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Original Article

Pathology Section

Analysis of Changes in Variation of Neutrophil and Monocyte Parameters, Including Volume, Conductivity and Scatter in Sepsis Patients and Healthy Controls: A Cross-sectional Study

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ABSTRACT

Introduction: Sepsis continues to be a leading cause of mortality and prolonged hospitalisation. The conventional method of blood culture, while considered the gold standard, has limitations such as contamination and delayed reporting. The examination of peripheral smears has uncovered signs suggestive of septicaemia; however, these findings suffer from inter-observer variability and reliance on staining quality.

Aim: To investigate the variation of neutrophil and monocyte parameters, including Volume, Conductivity, and Scatter (VCS), in sepsis compared to healthy controls.

Materials and Methods: A cross-sectional analytical study was conducted at the Himalayan Institute of Medical Sciences, Swami Rama Himalayan University in Dehradun, Uttarakhand, over the course of one year, from January 2021 to December 2021, involving patients over 18 years of age categorised into sepsis group based on clinical suspicion, sepsis screen, blood culture, and Sequential Organ Failure Assessment (SOFA) score (n=117). A group of healthy controls was also included (n=140). Haematological investigations were performed using the DXH 800 Haematology Analyser (Beckman Coulter, CA, USA) with VCS Technology. Categorical variables were analysed using the Chi-square test, while non parametric data was compared using the Mann-Whitney U test.

Results: The average age in the sepsis group was 50.17±13.17 years, while in the control group, it was 38.14±8.78 years. The results revealed higher White Blood Cell (WBC) counts (16.76±7.39)×10^3/cumm in the sepsis group compared to healthy controls (6.68±1.42)×10^3, absolute neutrophil counts (13.74±7.280)×10^3 in sepsis patients, and eosinopenia in the sepsis group (0.0114±0.0104)×10^3 compared to controls (0.23±0.116)×10^3. Moreover, mean neutrophilic volumes (158.00±14.840) and monocytic volumes (182.58±18.64) were higher in the sepsis group, while they were lower in healthy controls, which were (149.52±5.23 and 171.17±6.28), respectively. Axial light loss for neutrophil and monocyte was 142.40±11.78 and 121.50±17.93, respectively, while it was lower in healthy controls showing a value of 135.51±7.63 and 119.45±8.25, respectively. Furthermore, mean neutrophilic and monocytic conductivity and scatter were decreased in sepsis. The observed higher WBC counts and absolute neutrophil counts in sepsis patients suggest a premature release of neutrophils from the bone marrow. The alterations in cell volume reflect an immune response. Additionally, the overall scatter of neutrophils and monocytes was reduced, accompanied by increased cellular transparency.

Conclusion: The present study contributes valuable insights into the pathophysiological mechanisms underlying sepsis, emphasising the dynamic interplay between immune cells and their functional characteristics. Understanding these variations in cellular parameters could potentially aid in the development of more targeted diagnostic and therapeutic approaches for sepsis, ultimately improving patient outcomes. Further research is warranted to delve deeper into the specific mechanisms driving these observed changes and to explore their clinical implications in the context of sepsis management.

Keywords: Biomarkers, Eosinopenia, Haematology analyser

INTRODUCTION

Sepsis presents a significant challenge in both developed and developing countries, necessitating early detection to reduce morbidity and mortality rates. The conventional method of blood culture, considered the gold standard, has limitations such as contamination and delayed reporting. Peripheral smear examinations have revealed potential indicators of septicaemia, including leukocytosis, neutrophilia, morphological alterations, and toxic changes. However, these findings suffer from inter-observer variability and reliance on staining and microscope quality. Bandemia, once considered an early indicator, has faced doubts in previous studies. Early understanding of sepsis pathogenesis focused on the hyper-inflammatory aspect, investigating cytokines like Interleukin (IL)-1b, IL-6, Tumour Necrosis Factor (TNF), and C-reactive Protein (CRP) as potential biomarkers. This led to the inclusion of corticosteroids and immunosuppressants in treatment protocols. Procalcitonin emerged as a biomarker in the 1990s, and recent therapeutic focus has shifted to the anti-inflammatory phase of sepsis. However, there is no single ideal biomarker for sepsis, necessitating a combination of clinical suspicion and supportive biomarkers for early diagnosis [1-3]. In recent years, automated analysers with advanced technology have provided additional information beyond routine parameters. Parameters such as fluorescence, volume conductivity, and scatter are being explored as cost-effective means to assess sepsis early. These advancements hold promise for improving sepsis detection and aiding clinicians in timely interventions. Technology in the Coulter that measures volume conductivity and scatter, like the DxH 800 Haematology Analyser (Beckman Coulter), measures different morphological parameters, including the size and volume of the cells in the given biological sample. These are mainly computed

by employing three independent energy sources: direct current impedance, alternating current using radio frequency to assess the internal complexity, and Laser-mediated determination of the size, volume, and internal cellular structures. This equipment can analyse >8000 WBCs simultaneously and provide a mean value of all the parameters. This comprehensive dataset can be further explored for the identification of distinct cell types of WBCs. This segregation of different cell types is fully automated and results in a comprehensive differential count. In addition to this, the Coulter analyser assesses the morphological changes that are bona fide in reactive neutrophils, lymphocytes, and monocytes. The segregation of reactive and immature neutrophils is based on cellular volume, cytoplasmic granularity, and their relative sizes. These pieces of evidence can be promising tools in the diagnosis of sepsis [4].

To study the variations in neutrophil and monocyte parameters, including VCS, will be observed in patients with sepsis compared to healthy controls and to find any specific parameter with a diagnostic ability to differentiate sepsis patients from healthy controls. Specifically, it was hypothesised that sepsis patients will exhibit alterations in these parameters indicative of immune system activation and dysfunction.

MATERIALS AND METHODS

The present study was a cross-sectional analytical study conducted in the Department of Pathology at a tertiary care center, specifically the Himalayan Institute of Medical Sciences, Swami Rama Himalayan University in Dehradun, Uttarakhand, India. Over the course of one year, from January 2021 to December 2021, patients diagnosed with sepsis were recruited for the study. Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from each patient, and ethical approval from the Institutional Ethics Committee was obtained prior to conducting the study. The approval number is SRHU/HIMS/ETHICS/2022/283. All procedures performed in studies involving human participants were according to the ethical standards of Institutional Ethics Committee and in compliance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any regulated animal-related research. The patients were categorised based on clinical suspicion, sepsis screen, blood culture, and Sequential Organ Failure Assessment (SOFA) score, which included partial oxygen pressure, platelet count, bilirubin value, mean arterial pressure, central nervous system findings, and creatinine values. A score of 0 to 4 was given [5].

Inclusion and Exclusion criteria: The study included the following groups: **Sepsis:** This group consisted of patients with a positive blood culture and probable sepsis with a SOFA score of 2 or higher, along with a suspected source of infection and negative blood culture (n=117); **No sepsis:** This group comprised age-adjusted healthy volunteers (n=140) who showed no abnormalities in their health check and no signs or symptoms of infection. Patients with active haematological malignancy, pregnancy, oral corticosteroid use for less than 24 hours prior to enrollment, adults on GCSF, and those on immunosuppressants were excluded.

Study Procedure

Data was collected using a proforma that included information such as age, sex, address, and relevant medical history, including co-morbidities like diabetes mellitus, tuberculosis, and hypertension. A detailed clinical examination of each adult patient was recorded. Blood samples were drawn under full aseptic precautions immediately after making the diagnosis, and additional investigations such as CRP and blood culture were performed simultaneously. Haematological investigations, including haemoglobin, total leukocyte count, absolute leukocyte count, differential leukocyte count, platelet count, and VCS parameters of leukocytes, were included. Peripheral blood Ethylenediamine tetracetic acid (EDTA) samples were analysed on a UnicelDxH 800 (Beckman Coulter, CA, USA) automated haematology analyser.

The VCS technology, which is being used in the UniCel DxH 800 system by Beckman Coulter in California, USA, represents a cutting-edge approach to blood cell analysis. This technology is featured in the Coulter STKS, MAXM, and MAXM A/L systems. Leveraging VCS, this proprietary technology utilises a Laser-based flow cytometer with modifications for enhanced unstained cell analysis. The process begins with a precisely prepared sample that undergoes gentle lysis for Red Blood Cells (RBC) while preserving WBCs in their native state. Unlike traditional light scatter methods, VCS employs the Coulter Principle of (DC) Impedance, physically measuring cell volume in an isotonic diluent. The Laser beam striking cells generates scattered light, which is captured by proprietary detectors for median-angle light scatter signals. VCS compensates conductivity and scatter signals based on cell size information, resulting in unique measurements. Opacity corrections focus on internal cell structure independently of cell size, enabling differentiation between cells with similar sizes but distinct internal compositions. Rotated Light Scatter (RLS) eliminates size components, accurately separating mixed cell types without mathematical manipulations, showcasing the sophistication of VCS in blood cell analysis. This advanced technology provides unparalleled sensitivity, specificity, and efficiency for comprehensive insights into cellular characteristics [4]. Coulter-Latron controls were run every day to ensure the quality of VCS data obtained by the analyser. Blood cultures were also conducted for all subjects using the BacT or Alert method. Other investigations required for patient management were performed as needed.

STATISTICAL ANALYSIS

The collected data were entered into a Microsoft Excel sheet, and statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20.0. The Chi-square test was used to analyse categorical variables between groups. Two independent sample t-tests were used to compare the means of normally distributed variables, while the Mann-Whitney U test was used to compare the medians of non parametric data. A p-value of 0.05 was considered statistically significant. To evaluate the significance of cut-off levels, the power of variables, and the area under the curve for the Receiver Operating Characteristic (ROC) curve were used. Sensitivity, specificity, and the area under the curve were estimated using the ROC curve approach. The area under the curve was categorised as better when Area under Curve (AUC) was close to 1 and categorised as worse when it was 0.5 [6].

RESULTS

In the healthy group (140 controls), the highest number of patients fell within the 31-40 age range (54 individuals, 38.57%), followed by the 41-50 age range (38 individuals, 27.14%). Conversely, in the sepsis group, the majority of patients were over 60 years old (41 individuals, 29.28%). The average age in the sepsis group was 50.17±13.17 years, while in the control group, it was 38.14±8.78 years. Regarding gender distribution, out of the 117 sepsis cases, 68 (48.57%) were males and 49 (35%) were females. In the control group, there were 92 (65.71%) males and 48 (34.28%) females. Most of the subjects in the healthy group were from rural areas (77/114), 68%, while the remaining (37/114), 32%, were from urban areas. Of the 117 subjects, 31 (12.06%) were diagnosed with definite septicaemia (culture-positive), 86 (33.46%) had probable septicaemia. Among the 117 sepsis subjects, 31 had positive blood cultures, with *Escherichia coli* (E. coli) being the most common organism isolated in ten cases. Fungal growth was observed in two cases (6.45%). The study found that sepsis patients had significantly higher WBC counts (16.76±7.39)×10^3/

cumm compared to healthy controls (6.68±1.42)×10^3/cumm. Additionally, they had lower RBC counts (3.85±3.07)×10^6/cumm compared to healthy controls (4.53±0.52)×10^6/cumm. Haemoglobin (HGB) levels (11.05±9.03) g/dL and Haematocrit (HCT) levels (31.65±8.48)% were also lower compared to the control group. There were statistically significant differences in Mean Corpuscular Volume (MCV) (88.29±10.16) and Mean Corpuscular Haemoglobin (MCH) (28.71±3.50), which were lower in the sepsis group, while Red Cell Distribution Width (RDW) (16.72±2.50)% was higher. Platelet counts did not show a significant difference between the two groups [Table/Fig-1]. Neutrophil percentage (NE%) was higher in the sepsis group compared to the healthy controls. A cut-off percentage of >70.10% for neutrophils was found to be highly sensitive (78.60%) and specific (97.10%) in differentiating the sepsis group from healthy controls. Similar cut-off values were also determined for absolute lymphocyte count and eosinophil percentages, showing high sensitivity and specificity in differentiating sepsis from healthy controls, which were ≤1.03% and <1500/cumm, respectively. Absolute Neutrophil counts (NE#) and absolute Monocyte counts (MO#) were significantly higher in sepsis patients, while the mean absolute Lymphocyte count (LY#) was lower in the sepsis group compared to healthy controls [Table/Fig-2,3]. An absolute neutrophil count above >2,600/cumm showed a sensitivity and specificity of 94.9% and 86.10%, respectively, in differentiating sepsis patients from healthy controls [Table/Fig-3]. Various parameters related to neutrophilic and lymphocytic characteristics were measured. Mean neutrophilic volumes (158.00±14.840) and monocytic volumes (182.58±18.64) were higher in the sepsis group, while they were lower in healthy controls (149.52±5.23 and 171.17±6.28), respectively. Axial light loss for neutrophils and monocytes was 142.40±11.78 and 121.50±17.93, respectively; while it was lower in healthy controls, showing values of 135.51±7.63 and 119.45±8.25, respectively [Table/Fig-4]. No cut-off could be derived for any of the additional parameters, volume, scatter, or conductivity of leukocytes with

Routine haematological parameters (reference range)	Sepsis group (n=117) (Mean±SD)	Healthy group (n=140) (Mean±SD)	Units	p-value
TLC (4-10)	16.76±7.39	6.68±1.42	10^3/cumm	0.001
RBC (3.8-5.5)	3.85±3.07	4.53±0.52	10^6/cumm	0.001
HGB (12-17)	11.05±9.03	13.80±1.63	g/dL	0.001
HCT (36-50)	31.65±8.48	41.00±4.31	%	0.001
MCV (83-99)	88.29±10.16	91.07±9.10	Fl	0.017
MCH (27-32)	28.71±3.50	30.66±3.57	Pg	0.001
MCHC (31.5-34.5)	33.49±11.67	33.61±0.91	g/dl	0.001
RDW (10-15)	16.72±2.50	14.47±1.38	%	0.001
PLT (150-450)	218.32±151.85	195.68±75.75	10^3/cumm	0.801
MPV (8-10.5)	9.85±1.59	10.89±1.88	FL	0.001

[Table/Fig-1]: Routine haematological parameters in healthy and sepsis groups.
*Mean±standard deviation; p-value cut-off of <0.05 for statistical significance; independent t-test. TLC: Total leucocyte count; RBC: Red blood cell count; HGB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelet; MPV: Mean platelet volume; The Chi-square test was used to analyse categorical variables between groups. Two independent sample t-tests were used to compare the means of normally distributed variables, while the Mann-Whitney U test was used to compare the medians of non parametric data

Routine WBC parameters (reference range)	Sepsis group (n=117) (Mean±SD)x10^3	Healthy group (n=140) (Mean±SD)	Units	p-value
NE% (40-80)	78.49±17.25	56.70±8.05	%	0.001
LY% (20-40)	12.65±14.79	31.57±7.20	%	0.001
MO% (2-10)	6.66±5.28	7.57±1.84	%	0.001
EO% (1-6)	1.00±1.75	3.51±3.07	%	0.001
BA% (<2)	0.47±0.44	0.62±0.29	%	0.001
NE# (1-3)	(13.74±7.280)	(3.8±1.150)	10^3/cumm	0.001
LY# (2-7)	(1.60±1.45)	(2.7±0.510)	10^3/cumm	0.001
MO# (0.2-1)	(1.12±1.03)	(0.5±0.014)	10^3/cumm	0.001
EO# (.02-0.5)	(0.0114±0.0104)	(0.23±0.116)	10^3/cumm	0.001

[Table/Fig-2]: Routine WBC parameters in healthy and sepsis groups.
*Mean±standard deviation; p-value cut-off of <0.05 for statistical significance; independent t-test. WBC: White blood cells; NE%: Neutrophil percentage; LY%: Lymphocyte percentage; MO%: Monocyte percentage; EO%: Eosinophil Percentage; BA%: Basophil Percentage; NE#: Absolute Neutrophil Count/cumm; LY#: Absolute Lymphocyte Count/cumm; MO#: Absolute Monocyte Count/cumm; EO#: Absolute Eosinophil Count/cumm. The Chi-square test was used to analyse categorical variables between groups. Two independent sample t-tests were used to compare the means of normally distributed variables, while the Mann-Whitney U test was used to compare the medians of non parametric data

Variables	Area	p-value	Asymptotic 95% confidence interval		Cut-off	Sensi-tivity	Speci-ficity
			Lower bound	Upper bound			
NE%	0.897	0.001	0.055	0.151	>70.10	78.60	97.10
LY%	0.089	0.001	0.866	0.956	≤19.74	84.60	97.10
MO%	0.354	0.001	0.572	0.720	≤5.68	49.60	90.00
EO%	0.124	0.001	0.829	0.922	≤1.03	76.90	87.90
BA%	0.319	0.001	0.611	0.751	≤0.39	59.80	83.60
NE#	0.927	0.001	0.884	0.971	>2600	94.90	86.10
LY#	0.242	0.001	0.176	0.309	<1500	38.85	85.30
MO#	0.779	0.001	0.712	0.847	>260	88.00	94.60
EO#	0.879	0.001	0.837	0.923	<120	85.00	90.10

[Table/Fig-3]: Comparison of routine WBC parameters between healthy controls and sepsis group and the derived cut-off.
NE%: Neutrophil percentage; LY%: Lymphocyte percentage; MO%: Monocyte percentage; EO%: Eosinophil Percentage; BA%: Basophil Percentage; NE#: Absolute Neutrophil Count/cumm; LY#: Absolute Lymphocyte Count/cumm; MO#: Absolute Monocyte Count/cumm; EO#: Absolute Eosinophil Count/cumm

VCS parameters	Sepsis group (n=117) (Mean±SD)	Healthy group (n=140) (Mean±SD)	p-value
MN-V-NE	158.00±14.84	149.52±5.23	0.001
MN-C-NE	143.41±4.78	146.07±3.81	0.001
MN-MALS-NE	136.23±7.79	140.00±6.91	0.001
MN-UMALS-NE	137.43±7.25	140.95±6.64	0.001
MN-LMALS-NE	131.46±10.95	135.12±8.47	0.002
MN-LALS-NE	159.42±28.93	169.94±30.83	0.023
MN-AL2-NE	142.40±11.78	135.51±7.63	0.001
MN-V-LY	91.05±8.76	88.75±3.32	0.010
MN-C- LY	116.51±7.37	115.13±4.70	0.002
MN-MALS- LY	75.61±9.78	70.17±7.30	0.001
MN-UMALS- LY	79.97±12.47	73.76±9.10	0.001
MN-LMALS- LY	66.10±8.58	61.17±6.20	0.001
MN-LALS- LY	36.46±6.60	38.45±5.03	0.006
MN-AL2- LY	66.03±14.02	65.47±4.90	0.327
MN-V-MO	182.58±18.64	171.17±6.28	0.001
MN-C- MO	124.03±6.10	123.98±4.01	0.814
MN-MALS- MO	89.79±7.65	90.15±5.77	0.915
MN-UMALS- MO	98.85±9.81	99.81±6.66	0.719
MN-LMALS- MO	76.80±8.18	77.07±5.39	0.925
MN-LALS- MO	83.29±20.68	102.32±18.79	0.001
MN-AL2- MO	121.50±17.93	119.45±8.25	0.010

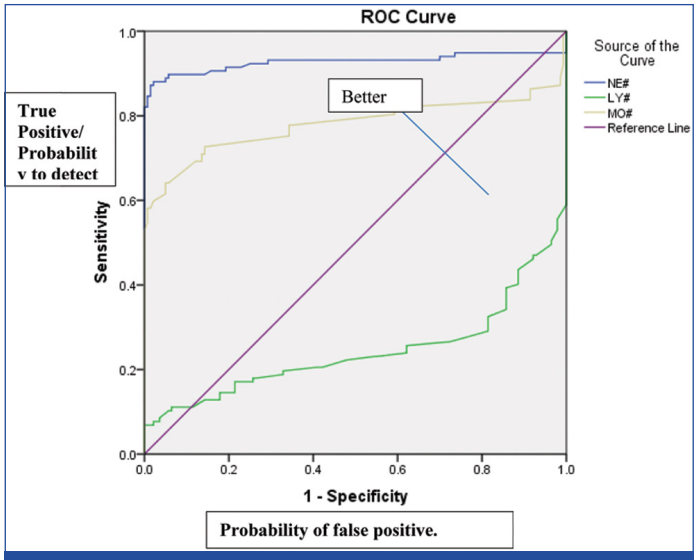
[Table/Fig-4]: Mean volume, conductivity and scatter parameters of neutrophil, monocyte and lymphocyte in the sepsis group and healthy controls.
Mean±standard deviation; p-value cut-off of <0.05 for statistical significance; independent t-test. MN-V: Mean volume; MN-C: Mean conductivity; MN- MALS: Mean median angle light scatter; MN-UMALS: Mean upper median angle light scatter; MN-LMALS: Mean lower median angle light scatter; MN-LALS: Mean lower angle light scatter, MN-AL-2- Mean axial light loss; NE: Neutrophil; LY: Lymphocyte; MO: Monocyte. The Chi-square test was used to analyse categorical variables between groups. Two independent sample t-tests were used to compare the means of normally distributed variables, while the Mann-Whitney U test was used to compare the medians of non parametric data

Variables	Area	p-value	95% Confidence interval		Cut-off	Sensitivity	Specificity
			Lower Bound	Upper Bound			
MN-V-NE	0.688	0.001	0.618	0.758	>158	41.10	95.70
MN-C-NE	0.668	0.001	0.265	0.399	≤141	38.50	92.10
MN-MALS-NE	0.647	0.001	0.286	0.420	≤138	53.80	69.30
MN-UMALS-NE	0.655	0.001	0.278	0.412	≤138	50.40	77.10
MN-LMALS-NE	0.613	0.002	0.318	0.456	≤132	49.60	72.10
MN-LALS-NE	0.582	0.023	0.347	0.489	≤199	97.40	27.90
MN-AL2-NE	0.705	0.001	0.635	0.775	>143	45.30	96.40
MN-V-LY	0.594	0.010	0.519	0.668	>92	35.90	89.30
MN-C-LY	0.614	0.002	0.545	0.684	>113	69.20	52.10
MN-MALS-LY	0.717	0.001	0.652	0.782	>75	57.30	81.40
MN-UMALS-LY	0.685	0.001	0.618	0.753	>82	48.70	87.90
MN-LMALS-LY	0.724	0.001	0.660	0.788	>64	65.80	70.00
MN-LALS-LY	0.600	0.006	0.330	0.470	≤33	29.90	90.00
MN-AL2-LY	0.535	0.328	0.460	0.611	> 72	27.40	94.30

[Table/Fig-5]: Comparison of volume conductivity and scatter parameters of neutrophil, lymphocytes between healthy controls and sepsis group and the derived cut-off. MN-V: Mean volume; MN-C: Mean conductivity; MN- MALS: Mean median angle light scatter; MN-UMALS: Mean upper median angle light scatter; MN-LMALS: Mean lower median angle light scatter; MN-LALS: Mean lower angle light scatter; MN-AL-2: Mean axial light loss; NE: Neutrophil; LY: Lymphocyte; MO: Monocyte

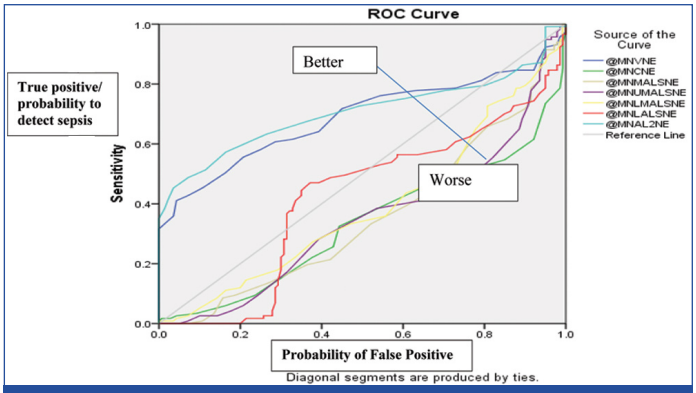
Variables	Area	p-value	95% Confidence Interval		Cut-off	Sensitivity	Specificity
			Lower Bound	Upper Bound			
MN-V-MO	0.728	0.001	0.661	0.796	>178	55.60	90.00
MN-C-MO	0.509	0.814	0.437	0.580	>123	57.30	53.60
MN-MALS-MO	0.504	0.915	0.432	0.575	>95	18.80	87.10
MN-UMALS-MO	0.513	0.719	0.415	0.559	≤105	78.60	12.10
MN-LMALS-MO	0.503	0.925	0.424	0.569	≤72	22.20	87.10
MN-LALS-MO	0.742	0.001	0.197	0.319	≤88	59.80	84.30
MN-AL2-MO	0.593	0.010	0.517	0.668	>130	36.80	94.30

[Table/Fig-6]: Comparison of volume conductivity and scatter parameters of monocyte between healthy controls and sepsis group and the derived cut-off. MN-V: Mean volume; MN-C: Mean conductivity; MN- MALS: Mean median angle light scatter; MN-UMALS: Mean upper median angle light scatter; MN-LMALS: Mean lower median angle light scatter; MN-LALS: Mean lower angle light scatter; MN-AL-2: Mean axial light loss; MO: Monocyte

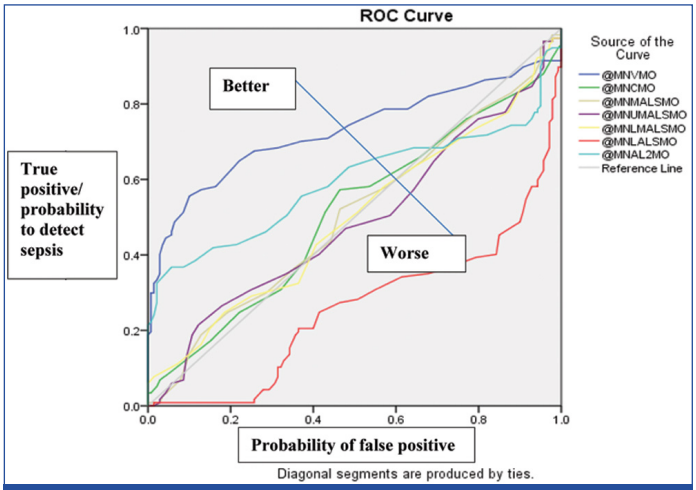


[Table/Fig-7]: The ROC curve to evaluate diagnostic ability of absolute WBC Counts (Ne#, Ly#, Mo#) to discriminate sepsis patients from healthy controls.

high sensitivity and specificity [Table/Fig-5,6]. The area under the curve can be better appreciated in [Table/Fig-7-9], explaining the diagnostic ability of neutrophil and monocyte volume scatter and



[Table/Fig-8]: ROC curve to evaluate diagnostic ability of neutrophil volume, conductivity and scatter to discriminate sepsis patients from healthy controls.



[Table/Fig-9]: ROC curve to evaluate diagnostic ability of monocyte volume, conductivity scatter parameters to discriminate sepsis patients from healthy controls.

conductivity parameters in deriving a cut-off to differentiate healthy controls from sepsis patients. None of the parameters showed an AUC close to 1 along with high sensitivity and specificity.

DISCUSSION

The present study represents a hospital-based cross-sectional descriptive study. It was conducted over a period of one year (January 2021-December 2021) at the study Institute. All adults with culture-positive sepsis or probable sepsis (suspected source of infection along with a >2 SOFA SCORE) were taken up for the study (as per the inclusion criteria). A total of 257 subjects in the hospital were enrolled. In the present study, the maximum number of patients in the healthy group was 31-40 years of age, i.e., 54 (38.57%), followed by the age group 41-50 years of age, i.e., 38 (27.14%). Whereas, in the sepsis group, the maximum number of patients were >60 years of age, i.e., 41 (29.28%). The mean age in the case group was 50.17±13.17 years, and in the control group, the mean age was 38.14±8.78 years. Most of the patients in the sepsis group were elderly, suggesting a predisposition to sepsis secondary to age-related immunodeficiency and other comorbidities. A sepsis screen and blood culture are important tools in determining the status of septicaemia in adults [5]. In present study, 31 (12.06%) subjects had a positive blood culture, while 86 (33.46%) had a negative blood culture. The percentage is variable in some studies done by Celik HT et al., and Shi razi H et al., where culture positivity ranges from 11% to 40% in patients with sepsis or septic shock [7,8]. Microbiological profiles were sought in these 31 cases, and the most common organism isolated was gram negative *Escherichia coli* in 32.2% of subjects. This was followed by fungal growth in 6.45 subjects. Among the gram-positive bacteria in the present study, coagulase-negative staphylococcus was the most common, i.e., 29.03%. *Staphylococcus aureus* was found to be positive in 9.67% of

cases. Umemura Y et al., and Mora-Rillo M et al., have also reported E.coli to be the most common organism isolated in adult sepsis [9,10]. Authors found that the mean Total Leucocyte Count (TLC) of the subjects with septicaemia was 16.76±7.39/mm^3, which was higher than that of healthy controls, which were 6.68±1.42/mm^3. The difference between the two groups was also statistically significant [Table/Fig-1]. Agnello L et al., also observed that the mean TLC of the subjects with sepsis was relatively higher than that of the healthy controls [11]. Authors also found that the MCV, MCH, MCHC, RDW, and MPV were higher in the control group. However, RDW was higher in the sepsis group. While no statistically significant difference was observed in PLT [Table/Fig-1]. A study done by Muady GF et al., showed that severe stress-induced gastrointestinal bleeding/haemodilution from fluid overload, frequent blood draws for lab testing, impaired iron metabolism, haemolysis as a component of the pathogenesis of some infectious processes, bleeding from DIC, and possibly increased red cell destruction due to changes in RBC membranes are all factors in the evaluation of haemoglobin reduction as well as relatively normal MCV, MCH, and MCHC [12]. Martins EC et al., evaluated that, in addition to other metrics, the neutrophil-lymphocyte ratio and band neutrophils may serve as indicators for the early recognition of sepsis in intensive care units. They discovered that sepsis patients had larger concentrations of granulocytes (neutrophils, eosinophils, and basophils) than controls. Both the NLR and band neutrophil concentrations were considerably greater in sepsis patients ($p<0.001$) as well [13]. Similarly, in the present study, significantly higher neutrophil percentage (NE%) in the sepsis patients than the healthy controls cut-off of >70.10% with a sensitivity of 78.60% and a specificity of 97.10%, while LY% <19.74% with a sensitivity and specificity of 84.6 and 97.1, EO% <1.03% with a sensitivity and specificity of 76.9 and 86.9, and absolute eosinophil count <120 has a sensitivity and specificity of 85 and 90.1, respectively, were able to differentiate the sepsis group from healthy controls [Table/Fig-3]. According to Shen XF et al., sepsis impairs neutrophil migration and antimicrobial activity. Sepsis is also closely related to a relative increase in the total number of circulating neutrophils or a rise in the proportion of immature forms. Neutrophils play a role in the development of sepsis. Dohle bodies and toxic granulations are two additional neutrophil products that are thought to be unique indicators of bacterial infection [14]. In the present study, both the absolute neutrophil (NE#) and monocyte (MO#) counts were significantly higher in the sepsis patients than in the controls, while the absolute lymphocyte count (LY#) was found to be lower in some cases compared with the controls [Table/Fig-2]. However, the cut-off for an absolute neutrophil count >2600/cumm has a significant overlap with the healthy population; therefore, it will not be a very reliable marker. The significantly higher WBC count, neutrophil percentage, and absolute counts in the sepsis group compared to healthy controls suggest the premature release of neutrophils from the bone marrow. Farkas JD also evaluated the same thing in their study [15]. Many studies in the past have pointed out the role of eosinopenia as a biomarker for the diagnosis of sepsis [16,17], and present study found concordant results. Arora P et al., also found a significantly higher mean neutrophilic volume in sepsis patients than in the controls, while the Mean Neutrophilic Conductivity and (MNC) Mean Neutrophilic Scatter (MNS) were significantly lower in cases than in the controls. Similar findings are found in other studies [18,19]. In the present study, a significantly higher Mean Neutrophilic Volume (MN-V-NE) and Mean Axial Light loss (MN-AL2-NE) were seen in sepsis patients than in the controls. While MN-C-NE, MN-MALS-NE, MN-UMALS-NE, MN-LMALS-NE, and MN-LALS-NE were significantly lower in cases compared to those in the controls [Table/Fig-4]. The VCS parameter of neutrophils, MN-AL2-NE, showed a cut-off of >143 with a sensitivity of 45.30% and a specificity of 96.40% to predict sepsis, followed by MN-V-NE at a cut-off of >158 with a sensitivity of 41.10% and a specificity of 95.70%, and MN-C-NE at a cut-off of ≤ 141 with a sensitivity of 38.50% and a specificity of 92.10 [Table/Fig-5]. The Coulter DxH 800 uses multiple angles of light scatter. It measures seven distinct scatter parameters in addition to volume and conductivity. Median angle light scatter, lower median angle light scatter, and upper median angle light scatter inform about granularity and membrane. Low-angle light scatter is a measure of the cellular complexity index. The measurement of AL2 provides a measure of the light absorbed by the cell and an indicator of cellular size and is influenced by cellular transparency [4]. A study by Lee AJ et al., investigated these parameters in the DXH 800 cellular analysis system and found that MN-V-NE and MN-UMALS-NE were significantly higher in the sepsis group compared to controls, while (MN-C-NE), MN-MALS-NE, MN-LMALS-NE, and MN-LALS-NE were significantly lower in cases than in controls. MN-AL2-NE was not measured in present study. They discovered that MN-V-NE, with a cut-off >156.5, exhibited a sensitivity of 83.3% and a specificity of 78% in prediction of sepsis [20]. Notably, in present study, MNV demonstrated a sensitivity of 92.7% and a specificity of 40% when using a cut-off value greater than 129.3 [21]. Conversely, the sepsis group demonstrated significantly higher levels of total leukocyte count, absolute neutrophil count, absolute monocyte count, MNV, and CRP compared to those without sepsis (negative-for-sepsis group). They suggested that an MNV greater than 154.2 exhibited a sensitivity of 95.5% and specificity of 82.1% (with an AUC of 0.93) for diagnosing neonatal sepsis [22]. In a study conducted by Kannan A and Selvam P using the LH 780 haematology analyser from Beckman Coulter, Fullerton, CA, significant differences emerged [22]. The study revealed a statistically significant variance in the MNV values between cases and controls, with the mean MNV in cases being higher than that in the control group. This difference in MNV remained significant even when the neutrophil count in cases was below 85% or the WBC count was less than 11,000/cumm. Additionally, the MNS values in cases were lower compared to those in controls, indicating a difference in neutrophil behaviour between the two groups. However, no significant disparity was observed in MNC values between cases and controls [22]. According to Bhargava M et al., among the various indices examined, individuals with sepsis exhibited notably lower levels of haemoglobin, platelet count, absolute lymphocyte count, as well as MNS and MNC [23]. The most helpful VCS metric with the highest specificity for sepsis, with a cut-off MNV >157 having 79% sensitivity and 82% specificity [23]. Another study by Celik IH et al., on the Coulter LH 780 haematology analyser (Beckman Coulter, Fullerton, CA) found that MNV and Volume Distribution Width (VDW) were higher in sepsis than in the control group, while conductivity and scatter were lower in the sepsis group compared to controls [24]. In the current study, sepsis patients had considerably higher MN-V-LY, MN-C-LY, MN-MALS-LY, MN-UMALS-LY, MN-LMALS-LY, and MN-AL2-LY than the controls, while MN-LAL-LY in sepsis is lower compared to the controls. MN-AL2-LY had a cut-off of >72 with a sensitivity of 27.40% and a specificity of 94.30%; MN-LALS-LY had a cut-off of ≤33 with a sensitivity of 29.90% and a specificity of 90.00%; and MN-V-LY had a cut-off of >92 with a sensitivity of 35.90% and a specificity of 89.30% [Table/Fig-5]. A study done in 2021 by Piva E et al., showed that the mean volume of lymphocytes, median angle light scatter of lymphocytes, and upper median angle light scatter of lymphocytes have good clinical practical value in distinguishing bacterial infection from viral infection and healthy controls because of their high sensitivity and specificity [25]. However, these parameters were not useful in present study. In present study, the mean volume (MN-V-MO) and MN-AL2-MO in sepsis patients were higher than in controls, while MN-MALS-MO, MN-UMALS-

MO, MN-LMALS-MO, and MN-LALS-MO were lower in cases when compared with the controls [Table/Fig-4]. The haematological parameter MN-V-MO, with a cut-off of >178, had a sensitivity of 55.60% and a specificity of 90.00%, while MN-AL2-MO showed a cut-off of >130 with a sensitivity of 36.80% and a specificity of 94.30% [Table/Fig-6]. Arora P et al., utilised the LH 750 analyser from Beckman Coulter for their study. They observed that monocytes displayed a significantly higher MMV in sepsis patients compared to controls (179.8±14.16 vs. 164.54±9.6, p=0.001) [19]. Conversely, Mean Monocyte Conductivity (MMC) was found to be lower in cases compared to controls (110.8±6.3 vs. 130.6±2.9, p=0.001). The study identified a cut-off value for MMV >168.3 with a sensitivity of 80.6% and a specificity of 77.5% for detecting sepsis. Additionally, the study noted that MNC, MMC, and MMS displayed increased values post-treatment, a change that was statistically significant (p<0.01) [18]. In a study conducted by Mammen J et al., utilising the Dx800 haematology analyser from Beckman Coulter, notable differences were observed between the sepsis group and the ICU control group. Specifically, the study found that MNV, Mean Neutrophilic Axial Light Loss, MMV, Mean Monocyte Median Angle Light Scatter, and Mean Monocytic Axial Light Loss were higher in the sepsis group compared to the ICU control group. Conversely, MNC was lower in the sepsis group. Importantly, no significant difference was noted between MNS values [16]. There were significantly higher WBC counts, neutrophil percentages, and absolute counts in the sepsis group as compared to healthy controls in present study, suggesting the premature release of neutrophils from the bone marrow. Changes in the volumetric parameters in leukocytes have been described in sepsis and other bacterial infections, pointing toward the fact that changes in volume are a manifestation of an immune response to a severe infection [25]. The overall scatter of the neutrophils and monocytes was reduced in present study, along with increased axial light loss, suggesting reduced overall granularity or increased transparency of the neutrophils and monocytes. This finding is peculiar and interesting and is possibly explained by the relative decrease in neutrophil and monocyte granularity in comparison to the nuclear size [26]. The findings were in concordance with the previous findings [21,23,24], but unlike them, no cut-off could be derived with high sensitivity and specificity.

Limitation(s)

In present study, authors found variation in individual VCS parameters in patients with sepsis compared to healthy controls; however, authors were unable to derive an algorithm with the most sensitive volumetric parameters to differentiate between both groups.

CONCLUSION(S)

Exploring volumetric and light scatter parameters to distinguish sepsis from healthy controls, Mean Neutrophilic Volume (MN-V-NE), Mean Axial Light Loss (MN-AL2-NE), and Mean Neutrophilic Volume of Lymphocytes (MN-V-LY) demonstrated promising sensitivity and specificity in predicting sepsis. However, overlaps in some parameters between sepsis and healthy groups, coupled with variations in cut-off values across studies, underscore the need for further research and validation. The present study enriches understanding of sepsis epidemiology, emphasising the relevance of haematological and volumetric parameters. Future investigations should focus on establishing standardised cut-off values to enhance the clinical utility of these parameters in sepsis diagnosis and management.

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