

Evaluation of use of Volume Conductivity Scatter Parameters as Early Indicator of Sepsis in Elderly Patients: A Hospital Based Case Control Study

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ABSTRACT

Introduction: Sepsis in elderly patients is a critical clinical issue due to high rates of morbidity and mortality. The modern age cell counters precisely measure volume, size and internal structure of the cell thereby assessing increase in volume and cytoplasmic granularity of leukocytes as seen in cases of sepsis. The present study was done to evaluate the use of Volume Conductivity Scatter (VCS) parameters as early indicators of sepsis in elderly patients and to overcome the shortcomings of blood culture by using a more precise VCS technology to assess morphologic changes in cell populations in sepsis.

Material and Method: The study included 51 patients above the age of 50 years which were divided into 2 groups Group 1, patients with localised infection (n= 39) and Group 2 with systemic infection (n=12). Group 3 included 30 controls without any signs of infection and with total leucocyte count and differential counts within normal limits. Coulter LH 750 cellular analysis system was used.

Result: Significant increase in both mean neutrophil volume (MNV) and mean monocyte volume (MMV) was reported among the patients with sepsis as compared to localized infections or control. With a cut off of 145 and 170 for MNV and MMV respectively, a sensitivity of 96% and 91.4% was obtained in predicting sepsis.

Conclusion: Both MNV and MMV have the potential to be used as early indicators of acute bacterial infections.

Keywords: Sepsis, Volume Conductivity Scatter, Mean Neutrophil Volume, Mean Monocyte Volume.

resistance.^{4,5} Other disadvantages of blood culture include low sensitivity, the need for sterile collection techniques to avoid contamination and false positivity. Since 1980s, many acute-phase reactants and other markers have been used to diagnose sepsis namely several interleukins (ILs), tumor necrosis factor, C-reactive protein (CRP), procalcitonin and immunoglobulins.^{6,7} However, there is no perfect marker to diagnose sepsis and combination of these markers strengthens the diagnosis.⁸

The complete blood cell count and peripheral blood smear have been the routinely ordered tests for the diagnosis of sepsis for many years. Sepsis is known to produce numerical and morphological changes in the leucocytes.⁹ There is increase in white blood cell (WBC) and absolute neutrophil count along with raised immature/total neutrophil ratio in cases of sepsis. Peripheral blood smears can give diagnostic information by identifying characteristic morphologic changes seen in reactive neutrophils and monocytes.^{10,11} Various morphological changes include change in size of cell, density of the nuclear chromatin, presence of the nucleolus, presence of toxic granulation, vacuolization, and Dohle bodies. Immature forms of neutrophils like band forms can be identified. Similarly, monocytes also show an increase in

INTRODUCTION

Septicemia is one of the leading causes of death in intensive care unit (ICU) patients. Sepsis in elderly patients remains a critical clinical issue due to the high rates of morbidity and mortality associated with it. More than 60% of sepsis patients are over 65 years of age.¹ Proper patient management can be achieved only with accurate and timely diagnosis of such cases. Early diagnosis of sepsis in elderly is difficult because of nonspecific signs and symptom and therefore, laboratory findings play an important role in the diagnosis of sepsis.^{2,3} Although septicaemia can have viral and fungal etiology, bacteria still remains the most common cause. Blood culture is the gold standard laboratory technique for the diagnosis of infection, but it has its own limitations. Culture results may take 48–72 hours. This delay leads the clinicians to start empirical antibiotic therapy that needs to be withdrawn and substituted by a sensitive antibiotic according to the culture results. This whole process often leads to antibiotic

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the cell size and presence of granules in the cytoplasm in cases of sepsis. However, evaluation of these morphological alterations by blood film examination is labor-intensive, time-consuming and requires an experienced haematologist. Furthermore, the results may be subjective because of human interpretation and less accurate as only a few hundred cells can be analyzed for any given sample.¹²

The advent of newer automated haematology analyzers has widened up the diagnostic horizon. The VCS technology of the Coulter LH 750 hematology analyzer (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.¹³ The data from VCS technology can thus be a comparable reflection of cell morphology.^{4,14,15} Therefore, volume, conductivity and scatter parameters can be used as a promising tool for the early diagnosis of sepsis. Although there have been previous studies from the west which support the same, but their different study population limits its relevance in India. In the present study, we tried to evaluate the use of VCS parameters to assess morphologic changes in cell populations in sepsis and to minimize the time duration of diagnosis of sepsis so that effective treatment can be given to the patients as early as possible.

MATERIAL AND METHODS

This was a hospital based case control study conducted over a period of three months in Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonapat. The study included total 51 patients. All the patients included in the study were above the age of 50 years. The patients were divided into 2 groups according to their clinical history and blood culture results i.e. Group 1 including patients with localised infection (LI) (n= 39) and Group 2 with systemic infection (SI) (n=12). Group 3 included 30 healthy blood bank donors as controls without any signs of infection such as fever and with WBC and differential counts within normal limits. Coulter LH 750 cellular analysis system was used. Samples were collected from all patients in the EDTA vacutainer for cell population data (CPD) measurements in the plane vacutainer for

qualitative measurement of C-reactive protein and in the blood culture bottles. The blood culture bottles were examined after 1,3 and 7 days of incubation at 37^o C and subcultures done.

The data collected included the total WBC count, the mean channel of neutrophil volume (MNV), mean channel of neutrophil conductivity (MNC), mean channel of neutrophil light scatter (MNS) and mean channel of monocyte volume (MMV), mean channel of monocyte conductivity (MNC) and mean channel of monocyte light scatter (MNS) obtained from the Coulter LH 750 Haematology analyser (Beckman Coulter. Inc).

Statistical analysis of data was performed using IBM SPSS Statistics 22.0 software. Results were expressed as the mean. Comparisons between mean values of the groups were performed by ANOVA and subsequent post-hoc range tests (Dunnett t tests). Multiple comparisons were performed in pairs to determine which mean values differ. A p-value<0.05 was considered statistically significant. Subsequently, the sensitivity and specificity were assessed.

RESULTS

VCS parameter data obtained from patients of localised infection cases (n=39), systemic infection cases (n=12) and control (n=30) was analysed. Most common bacteria isolated from blood culture in cases of systemic infections were Staphylococcus aureus (n=7) and Escherichia coli (n=5). Qualitative assessment of CRP was done and it was found positive for all patients of localised and systemic infections.

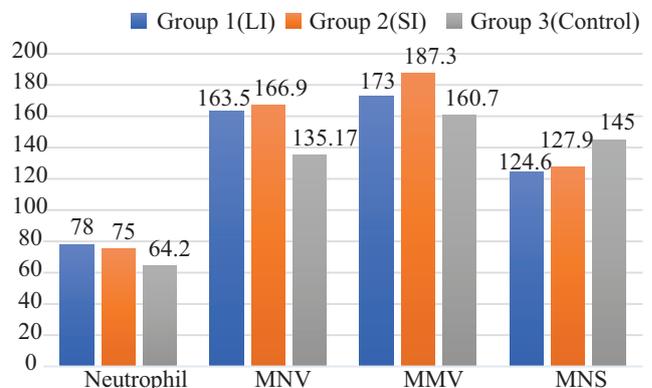


Figure-1: Comparison of neutrophil %, MNV, MMV, MNS values of group 1,2 and 3

	Localized infection (Group1)	Systemic infection (Group2)	Control (Group 3)
Number of cases(n)	39	12	30
Mean Age (yrs)	60.2	61.6	59.5
M:F Ratio	1:0.4	1:1.4	1:0.6

Table-1: Distribution of mean age and male to female ratio in the three groups

	Localized infection (Group 1)	Systemic infection (Group 2)	Control (Group 3)	LI vs. SI p-value	LI vs. C p-value	SI vs. C p-value
WBC /cumm	12030	12100	7906	0.982	0.016	0.101
Neutrophil%	78	75	64.2	0.714	0.016	0.048

p-value < 0.05 is considered significant

Table-2: Comparison of the mean TLC and mean neutrophil percentage in the three groups

	Group 1	Group 2	Group 3	p-value	p-value	p-value
VCS Parameters	Localized Infection (LI)	Systemic Infection(SI)	Control (C)	LI vs. SI	LI vs. C	SI vs. C
MNV	163.5	166.9	135.17	0.471	0.001	0.001
MNS	124.6	127.9	145.04	0.357	0.001	0.001
MNC	168.9	169.2	145.5	0.932	0.001	0.001
MMV	173	187.3	160.75	0.017	0.001	0.001
MMS	85.3	84.9	87.09	0.926	0.844	0.881
MMC	141.2	141.5	128.2	0.957	0.016	0.097

p-value < 0.05 is considered significant

Table-3: Comparison of VCS parameters of neutrophils and monocytes in three groups

Parameters	Cut off point	Sensitivity (%)	Specificity(%)
MMV	>170	91.4	58.6
MNV	>145	96	90.3

Table-4: Sensitivity and specificity of MMV and MNV in predicting sepsis

Hence, it was not included in the quantitative statistical analysis.

In group 1 the average age of 39 patients was 60.2 years and male to female ratio of 1: 0.4. Group 2 included 12 cases with an average age of 61.6 years and male to female ratio of 1: 1.4. A total of 30 controls were included in group 3 with mean age of 59.5 years and male to female ratio of 1: 0.6 (Table 1).

The mean total leucocyte count for patients of localized infection and systemic infection were 12,030/cumm and 12,100/cumm respectively while the control group had 7906/cumm. Mean neutrophil count of patients of localized and systemic infection were 78% and 75% respectively in contrast to 64.2% in control group.

Significant difference was seen in the mean TLC between group 1 and control group with a p value of 0.016. However, no significant difference was seen in the mean WBC count between group 1 and group 2 (p=0.982) and group 2 and control group (p=0.101). Significant difference in percentage of neutrophils was seen between group 1 and group 3 (p=0.016) and group 2 and group 3 (p=0.048) (Table 2).

On analyzing the VCS parameters of group 1 with controls, we found a significant increase in the MNV (163.5 vs 135.17) and MMV (173 vs 160.75) with p<0.05. Similarly, on comparing group 2 with controls, both MNV (166.9 vs 135.17) and MMV (187.3 vs 160.75) were found increased with p<0.05. Values of MNS were lower while MMC was increased significantly in both groups 1 and 2 as compared to controls(table 3).

The cut-off value for MNV ≥ 145 yielded a sensitivity of 96% and a specificity of 90.3%. Using 170 as the MMV cut-off, a sensitivity of 91.4% and a specificity of 58.6% were achieved. Thus, MNV was found to be a better predictive marker of acute bacterial infection than MMV (Table 4).

DISCUSSION

Sepsis in elderly patients is a critical clinical issue because of the high rates of morbidity and mortality. Bacteria is still the most common etiological agent for sepsis. Routinely, the WBC count, absolute neutrophil count, percentage of bands and immature neutrophils have been used to predict bacterial

infections. Manual peripheral blood film examination can also provide useful diagnostic information such as morphologic changes in neutrophils including toxic granulation, toxic vacuolization and the presence of Döhle bodies. But this is time consuming and labor-intensive approach.

Since the advent of automated analysers such as Coulter technology, the laboratory medicine practice has become very advanced, making the diagnosis quick and time saving. In the present study, the VCS parameters such as MNV, MNS, MNC, MMV, MMC and MMS were evaluated for their use as early indicators of sepsis in elderly patients. VCS parameters were calculated while the blood samples were run routinely through the coulter, without additional sample requirement. This approach saves time and decreases stress and workload on the pathologists who otherwise have to evaluate thousands of cells to look for the reactive features in WBC seen in cases of infections.¹⁶

In our study, there was significant difference in values of total WBC count and percentage of neutrophils between cases and controls as observed in few other studies.^{4,9,16-18} There was a significant difference in the mean MNV values between the cases and controls, with mean MNV of cases higher than the control. MMV was also significantly raised in cases compared to controls. Similar findings were seen in many previous studies.^{4,9,16-18} Our study also demonstrated higher values of both MNV and MMV among the patients with sepsis as compared to localized infections or control.

On comparing the values of MNC and MNS among all three groups, we observed significantly lower values in cases of both local and systemic infections as compared to controls. Lee et al¹⁶ also observed that in elderly patients with sepsis, MNC was reduced in sepsis patients when compared to control group. Other studies observed no significant difference in MNC values between cases and controls.^{4,9,17}

In the present study, we found a significant increase only in MMV among the patients with systemic infections as compared to localized infections suggesting the specificity of MMV for diagnosing systemic infections. A significant increase in MNV values was observed in both systemic as well as localized infections, however this parameter was found to be statistically insignificant in differentiating

the cause as systemic or localized. Hence, MMV can be a potential an early indicator of systemic infection and MNV as an indicator of any bacterial infection in the body. Our findings correlate well with the findings of study done by Suresh et al¹⁸ and Purohit et al.¹⁷ This was in contrast to the study by Mardi et al. where they found a significant increase in MNV as well as MMV.⁵ Our study revealed that with a cut off value of 145, MNV is 96% sensitive and 90.3% specific in predicting sepsis. Similarly, the study done by Purohit et al¹⁷ also found that with a cut off 149, MNV had a sensitivity of 91.4 % and specificity of 88.6% in predicting sepsis in elderly.

The current study was a prospective study with a small sample size and specific study group. All this might have introduced bias despite of all care. There may have been incorporation bias, overestimating the diagnostic accuracy of the investigated markers. Larger prospective cohort studies are needed to further validate the clinical usefulness of VCS parameters.

CONCLUSION

There is a need of a rapid, accurate and highly specific diagnostic method for sepsis which still remains a critical clinical issue due to the mortality and morbidity associated with it. The current routine and gold standard method used in diagnosis of sepsis are effective but have their own drawbacks. This study, along with many, done in the wake of the Beckmann Coulter Haematology Analyser, compare and establish the potential of using the VCS parameters of neutrophils as a diagnostic criteria. Through our study, we believe that MNV and MMV have the potential to be used as early indicators of acute non-systemic & systemic bacterial infection, we would like to highlight MMV as an emerging predictor of acute systemic infections.

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