International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

A Study of Neutrophil Volume, Conductance and Scattergram Parameters in early detection of Neonatal Sepsis

Dr. Vijay Kapse[1], Dr. Jayanti Chandrakar[2], Dr. Renuka Gahine[3], Dr. Girish Suman[4]

[1]Associate Professor Department of Pathology Pt. Jawaharlal Nehru Memorial Medical College, Raipur (C.G.) India, 492001

[2]Associate Professor Department of Pathology, Pt. Jawaharlal Nehru Memorial Medical College, Raipur (C.G.) India, 492001

[3]Director Professor and Head of the Department Pathology , Pt. Jawaharlal Nehru Memorial Medical College, Raipur (C.G.) India, 492001

[4]PG, Resident Department of Pathology, Pt. Jawaharlal Nehru Memorial Medical College, Raipur (C.G.) India, 492001

Abstract: ***Background**: Neonatal sepsis remains one of the main causes of mortality and morbidity despite the progress in hygiene, introduction of new and potent antimicrobial agents for treatment, and advanced measures for diagnosis .It is responsible for 30- 50% of the total neonatal deaths in developing countries. It is extremely important to make an early and accurate identification of neonatal sepsis for prompt antimicrobial therapy and better outcomes. **Materials and method**: A total Number of 350 neonates with clinical suspicion of septicemia were selected. Blood samples were collected within 24 hours of patient's admission and before the initiation of antibiotic therapy. Blood samples were tested to obtain culture and haematological results. **Results**: Out of 350 neonates we found 184 culture positive and 166 culture negative patients. Klebsiella pneumonae was the most common micro-organism inoculated. We observed significant increase of MNV and significant decrease of MNS in culture positive patient than culture negative group. However the changes in MNC were not significant. **Conclusion**: There are several markers to diagnose neonatal sepsis still the researchers are searching for the ideal and accurate marker. We suggest that neutrophil VCS parameters are useful for early diagnosis of neonatal sepsis.*

Keywords: Neonates, Septicaemia, Neutrophil Volume, Conductance, Scattergram, Culture, Microorganism, Haematological parameters

1. Introduction

Neonatal sepsis is a clinical syndrome of bacterial infection characterized by signs and symptoms of systemic involvement during first month of life. As per National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live birth. It is estimated that up to 20% of the neonates develop sepsis and approximately 1% die of sepsis related causes**.** [1] The clinical course of neonatal sepsis can be fulminant within hours of onset so early diagnosis and treatment of neonatal sepsis may help to decrease neonatal mortality. Thus, it is extremely important to make an early and accurate identification of neonatal sepsis for prompt antimicrobial therapy and better outcomes. [2]

The clinical diagnosis of neonatal sepsis is difficult because the signs and symptoms are not always specific. There is no laboratory test with 100% sensitivity and specificity.[3] Laboratory studies used to evaluate for early -onset and late-onset sepsis include a complete blood count (CBC), differential blood count and cerebrospinal fluid (CSF) cultures, and measurement of C-reactive protein (CRP) and possibly other infection markers. Review of peripheral blood smears can also yield important diagnostic information through the identification of the morphologic changes characteristically seen in reactive neutrophils during infection.[4],[5],[6] Such changes include the presence of toxic granulation, toxic vacuolization, and Döhle bodies in the cytoplasm. Younger forms (left shift), such as band cells and metamyelocytes, also can be identified.[7] However, this approach is not only labour-intensive and time-consuming, but also requires the technical expertise of a haematologist to identify correctly these changes.[9]With the introduction of newer automated analysers for determining haemogram, technology has a new role to play. The volume, conductivity and scatter (VCS) technology of the Coulter LH 750 haematology analyser (Beckman Coulter, Fullerton, CA, USA) can obtain data from more than 8000 white blood cells (WBCs) in a few seconds, using impedance to measure cell volume (V) for accurate size of all cell types, radio frequency opacity to characterize conductivity (C) for internal composition of each cell, and a laser beam to measure light scatter (S) for cytoplasmic granularity and nuclear structure. The data from VCS technology can thus be a comparable reflection of cell morphology [9] [10] Hence, we planned present study to identify cost-effective tests for the diagnosis of early neonatal sepsis; it could bring down the morbidity and mortality rate substantially. So that prompt treatment can be initiated and neonatal deaths can be minimized.

Aim

To determine any difference in mean neutrophil volume, conductance and scatter gram in patients diagnosed with neonatal sepsis by blood culture positive with those of blood culture negative.

Objectives

- To Investigate the Value of Volume, Conductance and Scatter gram Parameters in the Diagnosis of Neonatal Sepsis.

- To determine the most appropriate cut-off value of each parameter in neonatal sepsis.
- To determine the diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of VCS parameters.

Sample Size

Study size n is calculated using formula n=4pq/E2. Where, p is the prevalence of neonatal septicaemia, q is 1-p and E is allowable error taken as 5%. Based on prevalence of 30% obtained from previous studies sample size is worked out to be 336, but we have taken the sample size350 for convenience of statistical analysis.

2. Materials and Methods

A total Number of 350 neonates with clinical suspicion of septicemia were selected. This prospective study was conducted in the department of Pathology and department of Pediatrics in Pt. Jawaharlal Nehru Memorial Medical College and associated Dr. B.R.A.M. Hospital Raipur. Septicemia was suspected from clinical history, signs, symptoms, and presence of predisposing factors in mothers and neonates. The patient's medical records were reviewed for clinical correlation. Before collection of blood, the puncture site and adjacent area were sterilized by swabbing with spirit. Blood samples were collected within 24 hours of patient's admission and before the initiation of antibiotic therapy. Blood was collected by vein puncture and transferred into culture broth as well as into EDTA vial for the different hematological tests. Venous blood was used to estimate hemoglobin (Hb), total leukocyte count, differential leukocyte count, platelet count and the VCS parameters of neutrophils. The culture reports were correlated with the hematological parameters.

3. Statistical Analysis

Sensitivity, specificity, positive and Negative predictive values (NPVs) were calculated. All analyses were performed using IBM SPSS 20 (Statistical Package for the Sciences, SPSS Inc., Chicago, IL, USA).

Results were expressed as the mean ± SD.

Comparisons between means were performed
By analysis of variance. Comparison between 2
Means was performed

Table 1: Group distribution of study population by Sepsis

S.N.	Group	Number of Cases	Percentage (%)
1	Proven sepsis	184	52.57%
2	Negative for sepsis	166	47.43%
Total		350	

Mean VCS Parameters

Above table shows out of 350 cases, 184 cases were positive for blood culture (Proven sepsis) and 166 cases were culture negative. We considered blood culture positive as case group and blood culture negative as control group. In sepsis group majority of the patient were in early onset sepsis group 72.28%, late onset sepsis group make up the rest 27.72%. The most frequent micro-organism which was inoculated from blood culture of our study population is Klebsiella Pneumonae (35.33%) followed by Coagulase negative staphylococcus (27.17%).

Neutrophils plays major role in immune response to acute bacterial infections, we focused mainly on the VCS parameter changes in reactive neutrophils. As shown in Table 2, we observed a significant increase in the MNV for Sepsis cases compared with control Group. On the other hand, the MNS was decreased significantly in sepsis groupindicating left-shift transition with increased bands and other immature granulocytes that classically have hypolobated, less complex nuclear morphologic features. We did not observe any difference in the MNC between both the groups. These results indicate the VCS

Parameters could identify the morphological changes seen in neutrophils with acute bacterial-infection

Table 2: Mean Value of VCS parameters

Parameter	Control Group (n=166)	Case Group (n=184)	P - Value
MNV	146±9.8	171±17.5	<0.0001
MNC	144±16	143±16	0.89
MNS	145±13	121±17.6	<0.0001

Correlation of VCS with WBC count

We studied whether changes in VCS parameters correlated with patients' WBC counts. We initially subdivided the patients into 3 groups based on WBC count: group 1 (n = 14) WBC count <5000, group 2 (n = 53) WBC count 5000/µL to 25,000/µL and group 3 (n = 117) WBC count ≥25,000/µL. We observed a significant increase of the MNV in all 3 groups. The control group, indicating that the MNV may be a more sensitive and reliable indicator of acute bacterial infection than the classically used WBC counts method. However, we observed a significant decrease in the MNS in all 3 groups. There was no significant difference in MNC between control samples and patient groups (Table 3).

Table 3: Correlation of VCS Parameters with WBC Count

Parameters	Control Group (n=166)	Case Group (1) n=14 <5000	Case Group (2) n=53 5000 - 25000	Case Group (3) n=117 >25000	All Groups	P - Value Control vs 1	P - Value Control vs 2	P - Value Control vs 3
MNV	146±9.8	174±17.2	171±18.3	170±17.3	<0.0001	< 0.001	< 0.001	< 0.001
MNC	144±16	138±14.6	141±13.4	145±17.1	0.213	0.249	0.23	0.404
MNS	145±13	124±16.4	123±11.9	119±19.7	<0.0001	< 0.001	< 0.001	< 0.001

Correlation of VCS Parameters with Percentage of Neutrophils

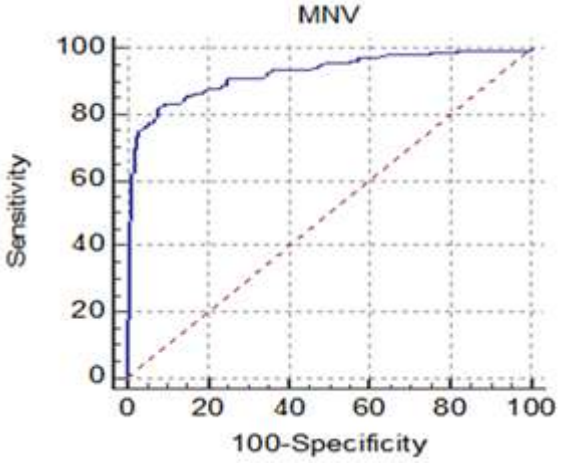
Further, we studied whether changes in VCS parameters are correlated with the percentage of neutrophils. Patient's samples were stratified based on the percentage of neutrophils into 2 groups: 1 (n = 148), neutrophils ≤ 85% (<0.85) and group 2 (n = 36) neutrophils ≥85%. We observed significant MNV increases in both groups compared with control Subjects. A significant MNS decrease was also noted in both Case groups (Table 4).

Table 4: Correlation of VCS parameters with Percentage of Neutrophils

Parameters	Control Group (n=166)	Case Group		P - Value	Control vs<85%	Control vs>85%
		<85% n=148	**>85% n=36**			
MNV	147±9.8	169±17.9	172±15.8	<0.0001	<0.0001	<0.0001
MNC	144±16	143±16	146±15.8	0.52	0.62	0.4
MNS	145±13	124±12.4	111±28.8	<0.0001	<0.0001	<0.0001

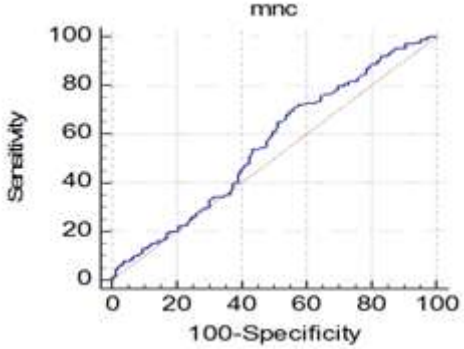
4. Assessment of Sensitivity and Specificity in Predicting Acute Infection

The sensitivity and specificity of the MNV and MNS, as well as the sensitivity of the MNC and percentage of neutrophils, for predicting infection were analyzed at selected cutoff points. When we selected a cutoff value of MNV equal to or greater than 157 (Table 5), we achieved a sensitivity of 82% and specificity of 92%. Thus, the MNV could be a more sensitive indicator for acute bacterial infection. When we selected a cutoff value of MNC equal to or greater than 136 (Table 5), we achieved a sensitivity of 70% and specificity of 45%. And when we selected a cutoff value of the MNS equal to or less than 131, the sensitivity and specificity were 63% and 78%, respectively (Table 5). Thus, Result shows the MNV was the single most predictable indicator of acute bacterial infection.



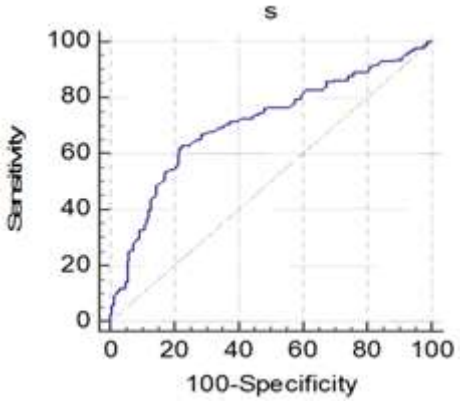
Graph 1: ROC Curve for MNV

ROC curve were constructed for MNV. Area under the curve value for predicting presence of sepsis was 0.924 with 95% confidence interval of 0.892 to 0.950 with p value <0.0001. Optimal cut-off value for predicting presence of sepsis was ≥157 with the sensitivity of 82% and specificity of 92%. The positive predictive value MNV was 81% and negative predictive value was 92%.



Graph 2: ROC Curve for MNC

ROC curve were constructed for MNC. Area under the curve value for predicting presence of sepsis was 0.557with 95% confidence interval of 0.503 to 0.610 with p value <0.0662. Optimal cut-off value for predicting presence of sepsis was ≥136.3 with the sensitivity of 70% and specificity of 45%. The positive predictive value MNC was 34% and negative predictive value was 77%.



Graph 3: ROC Curve for MNS

ROC curve were constructed for MNS. Area under the curve value for predicting presence of sepsis was 0.709 with 95% confidence interval of 0.659 to 0.756 with p value <0.0001. Optimal cut-off value for predicting presence of sepsis was ≤131.2 with the sensitivity of 63% and specificity of 77%. The positive predictive value MNS was 54% and negative predictive value was 82.7%.

Table 5: Prediction of Acute Bacterial Infection.

Parameter	Cut Off Point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MNV	≥157	82	92	81	92
MNC	≥136	70	45	34	77
MNS	≤ 131	63	78	54	83

Table 5 showing sensitivity specificity positive predictive value and negative predictive value of VCS parameters. Highest values are observed in MNV.

Table 6: Sensitivity and specificity of MNV and MNS in various studies

Studies	MNV	Sensitivity (%)	Specificity (%)	MNS	Sensitivity (%)	Specificity (%)
Chaves et al.	>145	83	54	<141	46	80
Mardi et al.	>150	76	63	-	-	-
Celik et al.	>157	79	82	<127.5	60	65
Bhargava et al.	>154	95.5	82	-	-	-
Present study	>157	82	92	<131	63	77

The sensitivity of MNV in prediction of neonatal sepsis in other studies is almost similar with our study.

5. Discussion

The WBC count, absolute neutrophil count, percentage of neutrophils, and, other immature neutrophils have been used to predict bacterial infection.[11] the automated hematology analyzers can fast examine the very large numbers of cells to provide all-inclusive haematological profiles. These parameters can be measured by the VCS technology. [12] The Morphologic changes in neutrophils, such as toxic granulation, and Döhle bodies, also provide important diagnostic information for neonatal sepsis. [13],[14],[15] This technology gives the results in few minutes, and no additional blood required to obtain other hematological parameters such as complete blood cell count (CBC)[16] It also provides measures of cell variability, such as the red cell distribution width, and cell size, mean corpuscular volume, that give valuable information for the differential diagnosis of anemia's.[15],[17] In this study, we included 350 neonates, 184 neonates with clinical proven sepsis, and 166 negative for sepsis neonates, evaluated neutrophil VCS parameters in the early diagnosis of sepsis and treatment efficacy, and sought to establish the cutoff levels, sensitivity, specificity, and NPV for each parameter.

We showed that the MNV was elevated significantly and the MNS was decreased significantly in septic patients (Cases). An elevation of the MNV was associated with positive blood cultures, higher WBC counts, and higher percentages of neutrophils. This result suggests that the MNV could be a more sensitive and reliable indicator of acute bacterial infection Clinical application of VCS parameters shows several advantages. They are more accurate than manual differential counts methods because more than 8,000 WBCs are evaluated automatically within a minute. [18]

In this study we observed statistically significant differences of the MNV and MNS between samples from bacteremic patient's group and control subjects. The clinical benefit of these findings was evaluated by comparing the sensitivity and the Specificity of parameters with other standard laboratory tests commonly used in the diagnosis of infection: The MNV shows the highest sensitivity for predicting infection as compare to MNS (Table 5). Furthermore, the MNV was elevated significantly even in samples from patients with normal percentages of neutrophils.

This shows the clinical importance of these findings, because a significant Number of infected individuals (184) are in this category, if the diagnosis of infection had been based on the WBC count or the percentage of neutrophils, the correct diagnosis easily could have been ignored.

Therefore, we consider that the MNV may play possible role and it can be used as an indicator of acute bacterial infection, particularly when the other hematologic parameters do not correlate well with the patient's clinical signs and symptoms. Large prospective cohort studies are needed to further validate the clinical worth of the VCS parameters in other causes of leukocytosis. Several other clinical applications of the VCS technology remain to be studied. With the vast amount of data that could be obtained relating to the morphologic features of neutrophils, eosinophils, monocytes, and lymphocytes.

Neonatal sepsis remains one of the most important clinical syndromes, especially in preterm infants, leading to high morbidity and mortality, despite advances in neonatology. Although early diagnosis and treatment is needed due to non-specific signs and symptoms and limitations of culturing procedures along with the lack of a perfect indicator/Marker of sepsis. We report cut-off levels of neutrophil VCS parameters in the diagnosis of sepsis, and these parameters seem to be useful in evaluating indicator ofsepticemia. The limitation of this study is that other markers like PCT, CD64, C - reactive protein, ESR, I/T ratio, morphological changes have not been included in the study.

In conclusion, there are several markers to diagnose neonatal sepsis still the Researchersare searching for the ideal and accurate marker. We suggest that neutrophil VCS parameters are useful for early diagnosis of neonatal sepsis.

References

[1] Shuveksharawat KN, Dr. Kothiyalpreeti, mathurprashant. A Review on Type, Etiological Factors, Definition, Clinical Features, Diagnosis Management and Prevention of Neonatal Sepsis.Journal of scientific and Innovation Reaserch. 2013;2(4):802-13.
[2] Fatma Mostafa Mahmoud NMD, Rania Ahmed Hassan, Nancy Mohamed Abou Shady evaluation of cd64 detection on neutrophils and tlr-2 on monocytes by flowcytometry as markers for early diagnosis of neonatal sepsis Int J of Adv Res. 2014;2 (7).
[3] kumarmondal SRN, Dipanwita&Bandyopadhyay, Ranjana&Chakraborty, Debdutta& Kumar Sinha, Swapan. Neonatal sepsis: Role of a battery of immunohematological tests in early diagnosis. International journal of applied and basic medical research. 2010;2. (43-7).
[4] Bagdasaryan RZ, Zhongren&Tierno, Bethany &Rosenman, Dara&Xu, Dongsheng. . Neutrophil VCS Parameters Are Superior Indicators for Acute Infection. Laboratory hematology. 2007;13. (12-6.).
[5] Xu D. Clinical Applications of Leukocyte Morphological Parameters. International Journal of Pathology and Clinical Research. 2015.
[6] Lila. Tawrmki. Diagnostic accuracy of septics markers for neonatal sepsis. paediatricaindonesiana,. 2016; Volume 48, Number 5,(pp. 299-305).
[7] P. K. Suresh JM, P. S. Rao and K. Ballal et al. "Volume Conductivity and Scatter Parameters as an Indicator of Acute Bacterial Infections by the Automated Haematology Analyser. " Journal of Clinical and Diagnostic Research. 2016; vol. 10,:pp. EC01-EC3.
[8] Purohit AH KP, Sharma S, Kapil A, Gupta A, Mukhopadhyay AK. Volume.conductivity, and scatter parameters as diagnostic aid to bacterial sepsis: A tertiary care experience. Indian J patholmicrobiol. 2015;58::459-63.
[9] Sankar MJ, Agarwal, R., Deorari, A.K. et al. Sepsis in the Newborn. Indian journal of pediatrics. 2008; 75:: 261-70.
[10] WE. B. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clinperinatol. 2010;

37::421–38.
[11] Mare, T.A., et al., The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. Crit Care, 2015. 19: p. 57.
[12] Celik, I.H., et al., Automated determination of neutrophil VCS parameters in diagnosis and treatment efficacy of neonatal sepsis. Pediatr Res, 2012. 71(1): p. 121-5.
[13] Suresh, P.K., et al., Volume Conductivity and Scatter Parameters as an Indicator of Acute Bacterial Infections by the Automated Haematology Analyser. J Clin Diagn Res, 2016. 10(1): p. EC01-3.
[14] Purohit, A.H., et al., Volume, conductivity, and scatter parameters as diagnostic aid to bacterial sepsis: A tertiary care experience. Indian J Pathol Microbiol, 2015. 58(4): p. 459-63.
[15] Lee, J.C., et al., Utility of hematologic and volume, conductivity, and scatter parameters from umbilical cord blood in predicting chorioamnionitis. Int J Lab Hematol, 2010. 32(3): p. 351-9.
[16] Ng, P.C., Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed, 2004. 89(3): p. F229-35.
[17] Abiramalatha, T., et al., Utility of neutrophil volume conductivity scatter (VCS) parameter changes as sepsis screen in neonates. J Perinatol, 2016. 36(9): p. 733-8.
[18] Celik IH, D.G., Aksoy HT, Erdeve O, Tuncer E, Biyikli Z, et al., Automated determination of neutrophil VCS parameters in diagnosis and treatment efficacy of neonatal sepsis. Pediatric Research, 2012. 71((1)): p. 121–25.