

Volume, Conductivity, and Scatter Parameters as a Diagnostic Aid in Neonatal Sepsis

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Abstract

Background: Neonatal sepsis remains an important clinical syndrome despite advances in neonatology. Early diagnosis and adequate antibiotic treatment are required because of high rates of mortality and morbidity, especially in developing countries. Early diagnosis of neonatal sepsis is difficult because of nonspecific signs and symptoms, and noninfectious diseases may mimic neonatal sepsis. Hence this study evaluates the role of certain new parameter in neonatal sepsis. *Material and method:* Before antibiotic therapy, blood samples for VCS parameters i.e. Volume, conductivity and scatter parameters of neutrophils and for culture were taken from patients with a diagnosis of sepsis. Data was analyzed by descriptive statistics. Student t test was used to compare 2 groups. Data included total WBC counts, percentage of neutrophils, and the VCS parameters of neutrophils, which were generated by each individual cell passing through the aperture and were optically and electronically measured by the Coulter LH 780 (Beckman Coulter, Fullerton, CA). VCS parameters were evaluated on the same day of sepsis diagnosis. *Results:* There were 55 cases and 10 controls. For each case total WBC counts, percentage of neutrophils, and the VCS parameters of neutrophils, CRP were noted. After the statistical analysis there was no statistically significant difference among cases and controls in relation to any of the parameters except for conductivity (C) of neutrophils. *Conclusion:* A high index of suspicion with or without lab evidences of infection is the key for early diagnosis. Prompt institution of antibiotic therapy and supportive care will save most of the cases of neonatal sepsis.

Keywords: Neonatal Sepsis; VCS Parameters; CRP.

Introduction

Neonatal sepsis remains an important clinical syndrome despite advances in neonatology. Early diagnosis and adequate antibiotic treatment are required because of high rates of mortality and morbidity, especially in developing countries. Early diagnosis of neonatal sepsis is difficult because of nonspecific signs and symptoms, and noninfectious diseases may mimic neonatal sepsis. Diagnosis is made by clinical and laboratory findings. Blood culture is the gold standard laboratory technique for the diagnosis of infection, but culture results may take 48–72 h. False-negative culture results may also occur.

Acute-phase reactants have been used to diagnose sepsis since the 1980s. Several interleukins (ILs), tumor necrosis factor, C-reactive protein (CRP), procalcitonin, immunoglobulins, and other markers have been used in the diagnosis of sepsis. Cutoff levels of IL-6 and CRP were determined in neonatal sepsis. Yet there is no perfect marker to diagnose sepsis and combination of these markers strengthens the diagnosis.

The complete blood cell count and peripheral blood smear were the most commonly ordered tests for the diagnosis of sepsis for many years. Higher white blood cell (WBC) and absolute neutrophil count and, immature/total neutrophil ratio are related to sepsis. Peripheral blood smears can give diagnostic information by identifying characteristic morphologic changes seen in reactive neutrophils and monocytes. However, this approach is labor-intensive and time-consuming because it requires manual examination and an experienced medical technologist.

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(Received on 02.10.2017, Accepted on 23.10.2017)

The volume, conductivity, and scatter (VCS) technology of the Coulter LH780 hematology analyzer (Beckman Coulter, Fullerton, CA) can obtain data from more than 8,000 WBC using direct current 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. It has been previously reported that VCS parameters can detect morphologic changes in immature and reactive neutrophils. This so-called VCS technology is analogous to microscopic evaluation of a peripheral blood smear but evaluates more than a microscope. VCS parameters were evaluated to diagnose sepsis and mean neutrophil volume (MNV), and volume distribution width (VDW) and mean neutrophil scatter (MNS) were found useful in an adult population. There are only two studies in a neonatal population and it showed that MNV and VDW were useful to diagnose neonatal sepsis. VCS parameters seem to be promising indicators for the diagnosis of sepsis [2].

Materials and Methods

The study was conducted in Department of Pathology, Bangalore medical college and research institute, Bengaluru.

Before antibiotic therapy, blood samples for VCS parameters and culture, CRP were taken from patients with a clinical diagnosis of sepsis.

Data included total WBC counts, percentage of neutrophils, and the VCS parameters of neutrophils, which were generated by each individual cell passing through the aperture and were optically and electronically measured by the Coulter LH 780 (Beckman Coulter, Fullerton, CA). The reagents that are used in the LH780 to prepare the sample for the analysis allow preserving WBCs in near-native state and removing all red blood cells with the lytic solution. Volume (V), as measured by direct current, is used to identify the size of the cell. Conductivity (C), or radio frequency measurement, provides information about the internal characteristics of the cell. Light-scatter (S) measurements, obtained as cell pass through the

helium-neon laser beam, provide information about cell surface characteristics and cell granularity. The raw data events collected from volume, conductivity, and light scatter are plotted three dimensionally on a data plot. All these measurements (mean and distribution width for volume) VCS parameters were evaluated on the same day of sepsis diagnosis.

Data was analysed by descriptive statistics. Student t test was used to compare 2 groups.

Results

There were 55 cases, 12 with proven sepsis and 43 of clinical sepsis and 10 healthy controls. Among them 12 were culture positive and 43 were culture negative. Thus variables such as total leucocyte count, neutrophil percentage, volume, conductivity and scatter parameters of neutrophils were compared between cases and controls. Cases were again divided into group 1a and group 1b depending on culture positivity. Thus we have 3 groups. Group Ia = 12 culture positive cases, group Ib = 43 culture negative sepsis, group II = 10 healthy controls. Table 1 shows that there was no significant difference among cases and controls with respect to sex, total count, differential neutrophilic count. Table 2 shows after analyzing the VCS parameters, statistically significant difference was found with regard to MNC (mean neutrophilic conductivity) $p < 0.05$. There was no statistical significant difference with regard to MNV and MNS among cases and controls $p > 0.05$. CRP was measured in every case of suspected sepsis. It was interesting to note that not all culture positive cases were showing high CRP levels. Only 3 cases were positive for both culture and high CRP levels. 9 were culture negative. Among culture negative 43 cases, 6 showed significant increase in CRP levels ($> 5 \text{ mg/dl}$) Table 3. Most common organism isolated was klebsiella pneumoniae (58.33%).

Different sensitivities and specificities were calculated at various cut off levels for the MNV, MNS and MNC as illustrated in Table 4. To note is MNC of less than 159 the sensitivity was 100%.

Table 1: Patient and control baseline characteristics

	Patient (55)	Controls(10)	p
Male :female ratio	1.2:1	1:1	
TLC	15260±7670	14710±8205	>0.05
Percentage of neutrophil	54±21	57±20	>0.05

Table 2: Comparison of VCS parameter between patient and control group overall.

	Control group Group II	Patient sample Group IA	Patient sample Group IB	p
MNV	150.63	160.29	159.64	0.154
MNC	150.56	127.88	134.89	0.015
MNS	123.22	126.65	128.10	0.765

Table 3: Distribution of cases according to test results.

	Culture positive	Culture negative	
CRP positive	3	6	9
CRP negative	9	37	46
	12	43	55

Table 4: Sensitivity and specificity for MNV,MNC,MNS at various cut-off levels between groups I and II

	Sensitivity (%)	Specificity (%)
MNV>150 au	75	29
MNC<159 au	100	19
MNS<127 au	50	51

Discussion

Our study is one of the few studies evaluating the role of VCS parameters of neutrophils in neonatal sepsis. we are co relating with the culture results and the CRP values of these neonates.

In a study done by Purohit A et al they concluded that volume, conductivity and scatter parameters are importantdiagnostic tools, which can serve as an adjunct to the clinicaldiagnosis of bacterial sepsis and can help in the early institutionof empirical antibiotic therapy [13].

Celik et al suggest that neutrophil VCS parameters and their DWs are useful both for early diagnosis and evaluation of treatment efficacy in neonatal sepsis without requirement for any extra blood collection [11].

In a study done by Hornik et al, although low WBC counts and ANCs and high I/T ratios are associated with infection, low sensitivities for CBC count cut-offs make these indices poor diagnostic markers to rule out infection. Thus, obtaining a CBC solely for the purpose of ruling out sepsis in neonates is not supported, and empiric antibiotic therapy will remain necessary in a large number of neonates with clinical suspicion of sepsis [5,6].

In a study done by Chaves et al an elevation of the MNV was associated with a higher WBC count and percentage of neutrophils and was present even in patients who did not have leukocytosis or neutrophilia. With a cutoff of 150 for the MNV, a specificity of 91% and sensitivity of 70% were achieved. As a quantitative, objective, and more

sensitive parameter, MNV has a potential to be an additional indicator for acute bacterial infection [2].

Thus in our study we didn't find MNV, MNS as indicators of infection, as other authors have found. Hence, large scale studies are required in our Indian scenario where in coulter are being increasing used.

Even though CRP levels were evaluated for neonates there was no significant co relation as the CRP was falsely negative in 9 out of 12 cases (75%) which is not acceptable. In a study done by Noha et al they concluded that CRP is particularly useful for monitoring the responseto treatment and for ruling out an infection. A repeated determination of CRP 24–48 h after the initiation of antibiotic therapy has been reported to carry a 99% negative predictive value in accurately identifying uninfected neonates, though nothing replaces a clinical impression and the gold standard (i.e. culture results) [11].

Conclusion

A high index of suspicion with or without lab evidences of infection is the key for early diagnosis. Prompt institution of antibiotic therapy and supportive care will save most of the cases of neonatal sepsis.

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