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Mean Cell Volumes of Neutrophils and Monocytes as Promising Markers of Sepsis in ICU Patients of Sri Ramachandra Medical Centre, Chennai

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ABSTRACT

Backround: VCS (Volume, Conductivity, and Light Scatter) parameters seem to be promising indicators for the diagnosis of sepsis. This study aimed at assessing the diagnostic significance of VCS parameters for sepsis in ICU patients and at comparing their reliability with that of inflammatory markers.
Material & Methods: Patients were divided into 3 groups according to their clinical history and Control (N=60), localized infection (N=40), and systemic infection (N=80).VCS parameters were obtained using a Beckman Coulter LH 780 system. Medical records of sepsis patients are perused by the investigator and Hematological parameters are archived from the laboratory data.
Results:The mean volumes of monocytes (MMV), conductivity and light scatter were higher in the sepsis group than in the control groups and localized infection (P=0.000).No difference was noticed in the mean cell volume, conductivity of neutrophils between groups, however the mean cell volume, scatter of neutrophils in sepsis (p=0.016) was lower than in localized infection and control groups (p=023).

Keywords: Volume of neutrophils, Volume of monocytes, VCS technology, Sepsis

1. INTRODUCTION

The word sepsis is derived from the Greek term for rot-ten or “to make putrid”. The term “severe sepsis” refers to the sepsis-associated failure of multiple organ systems. Sepsis is a serious medical condition caused by an overwhelming immune response to infection. In 1991, the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) agreed on a new definition of sepsis as the development of a systemic inflammatory response syndrome (SIRS) due to infection. The severity of sepsis was graded based on the development of hemodynamic compromise and associated organ dysfunction as follows: severe sepsis, septic shock, and multiple organ dysfunction syndromes (MODS).[1]
Morbidity and mortality remain unacceptably high despite increasing knowledge about the Pathophysiological pathways, processes involved in sepsis and the use of modern antibiotics and resuscitation therapies. Sepsis causes millions of deaths globally each year. It still is one of the most prevalent causes of intensive care units (ICU) morbidity and mortality worldwide. [2]
Sepsis is described as a syndrome consisting of complex pathophysiological and biochemical dysregulation, triggered by endogenous factors in response to the bacterial, viral, parasitic or fungal infections[3].Sepsis, a life-threatening condition characterized by systemic inflammation, often presents with thrombocytopenia, which at times, can be significant[4].Sepsis

is a major issue that has affected the history of healthcare and, to this day, remains a major cause of morbidity and mortality in the modern world.[5]

Infections are notably common among hospitalized patients' Early diagnosis of sepsis is of paramount importance for its appropriate management.[6]Sepsis is a major cause of morbidity and mortality in hospitalized patients worldwide, especially in a developing country like India. The early diagnosis of sepsis is extremely important to reduce the high mortality by prompt initiation of antibiotics.[7]Sepsis is a medical emergency that describes the body's systemic immunological response to an infectious process that can lead to end-stage organ dysfunction and death.[8]

The prevailing circumstances are notably exacerbated in low- and middle-income countries, where the occurrence and death rate of sepsis are markedly greater, and also in the locations that have the least resources to prevent, detect, or treat sepsis.Regardless of a country's economic and healthcare status, sepsis remains a persistent and universal medical concern, as evidenced by the World Health Organization (WHO) designating sepsis as a global healthcare issue.[9]The gold standard for diagnosing sepsis is blood culture but it has time constraints. Till now, pathologists rely on morphological examination of neutrophils in peripheral smears for the prediction of sepsis, but this process has person-to-person variability and is time-consuming.[10]

For patients in the ICU, sepsis is found in about 40% to 50% of patients with AKI in the ICU.1–4 A prospective cohort study including 1177 patients with sepsis across 198 ICUs in 24 European countries reported a 51% incidence of AKI with an ICU mortality rate of 41%. longer ICU and hospital stay, and increased mortality.[11]

The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non-specific nature of the signs and symptoms of sepsis.[12]

Therefore, early and accurate diagnosis is vital to ensure that appropriate medical decisions are made. To this end, clinicians in intensive care units and emergency departments need to predict systemic infection at an early stage. However, early diagnosis of sepsis is difficult because the signs and symptoms of sepsis in icu patients, such as fever, are nonspecific and may be blunted or absent. Therefore, laboratory findings are important in the diagnosis of sepsis[4]. The blood counts can be determined using an automated hematology analyzer. The latest automatic hematology analyzers, such as the Coulter LH 780 (Beckman Coulter, Fullerton, CA), can use a variety of volume conductivity scatter (VCS) technologies .[13]

Modern automated hematology analyzers, in addition to accurately and rapidly enumerating and classifying blood cells, also yield several quantitative leukocyte parameters. These have been shown to be valuable in myriad clinical settings. The volume, conductivity and scatter parameters (VCS, Beckman Coulter).[14]During sepsis, leukocytes especially circulating PMNs, undergo significant changes in number, size, roundness, granularity and chemotaxis, due to mobilization of immature, larger neutrophils from bone marrow, cell degranulation, activation by circulating bacterial fragments, activated complement proteins, and intracellular generation of reactive oxygen species. These changes in circulating leukocytes positively correlate with sepsis disease severity and prognosis. Currently, precise quantification of leukocytes changes during sepsis requires the use of specialized equipment such as flow cytometry dedicated methods such as, microfluidic-based cell migration, 'volume, conductivity and scatter' (VCS) measurements.[15]

The Beckman Coulter LH 780 cellular analysis system can evaluate mean neutrophil volume (MNV) , mean neutrophil conductivity (MNC) ,mean neutrophil scatter (MNC) and mean monocyte volume (mmv), conductivity (MNC), scatter (MNS). These parameters can be searched in the research population data by volume, conductivity, and scatter (VCS) technology. VCS parameters seem to be promising indicators for the diagnosis of sepsis. [16]

2. MATERIAL & METHODS

Patients were divided into 3 groups according to their clinical history and Control (N=60), localized infection (N=40), and systemic infection (N=80).VCS parameters were obtained using a Beckman Coulter LH 780 system. Medical records of sepsis patients are perused by the investigator and Hematological parameters are archived from the laboratory data.

Statistical analysis

Statistical analysis was performed using the SPSS software, version 13.0 (SPSS, Chicago, IL, USA). Results are expressed as mean±standard deviation. The CBC and VCS parameters were compared between different groups namely sepsis with controls, localized sepsis with controls, systemic with controls and localized sepsis with systemic sepsis.

3. RESULTS

The mean volumes of monocytes (MMV), conductivity and light scatter were higher in the sepsis group than in the control groups and localized infection (P=0.000).No difference was noticed in the mean cell volume, conductivity of neutrophils between groups, however the mean cell volume, scatter of neutrophils in sepsis (p=0.016) was lower than in localized infection and control groups (p=023).

Table: 1 STUDY POPULATION

	GROUP 1 NORMAL CONTROL (NO=60)	GROUP 2 LOCALISED INFECTION (NO=40)	GROUP 3 SYSTEMIC INFECTION (NO=80)	ALL GROUPS P value (between groups)
AGE	43.9±13.3	41.2±23.9	57.8±20.2	.000
GENDER M/F	28/32	19/21	48/32	

Table:2
COMPARISION OF PARAMETERS OF SEPSIS AND CONTROL GROUP

	ALL GROUPS	P value
NEUTROPHIL VOLUME	Between Groups	.725
NEUTROPHIL CONDUCTIVITY	Between Groups	.871
NEUTROPHIL SCATTER	Between Groups	.007
MONOCYTE VOLUME	Between Groups	.000
MONOCYTE CONDUCTIVITY	Between Groups	.009
MONOCYTE SCATTER	Between Groups	.125
DIFFERENTIAL COUNT NEUTROPHIL%	Between Groups	.000
DIFFERENTIAL COUNT MONOCYTE %	Between Groups	.448
TOTAL COUNT	Between Groups	.000

P value <0.05(ANOVA) was considered statistically significant.

Table: 3 Comparison of Parameters between Groups and Controls
Multiple Comparisons

DEPENDENT VARIABLE	Group 1	Group 2	P value
NEUTROPHIL VOLUME	Control	Localized	.772
		Systemic	.768
	Localized	Systemic	.993
NEUTROPHIL CONDUCTIVITY	Control	Localized	.997
		Systemic	.875
	Localized	Systemic	.933
NEUTROPHIL SCATTER	Control	Localized	**.023**
		Systemic	**.016**
	Localized	Systemic	.936
MONOCYTE VOLUME	Control	Localized	**.000**
		Systemic	**.001**
	Localized	Systemic	.497
MONOCYTE CONDUCTIVITY	Control	Localized	**.032**
		Systemic	**.015**
	Localized	Systemic	979

MONOCYTE SCATTER	Control	Localized	.214
		Systemic	.162
	Localized	Systemic	.986
DIFFERENTIAL COUNT NEUTROPHIL%	Control	Localized	**.000**
		Systemic	**.000**
	Localized	Systemic	.694
DIFFERENTIAL COUNT MONOCYTE %	Control	Localized	.503
		Systemic	1.000
	Localized	Systemic	.471
TOTAL COUNT	Control	Localized	.050
		Systemic	**.000**
	Localized	systemic	**.008**

P value <0.05(post hoc analysis) was considered statistically significant.

TABLE: 4 WBC AND VCS PARAMETERS IN THE STUDY GROUP AND CONTROL GROUP

PARAMETERS		N	MEAN	STD. DEVIATION
NEUTROPHIL VOLUME	Control (normal)	60	164.95	165.77
	Localized infection	40	151.47	9.65
	Systemic infection	80	153.55	13.85
	TOTAL	180	156.89	95.89
NEUTROPHIL CONDUCTIVITY	Control (normal)	60	134.28	5.61
	Localized infection	40	134.38	7.17
	Systemic infection	80	134.82	6.6
	TOTAL	180	134.54	6.39
NEUTROPHIL SCATTER	Control (normal)	60	141.7	4.66
	Localized infection	40	135.35	21.46
	Systemic infection	80	136.13	7.84
	TOTAL	180	137.81	11.93
MONOCYTE VOLUME	Control (normal)	60	162.02	19.29
	Localized infection	40	177.84	9.35
	Systemic infection	80	173.82	20.88
	TOTAL	180	170.78	19.37

MONOCYTE CONDUCTIVITY	Control (normal)	60	109.43	14.48
	Localized infection	40	114.61	6.95
	Systemic infection	80	114.23	6.51
	TOTAL	180	112.72	10.19
MONOCYTE SCATTER	Control (normal)	60	78.14	12.27
	Localized infection	40	81.37	10.72
	Systemic infection	80	81.08	5.26
	TOTAL	180	80.16	9.43
DIFFERENTIAL COUNT **NEUTROPHIL %**	Control (Normal)	60	57.92	6.24
	Localized infection	40	72.62	10.6
	Systemic infection	80	74.38	13.87
	TOTAL	180	68.5	13.38
DIFFERENTIAL COUNT **MONOCYTE %**	Control (normal)	60	6.29	1.08
	Localized infection	40	5.18	1.98
	Systemic infection	80	6.28	7.07
	TOTAL	180	6.04	4.85
TOTAL COUNT	Control (Normal)	60	7715	1851.59
	Localized infection	40	10640	5760.68

TABLE: 5 NEUTROPHIL SCATTER-STATISTICALLY SIGNIFICANT

		NO	MEAN	SD	P value
NEUTROPHIL SCATTER	Control	60	141.7	4.66	0.007
	Localized	40	135.35	21.46	
	Systemic	80	136.13	7.84	
	TOTAL	180	137.81	11.93	

FIGURE: 1

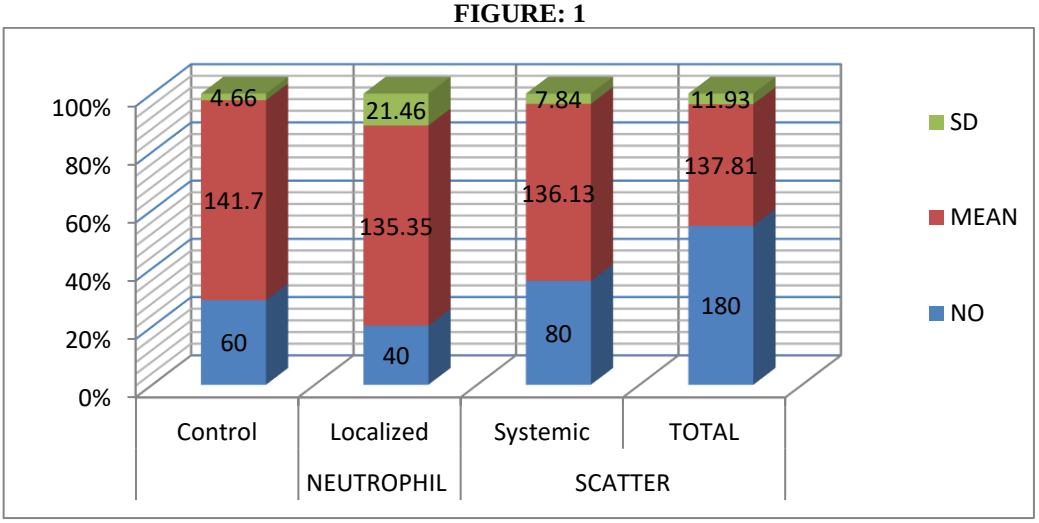


TABLE: 6 MONOCYTE VOLUMES - STATISTICALLY SIGNIFICANT

		NO	MEAN	SD	P value
MONOCYTE VOLUME	control	60	162.02	19.29	.000
	localized	40	177.84	9.35	
	Systemic	80	173.82	20.88	
	TOTAL	180	170.78	19.37	

FIGURE: 2

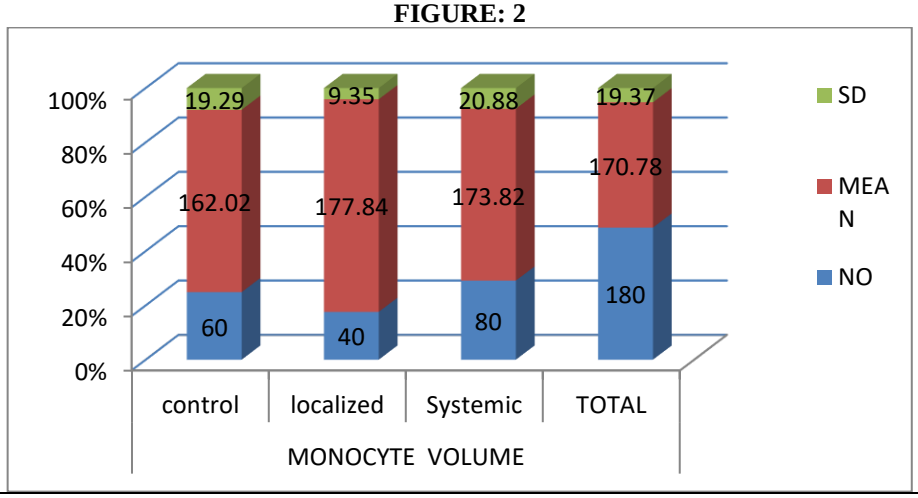


TABLE: 7 MONOCYTE CONDUCTIVITY- STATISTICALLY SIGNIFICANT

		NO	MEAN	SD	P Value
MONOCYTE CONDUCTIVITY	control	60	109.43	14.48	.009
	Localized	40	114.61	6.95	
	Systemic	80	114.23	6.51	
	TOTAL	180	112.72	10.19	

FIGURE: 3

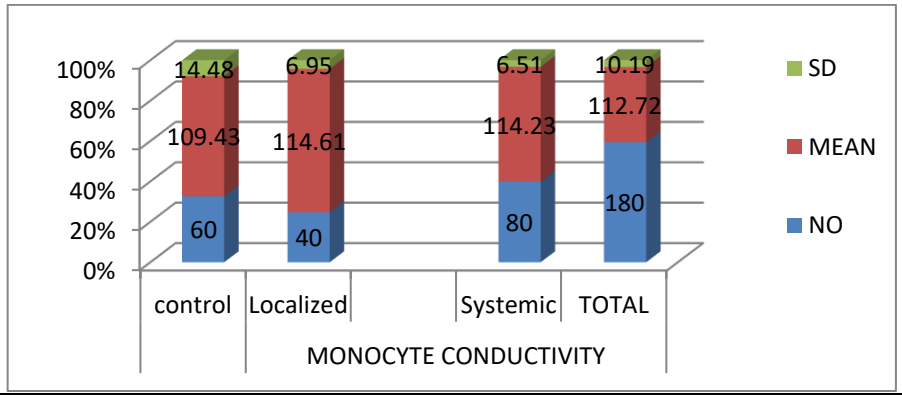


TABLE: 8 DIFFERENTIAL COUNT – STATISTICALLYSIGNIFICANT NEUTROPHIL%

		NO	MEAN	SD	P Value
DIFFERENTIAL COUNT NEUTROPHIL%	Control	60	57.92	6.24	.000
	Localized	40	72.62	10.6	
	Systemic	80	74.38	13.87	
	TOTAL	180	68.5	13.38	

FIGURE: 4

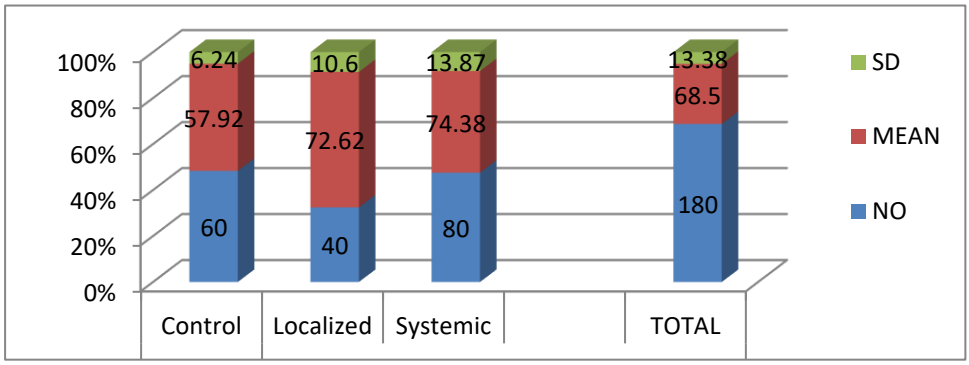


TABLE: 9 TOTAL COUNT - STATISTICALLY SIGNIFICANT

		NO	MEAN	SD	P Value
TOTAL COUNT	Control	60	7715	1851.59	.000
	Localized	40	10640	5760.68	
	Systemic	80	14242.5	8164.011	
	TOTAL	180	11266.11	6786.02	

FIGURE: 5

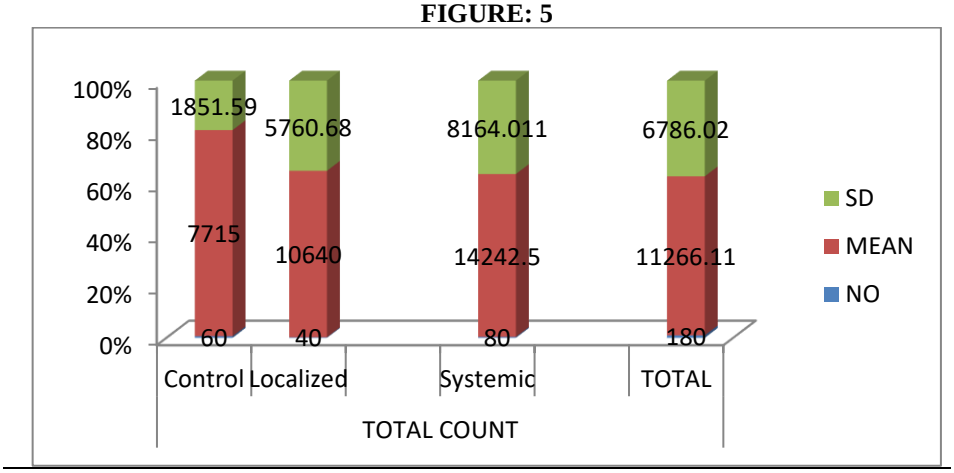
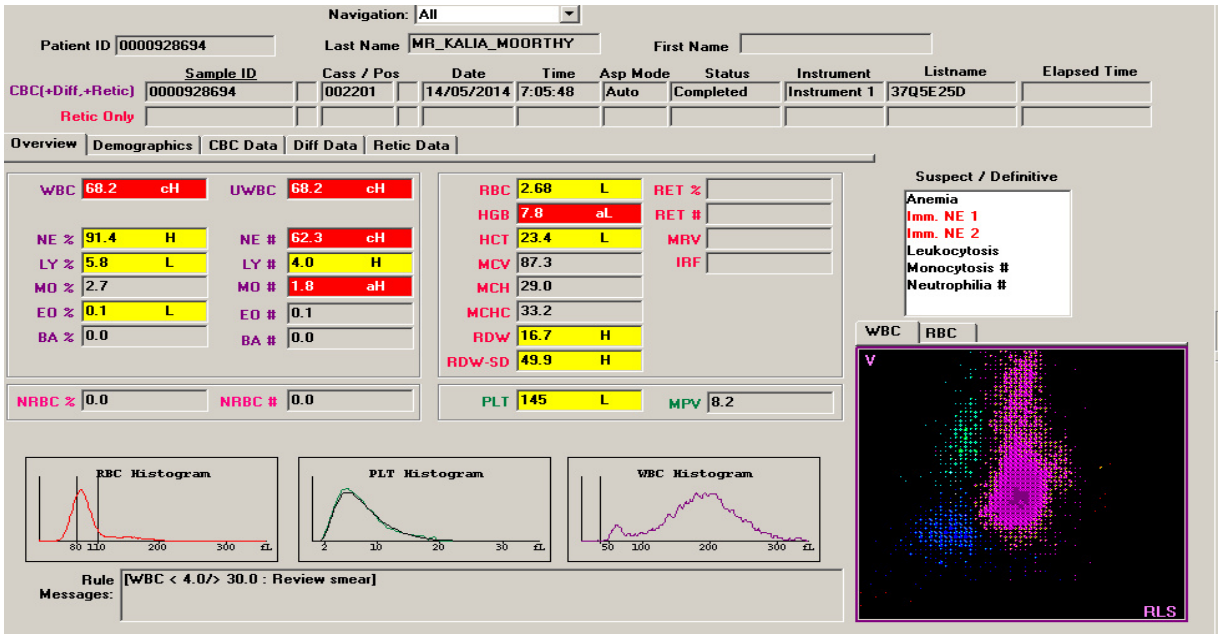
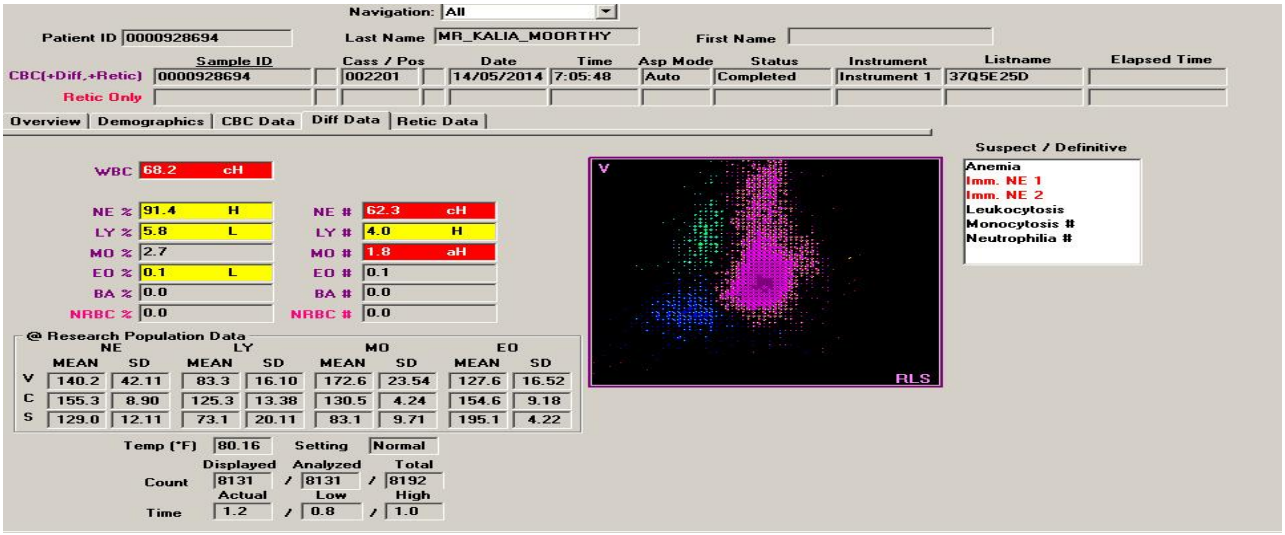


FIGURE: 6 ANALYSER DISPLAY OF CBC REPORT



The VCS technology parameters displayed in the research population mode is as follows

FIGURE: 7 ANALYSER DISPLAYS OF RESEARCH POPULATION DATA



4. DISCUSSION

WBC count as well as the percentage of neutrophils and immature granulocytes can be measured using automated hematology analyzers and have been used to predict infection. We demonstrated that sepsis patients had higher MNV values and lower MNC and LMALS of MNS values than non-sepsis patients. They concluded MNV and MMV may be useful as discriminators to distinguish between sepsis and non sepsis patients. however in our study the mean volume of monocytes (MMV) and monocyte conductivity (MMC) significantly higher in the localized and systemic sepsis group than in the control groups but the difference between localized and systemic infection was not statistically significant (p=0.001 for control groups) .No difference was noticed in the mean cell volume and conductivity of neutrophils between groups .The mean cell scatter of neutrophil in sepsis was lower in the localized infection and systemic infection than control group ; however the difference between localized infection and control group and localized infection and systemic infection were not statistically significant . Significant difference was noted between systemic infection and control group. The mean differential neutrophil count and the total WBC count for both localized and generalized sepsis was significantly higher compared to the other groups .The difference in values between localized and generalized infection groups was not statistically significant .Thus MMV, MMC and MNS may also be considered as distinguishing to separate sepsis and non-sepsis patients

5. CONCLUSION

The use of VCS parameters in the clinic setting can provide several advantages. First, these parameters are determined during differential analysis without additional specimen requirements. Second, determining these values is not labor-intensive or time-consuming, which are issues in manual differentiation. Finally, these values are more accurate, objective, and quantitative than manual differential counts because more than 8,000 WBCs are evaluated automatically with the VCS instrument. The VCS parameters can be included in the infectious disease screening investigation list in addition to the existing infectious laboratory panel to predict sepsis early.

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