared to the probable sepsis group. At the same time, MNC & MNS were lower in the culture-positive sepsis group. All three parameters had statistical significance ($p<0.05$) and are illustrated in Table II. The sensitivity and specificity of these parameters were analyzed using ROC curve analysis and shown in table III, figure 1, and figure 2. Using ≥ 0.20 as the CRP cut-off, the sensitivity was 100 %, and specificity was 46 %, with AUC 0.815. (Figure 3). Spearman correlation is a statistical measure used to show the strength of the relationship between two variables. The value of the coefficient ranges from -1 to +1, with 1 and -1 being positive and negative correlations, respectively. Correlation between the I /T ratio and VCS parameters in the present study shows correlation values of -0.22, -0.23, and +0.39 (P-values of 0.0198, 0.0153, and <0.0001) for MNC, MNS, and MNV respectively. Our results showed no association between the I /T ratio and VCS parameters. In an attempt to improve the diagnostic accuracy of the VCS parameters by combining them, the following were observed: MNV with MNS showed the highest specificity and NPV (table IV).

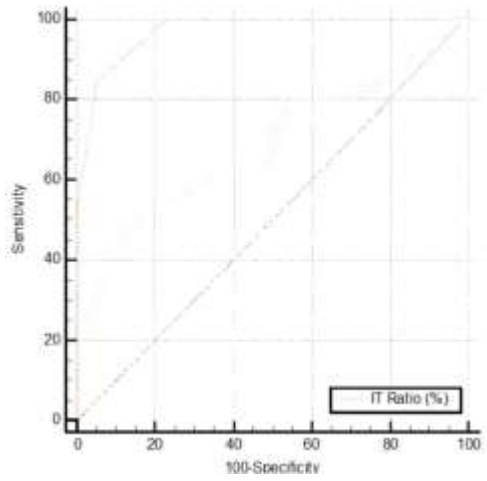

Figure 1. ROC curve analysis of I/T ratio

[DOI: 10.18502/ijpho.v12i3.10060]

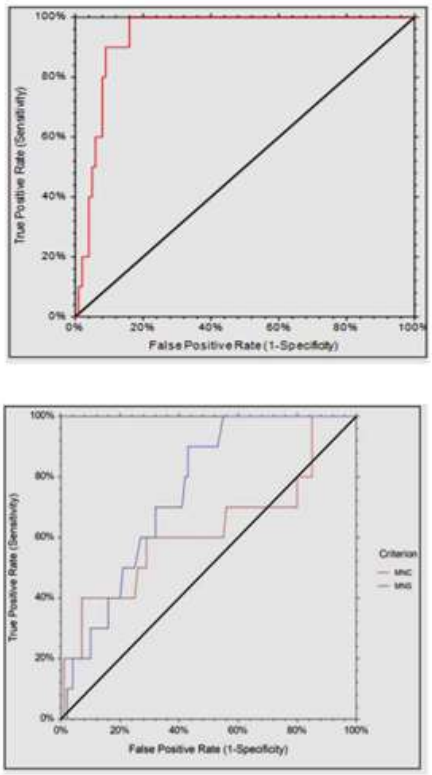

Figure 2: ROC curve analysis of MNV, MNC & MNS

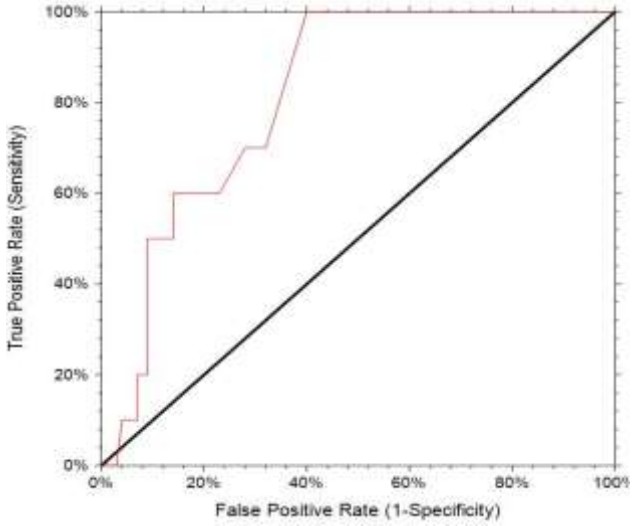

Figure 3: ROC curve analysis of CRP

Table I: Demographic profile and sepsis screen parameters of the study population

	Culture positive Sepsis (mean ± SD)	Probable Sepsis (mean ± SD)	P-value
Birth weight (kg)	2.2±0.84	2.47±0.69	0.2499
Gestational age (weeks)	32.5±3.9	35.53±3.3	0.0075
TLC (x10^3/ µl)	9.06±7.17	16.86±6.9	0.001
ANC (x10^3/ µl)	6.15±4.27	11.04±5.8	0.0109
I/T ratio	0.169±0.04	0.051±0.03	<0.0001
CRP (mg/dl)	1.91±1.9	0.84±1.9	0.0924

TLC =Total leucocyte count; ANC =Absolute neutrophil count; I/T ratio = Immature to mature neutrophil ratio; CRP = C-reactive protein; SD = standard deviation.

Table II: Results of VCS parameters of the study population

	Culture positive sepsis (mean ± SD)	Probable sepsis (mean ± SD)	Total (mean ± SD)	P-value
MNV	166.46 ± 9.33	148.1±10.64	149.78±11.75	<0.0001
MNC	143.16 ± 8.94	147.2±5.79	146.84±6.20	0.0489
MNS	126.38 ± 5.74	133.9±8.73	133.21±8.75	0.009

MNV= mean neutrophil volume; MNC = mean neutrophil conductivity; MNS = mean neutrophil scatter; SD = standard deviation.

[DOI: 10.18502/ijpho.v12i3.10060]

Table III: ROC analysis of VCS and sepsis screen parameters

	AUC	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
I/T ratio	0.9775	≥ 0.11	100	94	63	100
MNV	0.9370	≥ 156.4	100	66	38	100
MNC	0.6240	≤ 151.60	80	20	9.1	90.9
MNS	0.7510	≤ 131.10	90	57	17.3	98.3
CRP (mg/dl)	0.815	≥ 0.20	100	46	15	100

I/T ratio = Immature to mature neutrophil ratio; MNV= mean neutrophil volume; MNC = mean neutrophil conductivity; MNS = mean neutrophil scatter; CRP = C- reactive protein; AUC = area under curve; PPV = positive predictive value, NPV = negative predictive value.

Table IV: Combination of VCS parameters

	Cut-off values	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MNV+MNC	≥ 156.4, ≤ 151.60	50	91	34.78	97.7
MNV+MNS	≥ 156.4, ≤ 131.10	80	92	52.9	98.9
MNC+MNS	≤ 151.60, ≤ 131.10	50	82	14.6	95.2
MNV+MNC+MNS	≥ 156.4, ≤ 151.60, ≤ 131.10	50	95	46.7	96.8

MNV= mean neutrophil volume; MNC = mean neutrophil conductivity; MNS = mean neutrophil scatter; PPV = positive predictive value, NPV= negative predictive value.

Table V: Comparison of studies on VCS parameters.

Author	Year of study	MNV- cut-off value	Sensitivity (%)	Specificity (%)
Bhargava et al (16)	2014	>154.2	95%	82%
Abiramalatha et al (17)	2016	>151	71%	71%
Kelkar et al (18)	2017	143.5	90%	43%
Jacob et al (19)	2018	>152.8	96.9%	100%
Nesargai et al (20)	2020	>150.2	79.1%	95%
Present study	2021	≥ 156.4	100 %	66%

MNV= mean neutrophil volume.

Discussion

Early detection of neonatal sepsis is important as it prevents mortality and morbidity. The gold standard for diagnosing sepsis is blood culture. The changes in hematological parameters are leukocytopenia/leucocytosis, neutropenia, increased immature neutrophils in circulating blood, and cytotoxic changes in neutrophils and monocytes. Among biochemical parameters, an increase in CRP, fibrinogen, and haptoglobin and a decrease in pre-albumin and transferrin were observed (11). VCS parameters of neutrophils are automatically derived from the hematology analyzer, reflecting the morphological changes of the leucocytes. Earlier studies on the utility of VCS parameters in diagnosing sepsis were done by combining both the biochemical markers and the VCS parameters. The present study employs a combination of VCS parameters to obtain more accuracy and early diagnosis. It is the first time an investigation has been designed and done on this method. The role of the I/T ratio as a reliable marker in neonatal sepsis was accepted in many studies. I/T ratio, which is manually calculated, has high sensitivity and specificity (11) compared to the other

[DOI: 10.18502/ijpho.v12i3.10060]

parameters, but it requires a deck expert and might have subjective variations. Studies done by Celik et al. (10), and Raimondi et al. (13) observed that an I/T ratio with a cut-off of 0.20 showed a high NPV of 88.9% and 87.4%, respectively, in proven sepsis. This study also showed similar results with a high NPV of 84.8 % with a cut-off of 0.20 and 100% with a 0.11 cut-off value in proven sepsis. Thus, the I/T ratio is a reliable marker for the diagnosis of sepsis, especially in a resource-limited setting. Among the inflammatory markers, CRP was the most widely used parameter for diagnosing sepsis. Other markers are procalcitonin and Interleukin -6 (IL6). There have been studies analyzing the sensitivity and specificity of CRP and found to be 35-94% and 60-96%, respectively (14). The present study showed a sensitivity of 100% and specificity of 45%, with a cut-off of more than 0.20 mg/dl. The Coulter LH 780 hematology analyzer computes almost all of the hematological parameters, and its role in identifying sepsis in the adult population has been established. It indirectly represents the morphological changes in leukocytes during infection as indicated by changes in VCS parameters. Chaves et al. analyzed the VCS parameters in adult patients with proven sepsis. They observed higher MNV and lowered MNS in the sepsis group than in the control group, similar to the present study (15). This high MNV value in cases of sepsis could probably be explained by an increase in the number of immature neutrophils, which have large size nuclei and decreased granular cytoplasm. The comparison of the present study with other studies on neonatal sepsis from India published so far was highlighted in table V. Most of the studies analyzed the MNV parameter alone to diagnose neonatal sepsis. This is the first study that combined these parameters to yield more sensitivity and specificity. The MNV and MNS yielded the best results with a sensitivity and specificity of 80% and 92%, respectively. There were studies analyzing the combination of septic screen parameters with inflammatory markers in the diagnosis of early neonatal sepsis. They have stated that instead of using the single marker, a combination of markers and serial monitoring is warranted (10,20). This study shows the significant difference in TLC, ANC, and I/T ratio between the two groups. The small number of culture-positive cases is due to rigorous infection control protocols followed in our NICU. A large study was done by Celik et al. from Turkey (10) using both leukocyte parameters of neutrophils and monocytes against inflammatory markers such as CRP, procalcitonin, and interleukin (IL)-6 in the diagnosis of neonatal sepsis. They found that MNV seems to be the most helpful marker with the highest specificity, and MNC, MNS did not show a significant difference between sepsis and the control group. In contrast, this study showed a statistically significant difference between the two groups ($p < 0.005$). In Bhargava et al. (16) study, combined MNV+CRP showed 100% sensitivity and 85% specificity in predicting neonatal sepsis. In another study by Raimondi et al. (13), MNV+ CRP had 95% sensitivity and 97% specificity for diagnosing late-onset sepsis in very low birth weight infants. Compared to neutrophil parameters, the I/T ratio was the most potent predictor of sepsis. Therefore, the diagnostic value of neutrophil parameters is also essential in screening sepsis in conjunction with the I/T ratio. The current study infers no correlation between the I/T ratio and VCS parameters of neutrophils. A combination of septic screen parameters with Leukocyte parameters aids in the early diagnosis of sepsis without any additional cost, especially in resource-limited settings is suggested.

Conclusion

Considering the significant difference in VCS parameters of neutrophils in the sepsis group, this can be used as an

[DOI: 10.18502/ijpho.v12i3.10060]

additional marker for the early diagnosis of neonatal sepsis. These are easily obtained parameters from a hematology analyzer, and a combination of these parameters could increase the sensitivity and specificity in diagnosing sepsis. This simple and cost-effective tool may help clinicians identify newborn sepsis and decide on treatment. Further large-scale studies which include a healthy group for comparison are warranted to validate the VCS parameters.

Conflict of interest

The authors declare no conflict of interest.

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[DOI: 10.18502/ijpho.v12i3.10060]

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[DOI: 10.18502/ijpho.v12i3.10060]