Original Article

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Pathology Section

The Potential of Using VCS Parameters of Neutrophils and Monocytes as an Early Diagnostic Tool in Acute Bacterial Infections

AARTHI KANNAN, PAVITHRA SELVAM

ABSTRACT

Introduction: Infection and sepsis are known to produce numerical and morphological changes in the leucocytes. Morphological assessment of leucocytes by peripheral blood smear examination is time consuming and more subjective. The V (Volume), C (Conductivity), and S (Scatter) parameters of neutrophils are emerging as a sensitive predictor of acute bacterial infection and sepsis even in the absence of leucocytosis and neutrophilia. Volume, Conductivity and Scatter parameters (VCS) of leucocytes are obtained after analysis of about 8,000 leucocytes in a few seconds, using impedance to measure cell volume (V), radio frequency opacity to characterize conductivity (C) for internal composition of each cell and laser beam to measure light scatter (S) for cytoplasmic granulations and nuclear structure. The change in the morphology and in the number of these cells which are reflected in the VCS parameters of leucocytes proves to be a very accurate and sensitive method than the manual method.

Aim: To study the difference between the CBC and VCS parameters between bacterial culture positive and control cases and to assign a cut off value for these parameters that would aid in the diagnosis of acute bacterial infection.

Materials and Methods: CBC report of cases (positive bacterial culture with acute bacterial infections) generated from LH 780 automated analyser and CBC report of controls who doesn't have the disease is taken. CBC and VCS parameters of them were analysed with SPSS v.19

Results: Total 110 cases of acute bacterial infection with positive bacterial culture and 110 controls were taken into study. Mean age in the case group was 49.42±19.62 years and in the control group, the mean age was 39.77±12.14 years. There was significant difference in the WBC count (14.1 vs. 7.3; p<0.01), neutrophil percentage (74.2 vs. 59.0; p<0.01), MNV (139.4 vs. 131.9; p<0.01), MNS (141.4 vs. 136.8; p<0.01), MMV (157.4 vs. 154.3; p=0.04) and MMS (84.5 vs. 82.5; p<0.01) but there was no significance in the difference of MNC (157.7 vs 158.6; p=0.276) and MMC (131.3 vs. 130.6; p=0.25) between the case and control group. This significant difference in MNV was noted even when neutrophils count of the cases was <85% or WBC count <11,000/cumm. ROC curve was analysed for MNV values and it gave a criterion value of >129.3 with sensitivity of 92.7%. For MMV, the criterion value was >157.4 with a sensitivity of 47.27%.

Conclusion: Since, there is a significant difference in the VCS parameters of neutrophils and monocytes in acute bacterial infection, they can be used as sensitive indicators for diagnosing acute bacterial infections.

Keywords: Acute infections, Mean monocyte volume, Mean neutrophil volume, Positive bacterial culture

INTRODUCTION

Bacterial infections range from acute and life-threatening to chronic infections. Various morphological manifestations such as non inflammatory, granulomatous, and pyogenic or lymphohistocytic reactions are elicited by these organism [1]. Bacterial culture is considered gold standard in diagnosing bacterial infections [2], along with increase in neutrophils and its immature, precursor forms in the peripheral blood smear. The examination of neutrophilic series and its morphological changes are also studied [3], but all these methods vary in their sensitivity and are time-consuming and subjective.

VCS parameters of neutrophils have been studied extensively in sepsis in western population and have been proved to be a sensitive parameter [1,4-5]. The data is obtained after analysis of about 8,000 leucocytes in a few seconds, using impedance to measure cell volume (V), radio frequency opacity to characterize conductivity (C) for internal composition of each cell and laser beam to measure light scatter (S) for cytoplasmic granulations and nuclear structure [6]. Since, a large number of cells are counted per second,

this proves to be a very accurate and sensitive method than the manual method. During bacterial infection, along with increase in the total neutrophil count and presence of band forms due to left shift, there are some morphological changes in the neutrophils such as increase in the volume and size of the cells with marked variation in their morphology. In some cases, these minute morphological changes in neutrophil volume and neutrophil distribution width are seen even in the absence of other haematological abnormalities. The Coulter LH 780 can detect these changes early in disease and thus, proves to be a sensitive, cost effective, accurate and early diagnostic method of in all acute bacterial infections. This study was done to assess the VCS parameter of neutrophils and monocytes in all acute bacterial infections including sepsis and if possible to assign a cut-off values for these VCS neutrophil parameters that will aid in the diagnosis of acute bacterial infections.

MATERIALS AND METHODS

A retrospective case-control study was conducted between July 2016 to September 2016 for a period of 3 months in Pathology Department of Chettinad Hospital and Research institute, Kelambakkam, Tamil Nadu, India. Total 110 cases and 110 controls were taken into study. Results of bacterial culture done from samples of blood, body fluids, throat/nasal swab, wound swab of subjects suspected to have acute bacterial infection were taken from Microbiology Department. Subjects with positive bacterial culture were taken as cases. CBC reports of these cases were collected from LH 780 haematology analyser (Beckman Coulter, Fullerton, CA). Subjects with negative bacterial culture, bacterial contamination, children, neonates and those with chronic inflammation were excluded from the study. (Since neonates and children have different reference ranges for WBC count and differential count and vary significantly from adult population, they were not taken in to study. This was done to avoid confounding bias due to age).

Age and gender matched subjects who had CBC counts within the prescribed normal limits was selected randomly and taken as controls. CBC data and VCS parameters were compared between these two groups (case–control). VCS parameters were not studied between gram positive and gram negative cases, as few of them had mixed infections. Results were analyzed using SPSS statistical software 19.0 version.

RESULTS

Total 110 cases of acute bacterial infection and 110 controls who didn't have any acute bacterial infection were taken into study. Mean age in the case group was 49.42±19.62 years and in the control group, the mean age was 39.77±12.14 years. Out of 110 cases, there were 87 males and 23 females and 85 males and 25 females were considered in the control group. The CBC values from cases and controls were analyzed. The descriptive statistics including the mean and standard deviation of the haematological parameters of both cases and controls are shown in [Table/Fig-1,2].

The comparison of means between the haematological parameters among cases and control was done using SPSS software independent Student's 't'-test [Table/Fig-3].

There was significant difference in the WBC count (14.1 vs. 7.3; p<0.01), neutrophil percentage (74.2 vs. 59.0; p<0.01), MNV (139.4 vs. 131.9; p<0.01), MNS (141.4 vs. 136.8; p<0.01), MMV (157.4 vs 154.3; p=0.04) and MMS (84.5 vs. 82.5; p<0.01).

Those who had acute bacterial infection was further divided into two groups, Group 1 with those having neutrophils percentage less than 85% (Group 1; n = 90) and Group 2 with neutrophils percentage greater than 85% (Group 2; n = 20). The VCS parameter of these two groups was compared with control group. There was significant difference in the MNV values between cases and control group [Group 1(138.7 vs 132.0; p<0.01); Group 2 (142.7 vs 132.0; p<0.01)] [Table/Fig-4]. Even though cases with acute bacterial infections had neutrophils less than 85%, their MNV was still higher than control cases and the difference was statistically significant. This shows that changes in MNV occur irrespective of the neutrophil count. Case group having neutrophils more than 85% had higher MNV when compared to cases with neutrophils count less than 85%.

There was no significant difference in the MNC values between cases having neutrophils count <85% and >85% with the control group [Group 1 (157.5 vs. 158.5;p=0.235);

	WBC Case /cumm	WBC CTRL /cumm	NEU% Case	NEU% CTRL	MNV Case	MNV CRTL	MNC Case	MNC CRTL	MNS Case	MNS CRTL
Sample Size	110	110	110	110	110	110	110	110	110	110
Mean ± SD	14.2±5.8	7.4±1.4	74.2±11.6	59.1±5.7	139.4±9.2	131.9±7.4	157.8±6.0	158.5±5.0	141.4±7.6	136.8±8.0

[Table/Fig-1]: Descriptive statistics of the WBC and VCS parameters of neutrophils.

	MON% Case	MON% CTRL	VMON Case	VMON CTRL	CMON Case	CMON CTRL	SMON Case	SMON CTRL
Sample Size	110	110	110	110	110	110	110	110
Mean±SD	0.9±0.4	0.5±0.7	157.4±8.8	154.3±7.0	131.3±5.7	130.6±4.1	84.5±5.0	82.5±3.2

[Table/Fig-2]: Descriptive statistics of the VCS parameters of monocytes.

Parameters		Number of Cases	Mean	Standard Deviation	p-value
WBC Count Cells/cumm	Control	110	7.3	1.3	<0.01
	Case	110	14.1	5.8	
Neutrophil %	Control	110	59.0	5.7	<0.01
	Case	110	74.2	11.5	
MNV	Control	110	131.9	7.4	<0.01
	Case	110	139.4	9.2	
MNC	Control	110	158.5	5.0	0.37
	Case	110	157.8	6.0	
MNS	Control	110	136.8	7.8	<0.01
	Case	110	141.4	7.6	
MMV	Control	110	154.3	7.0	0.04
	Case	110	157.4	8.8	
MMC	Control	110	130.6	4.1	0.25
	Case	110	131.3	5.7	
MMS	Control	110	82.5	3.2	<0.01
	Case	110	84.5	5.0	

[Table/Fig-3]: The comparison of haematological parameters between the cases (n=110) and control (n=110).

Parameters		Number of Cases	Mean	Standard Deviation	Signi-ficance p-value (cases vs. control)
MNV	Control	110	131.9	7.4	
	Cases with Neutrophil % < 85%	90	138.7	8.4	<0.01
	Cases with Neutrophil % > 85%	20	142.7	11.8	<0.01
MNC	Control	110	158.5	5.0	
	Cases with Neutrophil % < 85%	90	157.5	6.1	0.235
	Cases with Neutrophil % > 85%	20	159.0	5.5	0.672
MNS	Control	110	136.8	8.0	
	Cases with Neutrophil % < 85%	90	141.9	7.0	<0.01
	Cases with Neutrophil % > 85%	20	138.8	9.5	0.32

[Table/Fig-4]: The comparison between the MNV, MNC & MNS values of control group and cases with neutrophil percentage less than 85% and greater than 85%.

Group 2(159.0 vs. 158.5; p=0.672) [Table/Fig-4].There was a significant difference in the means of MNS between control group and case group when neutrophils count is less than 85%. But there was no statistically significant difference between MNS of control and cases who had neutrophils count>85% Group 1(141.9 vs.136.8; p<0.01); Group 2 (138.8 vs. 136.8; p=0.32)]. This might be due to mature neutrophils in the case group with neutrophils >85%, which led to increase in MNV without any changes in MNS values.

The cases were again divided in to two groups based on the WBC count. (Group I: WBC count ≤11,000/cu.mm (n = 33); Group II: WBC count >11,000/cumm (n=77). There was a significant difference in MNV and MNS values between the two groups and control group [MNV values: Group 1 (136.8 vs. 132.0; p<0.05); Group 2(140.5 vs. 132.0; p<0.01)] & [MNS values: Group 1 (142.0 vs. 136.8; p<0.01); Group 2 (140.4 vs. 136.8; p<0.01)] but there was no statistically significant difference between MNC values between the groups and controls [Group 1 (158.2 vs. 158.5; p=0.79); Group 2 (157.7 vs. 158.5; p = 0.30)] [Table/Fig-5]. There was a significant difference in the MMV and MMS when the cases had WBC count more than 11,000/cumm [Table/Fig-6].

Parameters		Number of Cases	Mean	Standard Deviation	Signi-ficance p value cases vs. control
MNV	Control	110	132.0	7.4	
	Cases with Wbc Count ≤ 11,000	33	136.8	8.3	<0.01
	Cases with Wbc Count > 11,000	77	140.5	9.4	<0.01
MNC	Control	110	158.5	5.0	
	Cases with Wbc Count ≤ 11,000	33	158.2	5.4	0.79
	Cases with Wbc Count > 11,000	77	157.7	6.3	0.30
MNS	Control	110	136.8	8.0	
	Cases with Wbc Count ≤ 11,000	33	142.0	7.7	<0.01
	Cases with Wbc Count > 11,000	77	140.4	7.7	<0.01

[Table/Fig-5]: The comparison between the MNV, MNC & MNS values of control group and cases with white blood cell count lesser than and equal to 11,000/cumm and greater than 11,000/cumm.

ROC curve for MNV values gave a criterion value of >129.3; at which the sensitivity was 92.7% and specificity was 40.0%. Area under the curve was 0.73 with a p-value of

Parameters		Number of Cases	Mean	Standard Deviation	Signi-ficance p-value (cases vs. control)
MMV	Control	110	154.3	7.0	
	Cases with WBC Count ≤ 11,000	33	156.5	7.6	0.115
	Cases with WBC Count > 11,000	77	157.8	9.4	<0.01
MMC	Control	110	130.6	4.1	
	Cases with WBC Count ≤ 11,000	33	131.4	4.6	0.35
	Cases with WBC Count > 11,000	77	131.4	6.1	0.33
MMS	Control	110	82.5	5.7	
	Cases with WBC Count ≤ 11,000	33	83.5	4.6	0.25
	Cases with WBC Count > 11,000	77	84.9	5.1	<0.01

[Table/Fig-6]: The comparison between the MMV, MMC and MMS values of control group and cases with white blood cell count lesser than and equal to 11,000/cumm and greater than 11,000/cumm.

<0.001 [Table/Fig-7]. ROC curve for WBC count of cases with controls gave a criterion value of >8.9; where the sensitivity was 90.9% and specificity was 86.4%. When the count of WBC was taken as >11; the sensitivity dropped to 68.2% but specificity was 100% [Table/Fig-8]. For MMV, the criterion value was >157.4 with a sensitivity of 47.27% and specificity of 70.91 % [Table/Fig-9].

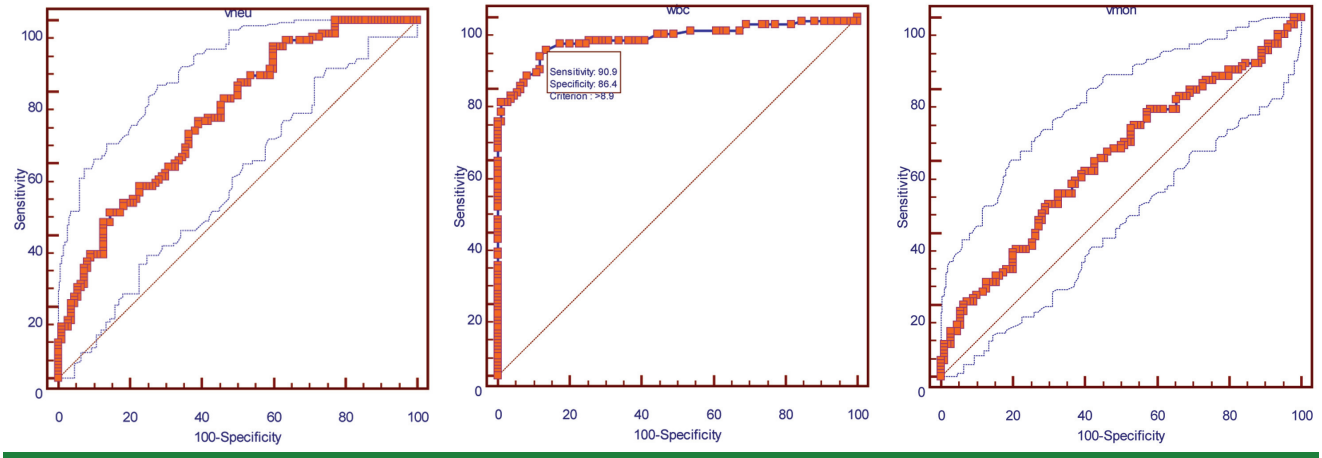
DISCUSSION

This study was done to establish the potential of using the VCS parameters of neutrophils as diagnostic criteria in acute bacterial infections. Though, bacterial culture is gold standard and CBC report aids in its diagnosis, these VCS parameters can be used as sensitive marker for diagnosing infections even when the WBC count and or neutrophils are within normal limits.

The necessity of a rapid, accurate and highly specific diagnostic method for a very common and greatly prevalent disease such as acute bacterial infections is clear. The current routines and gold standard methods used in diagnosis of this disease 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.

There are several studies based on the morphological changes that occur in the neutrophils and other leucocytes following infection, in some studies these morphological changes are also found to precede the quantitative increase and proliferation of the leucocytes. Based on this principle, the Coulter LH780 analyse the volume, conductivity and scatter of the white blood cells, which cannot be seen in peripheral smear, and can analyse up to 7,000 cells to 8,000 cells at once. Not only are these values accurate, rapid and do not require additional sample or tests, the results of various studies based on the effectiveness of this method has been promising [3].

In our study, there was significant differences in values of total WBC count and neutrophils between cases and controls as noted in few other studies [4,5,7,8]. There was a significant difference in the mean MNV values between the cases and controls, with mean MNV of cases higher than the control. This significant difference in MNV was noted even when neutrophils count of the cases was <85% or WBC count <11,000/cumm. These findings were similar to that obtained



[Table/Fig-7]: ROC curve for MNV values. **[Table/Fig-8]:** ROC curve for WBC values. **[Table/Fig-9]:** ROC curve for MMV.

Study	No. of Cases	MNV Value	Sensitivity	Specificity	WBC Value	Sensitivity	Specificity
Purohit AHL et al.,[4]	162	>149	91.4%	88.6%	>9	66.7%	66.7%
Chaves F et al.,[5]	69	>150	70%	91%	>11,000	55%	-
Suresh PK et al.,[7]	94	>150	72%	70%	-	-	-
Lee AJ et al.,[9]	85	>146.5	94.4%	44.1%	-	-	-
Mardi D et al.,[10]	76	>150	76%	63%	-	-	-
Celik IH et al.,[11]	206	>157	79%	82%	-	-	-
Our study	110	>129.3	92.7%	40.0%	>9	90.9%	86.4%

[Table/Fig-10]: Sensitivity and specificity of MNV at criterion value from different studies.

by other studies [4-5]; though Purohit AHL et al., used 75% of neutrophils as cut-off for cases [4]. These findings suggest that even though increase in WBC count and neutrophilia are sensitive markers for diagnosing acute bacterial infections, the MNV value is useful even when WBC or neutrophil count is normal. In a study conducted by Lee AJ et al., on elderly patient with sepsis, they found that MNV increased from normal to localized infections to sepsis with highest value in sepsis patients [9].

A significant difference in MNS value between cases and controls was seen with MNS of cases higher than control. There was a significant difference in MNS between cases and controls when WBC count was less than 11,000/cumm or when neutrophil count was <85%. Purohit AHL et al., [4] and Chaves F et al., [5] stated that there was a significant difference in the MNS value of cases and controls but MNS of cases were lower than MNS of controls. They suggested that the decrease in MNS was due to left shift in neutrophils. The increase in MNS value in our cases may be due to increase or presence of toxic granules in cases.

There was no significant difference in MNC values between cases and controls which were similar to other studies [4,5,7]. But in a study conducted by Lee AJ et al., in elderly patients with sepsis, they found that MNC was reduced in sepsis patients when compared to control group [9].

Chaves F et al., have described neutrophil distribution width as a sensitive indicator with increased NDW in cases when compared to controls [8]. Few studies even suggested the role of Mean Monocyte Volume (MMV) as being more sensitive in sepsis than MNV [9]. In our cases too, there was a significant difference in MMV and MMS value between cases and control with cases having greater value of these parameters than control. This suggests that there are significant changes in monocyte VCS parameters in acute bacterial infections and they can be used along with the VCS parameters of neutrophils in predicting acute bacterial infection.

Since, MNV appears to have a predictive role in acute bacterial infection and has a significant difference with the control values, even with normal WBC and neutrophils count, many studies have determined a cut-off value for MNV for diagnosing acute bacterial infection. Few of the studies have compared the sensitivity and specificity of WBC count in acute bacterial infections with MNV values and stated that MNV was superior to WBC count in predicting bacterial infection. [Table/Fig-10] describes the sensitivity and specificity for MNV values from different studies.

In our study, MNV had a sensitivity of 92.7% at the criterion value of ROC 129.3. This difference might be due to difference in the study population and in number of cases. Further studies need to be done before establishing the criterion value.

The VCS parameters of neutrophils are emerging as a sensitive predictor of acute bacterial infection and sepsis even in the absence of leucocytosis and neutrophilia. Since, it reflects the morphological changes in neutrophils, they can be used to predict the occurrence of sepsis and acute bacterial infection and follow-up of these patients. Since examination of peripheral smear is time consuming and labour intensive, these parameters can be used as a sensitive indicator similar to HS-CRP and procalcitonin in predicting acute bacterial infections.

Various studies are being done on these VCS parameters of white blood cells to understand their utility in the diagnosis of various infective and non-infective disease conditions.

LIMITATIONS

There is significant difference in the MNV values and the criterion value of the ROC curve for MNV values similar to other studies. But a large scale cohort studies on this subject is needed to establish the criterion value.

CONCLUSION

In our study, we analysed the VCS parameters and WBC count of cases having acute bacterial infection and found that the MNV and MNS of neutrophils along with total WBC count and neutrophil count had a significant difference in their mean values between control and cases group. There was also a significant difference in MMV and MMS between cases and controls. The significant difference in MNV values were noted even in the absence of leucocytosis or neutrophilia. ROC curve was analysed for MNV values and it gave a criterion value of >129.3 with sensitivity of 92.7%. So, these VCS parameters, especially MNV and MMV can

be used to predict sepsis and acute bacterial infections and can be used as a sensitive parameter for early diagnosis.

ABBREVIATIONS

WBC – White Blood Cell, MNV – Mean Neutrophil Volume, MNS – Mean Neutrophil Scatter, MNC – Mean Neutrophil Conductivity, MMV – Mean Monocyte Volume, MMC – Mean Monocyte Conductivity, MMS – Mean Monocyte Scatter, ROC – Receiver Operator Characterisitic.

ETHICAL CLEARANCE

This study was done after obtaining ethical clearance from the ethical committee of our institute. The patient identity have not been revealed at any time of the study and in the manuscript.

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AUTHOR(S):

1. Dr. Aarthi Kannan
2. Dr. Pavithra Selvam

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Pathology, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu, India.
2. Student, Department of Pathology, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Aarthi Kannan,
E1, Park Side Villas,148, Ist Cross Street, Annai Indira Nagar, Thoraipakkam, Chennai-600097,
Tamil Nadu, India.
E-mail: aarthi.kanna@gmail.com

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