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RESEARCH ARTICLE

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Discriminant value of automated leucocyte VCS parameters in the detection of tropical infections

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Introduction: In India, infectious diseases are a leading treatable cause of morbidity and mortality. Mangalore being endemic to many vector-borne diseases, their incidence is known to show seasonal variations with sharp increase during monsoon. Leucocytes have substantial role in the immunological pathogenesis of infections.

Methods: The present series was a hospital-based cross-sectional study performed in a tertiary care hospital for a period of three months from June-August wherein the cell population data of cases of malaria, dengue, leptospirosis, typhoid and rickettsial infections along with equal number of healthy controls were collected and analysed. Effectiveness of leucocyte-related volume (V), conductivity (C) and scatter (S) parameters by Coulter®DXH800 haematology analyser in predicting these infections was appraised.

Results: A total of 324 cases comprising of malaria (50%), dengue (30.9%), leptospirosis (13.9%), typhoid (4.0%) and rickettsial infections (1.2%) were included. There was statistically significant differences ($P < 0.05$) in the mean values of complete blood count parameters—haemoglobin, total leucocyte count, red blood cell count, haematocrit, red cell distribution width, differential leucocyte count, platelet count and plateletcrit between cases and controls and also between specific infections. The mean volumes of neutrophil, monocyte and lymphocyte were considerably increased in malaria and dengue fever compared to leptospirosis, typhoid and rickettsial infections. VCS parameters were the least altered in typhoid fever, except for a strikingly high conductivity and scatter of eosinophils.

Conclusions: Haematological analysis is a part of routine evaluation of any case of febrile illness. This study showed that there are specific alterations in VCS parameters in different types of infections such as malaria, dengue, leptospira, typhoid and rickettsia, the information and analysis of which comes without any additional cost.

Abbreviations: AL2, Axial light loss; C, Conductivity; CBC, Complete blood count; CPD, Cell population data; DLC, Differential leucocyte count; F, Fraction; fL, Femtolitre; Hb, Haemoglobin; HCT, Haematocrit; IgM, immunoglobulin M; LALS, Low-angle light scatter; LD, Lower discriminator; LMALS, Lower median-angle light scatter; MALS, Median-angle light scatter; MCH, Mean cell haemoglobin; MCHC, Mean cell haemoglobin concentration; MCV, Mean cell volume; MLV, Mean lymphocyte volume; MMV, Mean monocyte volume; MNV, Mean neutrophil volume; MPV, Mean platelet volume; PC, Platelet count; PCT, Plateletcrit; PDW, Platelet distribution width; QBC, Quantitative buffy coat; RBC, Red blood cell; RDW, Red cell distribution width; S, Scatter; SD, Standard deviation; T1, Trough 1; T2, Trough 2; UD, Upper discriminator; UMALS, Upper median-angle light scatter; V, Volume; WBC, White blood cell.

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KEYWORDS
cell population data, mean neutrophil volume, tropical, vector-borne, volume-conductivity-scatter data

1 | INTRODUCTION

Malaria and dengue are highly endemic in most parts of India. Mangalore, a city in coastal Karnataka, India, has high prevalence of both these vector-borne infections with extensive range of clinical presentations. The incidence of these infections peak during monsoon and are associated with increased mortality and morbidity.[1,2] In 2018, the reported number of malaria cases was 4 29 928, from India, accounting for 4% of malaria burden worldwide and 87% of burden from southeast Asia.[3] Karnataka reported 8174 cases, out of which 7042 (86.2%) cases were reported from Mangalore alone.[4] National Vector Borne Disease Control Programme under union health ministry site has recorded a provisional number of 3,38,513 positive malaria cases with 50 deaths reported from India in 2019.[5] The outbreak of these infections is common during and after the rainy season. Clinical symptoms of these infections intersect each other and those seen in leptospirosis, typhoid fever and rickettsial infections.[1,2,6-9] The latter three, though not as common as malaria and dengue fever, pose diagnostic difficulties in their early phases. The clinical presentation can vary from a mild febrile illness to lethal complications such as disseminated intravascular coagulation.[1,10]

Early suspicion and specifically directed investigations leading to timely diagnosis of these infection is crucial and can reduce the disease-related morbidity and mortality considerably. This necessitates an inexpensive tool that can predict the disease. Further specific investigations can quickly confirm the infection. This can substantially improve the disease outcome by promoting early initiation of judicious and timely supportive therapy, evading unwanted drugs.

There is a battery of laboratory tests for the infections in questions but they can be expensive and time-consuming if all are done together or randomly, and above all expertise at various is required to interpret them. Even though leucopenia or thrombocytopenia is common in these infections and some cases do present with normal blood counts, which can be misleading and thus adjourning the diagnosis, increasing the number of other unrelated investigations as well as the chance of developing complications.[10,11]

Newer versions of Coulter analysers have incorporated advanced technology that analyse and measure the various morphological features of leucocytes (neutrophils, lymphocytes, monocytes and eosinophils), thus giving additional information. This technology quantifies volume of the cells by voltage impedance (V); nuclear-cytoplasmic ratio by radiofrequency conductivity (C); and internal cellular features such as cytoplasmic granularity and nuclear complexity by laser light scatter (S) and hence known as VCS technology. These are measured as numerical values and are called cell population data (CPD). This technology has tremendously improved the diagnosis of various infectious and non-infectious diseases at a very early stage of presentation.[10,11]

We propose to compare automated parameters by VCS technology that can predict and aid in rapid and reliable prediction of specific infections such as malaria, dengue, leptospirosis, typhoid and rickettsial infections.

There are various studies in the literature that have discussed the changes in VCS parameters in malaria and dengue fever. Nevertheless, the data of VCS for other causes of fever such as rickettsia, leptospirosis or typhoid fever have not been explored or published. Besides an observational pilot study conducted by Kalra et al (Uttarkhand, North India) on 200 cases of acute undifferentiated febrile illness,[12] there is no similar study from Indian subcontinent and none from Southern India that addresses the utility of VCS parameters in bacterial (typhoid fever and leptospirosis), zoonotic bacterial (rickettsia), viral (dengue fever) and parasitic (malaria) infections. To the best of our knowledge, this is the largest study from India that includes 648 subjects (cases and controls) where five different infections have been compared with equal number of controls. As a continuation to this study, the authors are working to create an algorithmic approach based on VCS parameters in differentiating bacterial, viral and parasitic infections.

2 | MATERIALS AND METHODS

2.1 | Study setting

The present hospital-based cross-sectional study was performed in a tertiary care hospital in the urban area of Mangalore. The current study included cases of acute febrile illness diagnosed with malaria, dengue, leptospirosis, typhoid fever and rickettsial infections during the monsoon period over 3 months from June to August (peak monsoon season in Mangalore). The sample size was calculated with an assumption of 30% of the subjects in febrile illness population have the various infections of interest, with 5% absolute precision and 95% confidence interval. With that the sample size came to 324 for estimating the expected proportion. Institutional Ethical Committee (IEC) approval was obtained for the study.

2.2 | Selection of cases and methodology

The peripheral blood samples from cases with infections (malaria, dengue, leptospirosis, typhoid and rickettsial diseases) were studied in the Coulter ® DXH800 haematology analyser (Beckman Coulter Inc., Miami, FL, USA). The inclusion criteria were as follows: 1) patients of age 18 years and above; 2) serologically positive cases of

dengue, leptospirosis, typhoid and rickettsia; and 3) malaria cases diagnosed by florescence microscopy- quantitative buffy coat (QBC) method and were confirmed by peripheral smear examination which is the gold standard method of evaluation. Based on smear examination, the malaria cases were further categorized as vivax, falciparum and mixed malaria. The dengue cases were taken as positive based on positivity using either dengue NS1 test (immunochromatography card) or dengue IgM panbio ELISA. The leptospirosis cases were considered positive based on IgM panbio ELISA test. Positivity of either Widal test or blood culture and Weil Felix test were considered for selection of typhoid and rickettsial infections, respectively. The blood samples from an equal number healthy controls (data anonymised) who were seeking routine health check-up at the study hospital at the same time as those with febrile illnesses were included. Cases with co-infections and cases where complete blood count analysis by using the Coulter ® DXH800 haematology analyser were not performed, were excluded from the study.

2.3 | Variables analysed

The patients' clinical and demographic data were obtained from medical records. The parameters studied were the complete blood count that includes haemoglobin (Hb), total leucocyte count (TLC), platelet count (PLT), differential leucocyte count (DLC), red blood cell indices [RBC indices- mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC)], RBC count, red blood cell distribution width (RDW), platelet indices [mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW)]. The VCS parameters included in the study are volume (V), conductivity (C), MALS (median-angle light scatter), UMALS (upper median-angle light scatter), LMALS (lower median-angle light scatter), LALS (low-angle light scatter) and AL2 (axial light loss) in neutrophils, lymphocyte, monocyte and eosinophils.

2.4 | WBC histograms and DLC scatterplots

The WBC histograms and the differential leucocyte count scatterplots were analysed for five different infections and compared with healthy control group. In a WBC histogram, there are 2 discriminators, the first being lower discriminator (LD) towards 35fL and upper discriminator (UD) towards 450fL and the cells falling between these two discriminators are the WBCs. There are two troughs, valley discriminators, T1 (78-114fL) and T2 (less than 150fL). The area between LD and T1 has lymphocytes (F1- 35 to 90fL); between T1 and T2 has monocytes, basophils and eosinophils along with immature granulocytes—blasts, promyelocytes, myelocytes and metamyelocytes (F2- 90 to 160fL) and area between T2 and UD has neutrophils (F3- 160 to 450fL) [F stands for fraction; Figure 1A].[13,14] The DLC scatterplots in healthy controls is explained in Figures 2A, 3A and 3B.

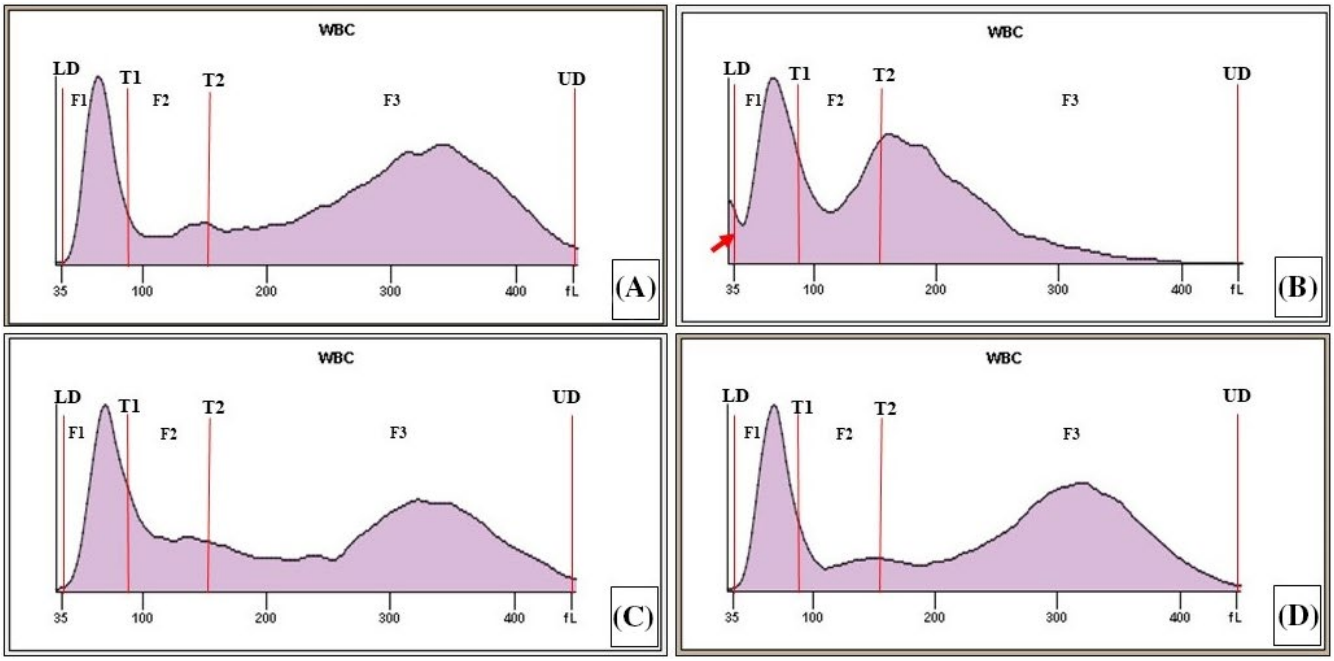

FIGURE 1 WBC Histogram: A: Healthy control: LD and UD discriminators at 35fL and 450fL; first imaginary discriminator T1 at 78-114fL and second imaginary discriminator T2 at 150fL. F1 peak (between LD and T1) consists of lymphocytes, F2 (between T1 and T2) represents monocytes, eosinophils, basophils and immature granulocytes and F3 (between T1 and LD) represents mature neutrophils. B: Malaria: Presence of pre-threshold peak at LD (35fL) representing parasitized un-lysed RBCs that enters WBC chamber is highly characteristic (arrow). There is a shift to right of T1 and obliteration of T2. C: Dengue: There is shift to right of T1 and T2 with widening of F1 peak. D: Typhoid: The shift to right of T1 is mild. The WBC histogram is almost comparable to that seen in healthy control. Similar histograms were noted in leptospirosis and rickettsia

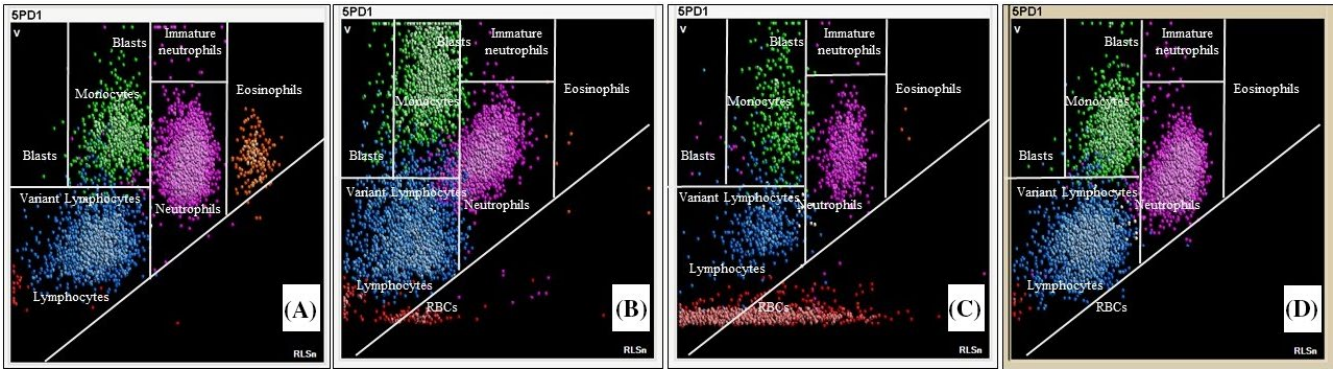

FIGURE 2 WBC differential count scatterplot: A: Healthy control scatterplot with separate windows for lymphocytes (blue), monocytes (green), neutrophils (purple) and eosinophils (orange). Note the windows where blasts, variant lymphocytes, immature neutrophils and un-lysed RBCs can fall. B: Malaria: Lymphocytes plots extend into variant lymphocyte window and monocyte plots extend into blast window. Note the obliteration of clear demarcation between the windows of lymphocytes, neutrophils and monocytes. C: Dengue: Lymphocytes plots are seen extending into variant lymphocyte window and monocyte plots into blast window. Leucopenia was noted in this patient. D: Typhoid fever: The WBC differential count scatterplot was comparable to that seen in healthy control. Similar scatterplots were noted in leptospirosis and rickettsia

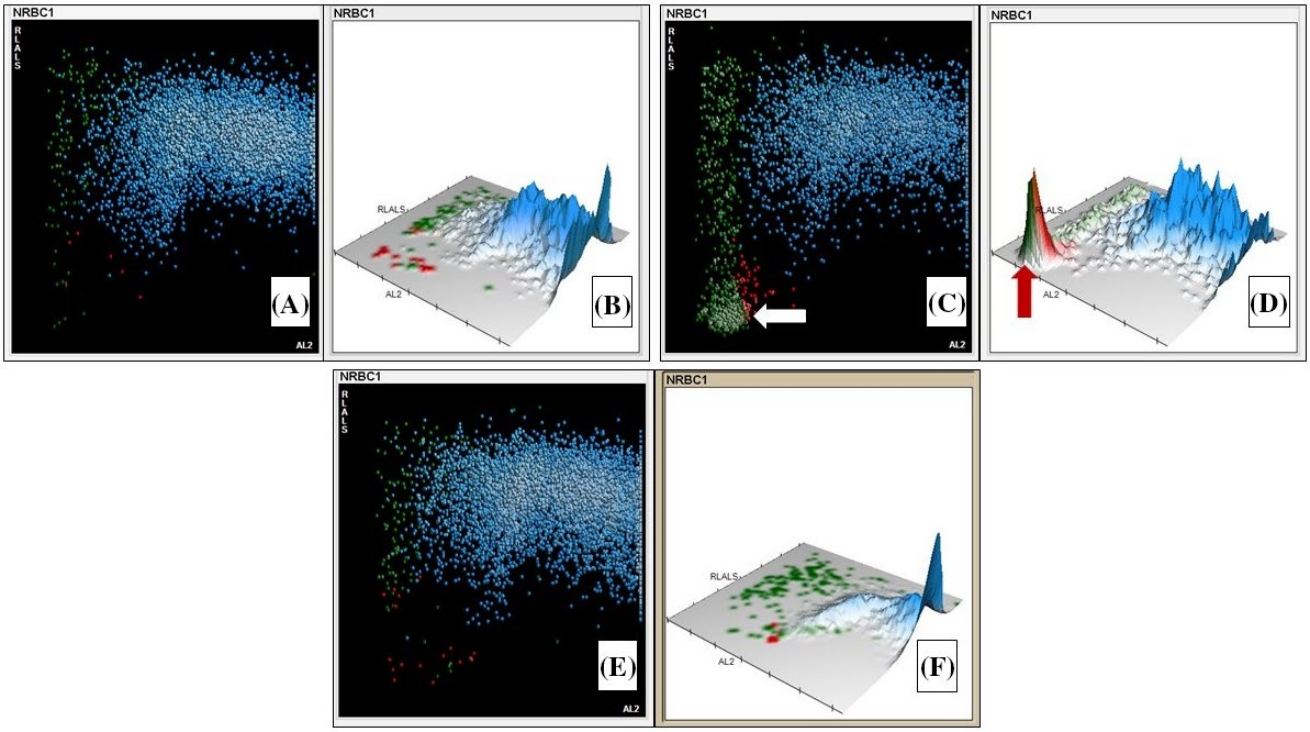

FIGURE 3 Nucleated RBC plot: A (2D plot), B (3D plot): Healthy control: Axial light loss is plotted on x-axis and low-angle light scatter on y-axis. Measurement of AL2 separates the leucocytes from nucleated red blood cells. Nucleated RBC plot can be used in discrimination of leucocytes (blue) from presence of NRBC (red), platelet (green) clumps, giant platelets and parasitized un-lysed RBCs. C,D: Malaria: The white arrow (C) and red arrow (D) shows tight green cluster representing parasitized un-lysed RBCs. E,F: In dengue fever, leptospirosis, typhoid fever and rickettsia, nucleated RBC plot was comparable to that seen in healthy control

2.5 | Data analysis

The collected data were coded and entered onto IBM SPSS Statistics for Windows, version 25.0 (Armonk, NY: IBM Corp.). Results were expressed as proportions and summary measures (median with inter quartile range) using appropriate tables. The values obtained and computed during the data collection did not follow normal distribution. Hence, non-parametric tests were used to compare these variables across the groups. Kruskal-Wallis test was used to compare the data across the five groups.

Mann-Whitney's test was used to analyse the variables in between the groups, post hoc. A $P < 0.05$ was considered to be statistically significant.

3 | RESULTS

A total of 324 patients fulfilled the inclusion criteria of infection group and equal number of controls were included in the study.

3.1 | Distribution of cases

Among the infection group, there were 162 cases of malaria (50.0%), 100 cases of dengue (30.9%), 45 cases of leptospirosis (13.9%), 13 cases of typhoid (4.0%) and 4 cases of rickettsial infections (1.2%) diagnosed during the study period.

3.2 | Demographic details and complete blood count parameters

The mean age among 324 patients was 41.6 years with a range of 18–85 years. The mean age of presentation in individual infection groups is as follows: malaria-39.2, dengue-43.4, leptospirosis-47.9, typhoid-33.5 and rickettsia-49.8 years. There were 232 men (71.6%) and 92 women (28.4%). The data regarding the demographic and complete blood count (CBC) among the five different infections are given in Table 1. There were 324 healthy controls of which 192 were men (59.3%) and 132 were women (42.74%).

TABLE 1 Comparison of the demographic details and CBC among the five infections

	Control group#	All infections	Malaria	Dengue	Leptospirosis	Typhoid	Rickettsia
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total No.	324	324	162	100	45	13	4
M:F ratio	1.5:1	2.5:1	4.6:1	1.1:1	4:1	1.6:1	1:1
RBC parameters							
RBC	4.6 ± 2.3 ^{M,D,L}	4.6 ± 0.8	4.7 ± 0.6^{D,L,T}	4.9 ± 0.8^{L,T}	4.1 ± 0.8	4.3 ± 0.7	4.3 ± 0.5
Hb	12.7 ± 1.9^{A,M,D}	13.4 ± 2.1	13.5±2^{L}	14 ± 2.2^{L,T}	12.2 ± 2	12.7 ± 1.9	12.5 ± 1.9
HCT#	NA	39.1 ± 6.5	39.3 ± 5.6^{D,L}	41 ± 7.3^{L,T}	34.8 ± 6.2	36.6 ± 4.6	36.1 ± 5
MCV#	NA	85 ± 7.4	84.9 ± 7.4	84.6 ± 7.8	86.1 ± 7.3	84.6 ± 5.4	86.8 ± 4
MCH#	NA	29.2 ± 2.9	29.2 ± 2.9	28.9 ± 3.1^{L}	29.7 ± 2.9	29.1 ± 2.3	29.7 ± 2.6
MCHC	NA	34.2 ± 2	34.3±2^{D}	34 ± 1.3^{L}	34.5 ± 0.9	32.8 ± 6.1	34.4 ± 1.1
RDW#	NA	14.3 ± 1.7	14.2 ± 1.8^{L}	14.2±2^{L}	14.7 ± 1.2	14.1 ± 0.6	14.2 ± 0.6
RDW-SD#	NA	42.4 ± 4.6	42.3 ± 4.5^{L}	41.6 ± 4.7^{L}	44.2 ± 4.6	42 ± .3.2^{L}	43 ± 1.1
Platelet parameters							
Platelet	295 ± 82.1^{A,M,D,L,T,R}	94.6 ± 71.1	99.4±55^{D,L,T,R}	77 ± 76.8 ^{T,R}	80 ± 79.9	190.4 ± 78.8^{L}	176.5 ± 81.2^{L}
MPV	8.0 ± 0.9^{A,M,D,L,T,R}	8.9 ± 1.1	8.9 ± 1.1	9±1^{L}	8.5 ± 1.2	8.8 ± 1.2	9.4 ± 1.3
PCT#	NA	0.06 ± 0.06	0.06 ± 0.05^{D}	0.5 ± 0.06	0.06 ± 0.07	0.4 ± 0.07^{L}	0.2 ± 0.05
PDW#	NA	17.5 ± 1.2	17.4 ± 1.1^{D}	17.7 ± 1.2	17.6 ± 1.4	17.1 ± 0.7^{L}	17.6 ± 0.5
WBC parameters							
WBC	7.6 ± 1.7^{A,M,D,L,T,R}	6.7 ± 4.3	5.5 ± 1.9^{L,T,R}	6.7±5^{L,R}	10.7 ± 6.2	6.4 ± 1.4 ^{L,R}	11.9 ± 5.7
Neutrophil (%)	60.2 ± 7.5 ^{M,D,L,R}	62.2 ± 18.8	67.9 ± 14.8^{D,L,T,R}	49 ± 17.7^{L,T}	73.4 ± 18.9	60.2 ± 14.4^{L,R}	44.9 ± 0.00^{L}
Lymphocyte (%)	28.4 ± 7.0^{A,M,L,R}	21.9 ± 12.6	19.1 ± 10.3^{D,L,T}	28.7 ± 12.6^{L,R}	14.3 ± 12.4	30.7 ± 12.1^{L}	15.7 ± 10.8
Monocyte (%)	7.6 ± 1.5^{A,M,D}	13.2 ± 8.4	10.8 ± 5.1 ^{D,L,T}	20.1 ± 10.1^{L,T,R}	8.8 ± 4.8	8±3^{R}	6.6 ± 4.5
Eosinophil (%)	3.2 ± 1.9^{A,M,D,L,T,R}	1.2 ± 1.7	0.9 ± 1.3^{L,T}	1.5 ± 2.2 ^{T}	1.3 ± 1.4	0.7 ± 1.6^{L}	1.1 ± 1.3
Basophil (%)	0.6 ± 0.3^{L,T}	0.7 ± 0.7	0.6 ± 0.5^{L,T}	0.8 ± 1.1^{L,T}	0.5 ± 0.6	0.2 ± 0.3^{L}	0.3 ± 0.5

Abbreviations: A-all infections; C-control; D-dengue; L-leptospirosis; M:F-male:female; M-malaria; No-number; R-rickettsia; SD-standard deviation; T-typhoid.

#The control group was not analysed for these parameters. The control group was used mainly to compare the data related to VCS parameters.

*Significantly different among the different groups.

The mean of Hb, PC, MPV, WBC count, lymphocyte, monocyte and eosinophil differential leucocyte count showed statistical significant differences between the case and control groups. The cases were sub-categorized based on specific aetiology and analysis of haematological parameters, and VCS data were performed in relation to controls. When the results were compared between the specific aetiological groups, there were statistical differences in the mean values of Hb (0.0), WBC count (0.0), RBC count (0.0), HCT (0.0), RDW (0.01), RDW-SD (0.0) PC (0.0), DLC (0.0) and PCT (0.03) between them (*P* value is stated within the brackets for each parameter which all are <0.05). However, no statistical significant difference was observed with RBC and platelet indices (MCV, MCH, MCHC and MPV, and PDW) between the groups (Table 1).

3.3 | Histograms, scatter plots, CBC and VCS parameters in individual infection

The various VCS parameters were found to have statistically significant differences between the five aetiological groups. There were significant differences in the volume, conductivity and scatter plots and data obtained in various infections. Tables 2 and 3 represents the various VCS parameters across the specific aetiological groups.

TABLE 2 Comparison of mean value of the VCS parameters among the five infections

VCS	Control	All	Malaria	Dengue	Leptospirosis	Typhoid	Rickettsia
Neutrophil parameters							
NV	143.2 ± 9.2^{A,M,D,L,R}	151.5 ± 10.3*	152.5 ± 9.6^{D,L}	147.2 ± 8.5^{L}	157.1 ± 12.9	152.1 ± 11	154.5 ± 12.8
NC	149.2 ± 8.5^{A,M,D,L}	138.2 ± 5.7*	138.6 ± 5.3^{D}	137.3 ± 3.3	140.0 ± 8.9	141.1 ± 9.6	138.6 ± 2.8
NMALS	139.7 ± 8.6^{A,M,L,T}	137.1 ± 6.2*	136.3 ± 6.5^{D,L}	140.2 ± 4.9^{L,T,R}	134.0 ± 5.2	135.9 ± 5.5	133.5 ± 5.1
NUMALS	135.2 ± 8.3^{A,M}	137.8 ± 7.2*	138.1 ± 8.1^{D,L}	139.7±6^{L,T}	134.2 ± 6.2	134.9 ± 5.7	134.0 ± 7.2
NLMALS	137.2 ± 8.4^{A,M,D,L,R}	131.9 ± 7.4*	130.3 ± 7.9^{D}	135.6 ± 5.6^{L,R}	129.4 ± 16.8	132.2 ± 7.1	128.5 ± 7.2
NLALS	165.2 ± 10.8^{D}	163.8 ± 15.3	163.0 ± 16.8	162.7 ± 11.8^{L,T}	167.9 ± 3	169.2 ± 13.5	161.0 ± 15.4
NAL2	141.9 ± 9.2^{D,L}	141.1 ± 6.2*	142.2 ± 5.1^{D,L}	136.3 ± 5.6^{L,T,R}	144.0 ± 7.5	144.5 ± 5.3	144.8 ± 2.6
Lymphocyte parameters							
LV	85.7 ± 5.4^{A,M,D,L,T}	91.5 ± 8.5	91.7 ± 9.2	90.9 ± 7.3	92.0 ± 9.3	92.5 ± 5.3	89.0 ± 3.2
LC	116.3 ± 6.8^{A,M,D,L,T}	108.7 ± 5.2	108.7 ± 4.7	108.4 ± 2.7	108.2 ± 9.1	110.7 ± 8.1	111.0 ± 3.6
LMALS	66.5 ± 13.1 ^{M,D}	66.9 ± 6.9	66.2 ± 6.1^{L}	67.6 ± 6.8	68.2 ± 9.3	66.2 ± 6.9	67.3 ± 5.7
LUMALS	64 ± 14.6^{A}	67.5 ± 9.4*	68.6 ± 9.6^{D}	65.2 ± 7.7^{L}	69.1 ± 11.5	65.3 ± 8.5	67.3 ± 6.1
LLMALS	60.4 ± 9.7 ^{M}	60 ± 7.6*	58.1±8^{D,L}	62.2 ± 6.7	61.5 ± 7.3	60.3 ± 4.9	61.5 ± 4.7
LLALS	35.9 ± 3.9^{A,M,D,L,T}	41.5 ± 7.5*	41.5 ± 6.1^{D}	41.1 ± 19.4^{L,T}	42.7 ± 5	42.2 ± 2.6	40.3 ± 2.9
LAL2	71.6 ± 6.6^{A,M,D,L,R}	68.0 ± 7.6*	68.4 ± 9.6^{D}	66.8 ± 6.7	67.8 ± 6.4	71.2 ± .7.2	69.5 ± 4.2
Monocyte parameters							
MV	167.5 ± 11.3^{A,M,D,L,R,T}	188.5 ± 14.5*	191.4 ± 13.1^{D}	183.8±16^{L}	188.3 ± 13.9	189.9 ± 15.4	185.3 ± 6.7
MC	125.2 ± 7.1^{A,M,D,L,R,T}	117.6 ± 9.1*	118.5 ± 6.4^{D}	117.2 ± 3.6	114.9 ± 19.1	121.1 ± 7.6	108.3 ± 18.2
MMALS	84.2 ± 10.4 ^{M,D,L,T}	84.0 ± 5.2	83.9 ± 4.4	84.7 ± 5.1^{L,T}	83.2 ± 7.9	82.5 ± 2.9	82.7 ± 8.8
MUMALS	92 ± 10.7^{A,M,D,L,T}	89.0 ± 7.1*	90.5 ± 5.3^{D}	86.1 ± 7.5^{L,T}	89.8 ± 10.2	90.2 ± 4.2	90.0 ± 8.7
MLMALS	72.1 ± 10.2^{A,D}	74.9 ± 5.8*	73.6 ± 4.5^{D}	78.3 ± 5.8 ^{T}	73.3 ± 6.8	72.1 ± 3.6	71.8 ± 10.1
MLALS	84.3 ± 14.2^{A,M,D,L}	95.0 ± 13.9*	97.9 ± 13.8^{D,L}	91.7 ± 11.8	93.6 ± 14.7	91.9 ± 14.6	84.6 ± 31.5
MAL2	128.4 ± 12.3^{A,M}	130.3 ± 12.8*	132.6 ± 10.9^{D,L}	129.1 ± 13.7	126.7 ± 15	126.9 ± 13.2	121.0 ± 18.2
Eosinophil parameters							
EV	154.3 ± 9.8^{A,L,D}	157.3 ± 14.5*	159.0 ± 13.1^{L}	157.2 ± 15.8^{L}	152.1 ± 12.5	157.0 ± 24.3^{L}	152.0 ± 10.2
EC	152.3 ± 9.7^{A,M,D,L,R}	147. ±16.7*	147.2 ± 10.1^{D}	144.7 ± 10.3	146.8 ± 9	170.0±65^{L}	144.3 ± 2.9
EMALS	2011.2±7^{A,M,L}	199.4 ± 13.6*	199.0 ± 11.7^{D,L}	200.8±8^{L}	194.1 ± 11.5	214.1 ± 41.3^{L}	195.3 ± 6.9
EUMALS	211.8 ± 7.8^{A,M,L}	209.1 ± 13.8*	208.9 ± 9.5^{D}	210.8 ± 10.2^{L}	201.9 ± 16.8	224.4 ± 38.3^{L}	207.0 ± 6.2
ELMALS	186 ± 7.4^{L}	186.1 ± 12.2*	186.5 ± 8.5^{L}	185.9 ± 7.6^{L}	182.2 ± 7.7	197.8 ± 45.2^{L}	179.8 ± 7.6
ELALS	167.7 ± 12.1^{D}	168.8 ± 21.9	167.9 ± 18.4	168.3 ± 23.3	172.2 ± 18.1	174.5 ± 49.4^{L}	158.0 ± 23.9
EAL2	120.6 ± 7.7^{A,M,L,D}	122.9 ± 7.9*	121.5 ± 7.9^{D,L}	124.1 ± 7.1	125.3 ± 7.4	124.1 ± 10.7^{L,R}	121.5 ± 9.1

TABLE 3 Comparison of SD value of the VCS parameters among the five infections

VCS	Control	All	Malaria	Dengue	Leptospirosis	Typhoid	Rickettsia
Neutrophil parameters							
NV-SD	16.6 ± 1.7^{A,M,D,L,R}	19.9 ± 4.8*	19.3 ± 5.6^{D}	20.1 ± 2.7 ^{T}	22.1 ± 5.3	17.8 ± 2.4^{L}	19.9 ± 2.6
NC-SD	5±1^{D,T}	5.1 ± 1.4*	5.08 ± 1.6^{D,T}	5.3 ± 1 ^{T}	5.1 ± 1.3	4.4 ± 0.5^{L}	4.9 ± 0.7
NMALS-SD	11 ± 1.8^{A,D,L}	11.6 ± 2.7*	11.1 ± 2.2^{D,L}	12.2 ± 2.8 ^{T}	12.8 ± 3.5	10.2 ± 1.2^{L}	11.4 ± 2.2
NUMALS-SD	11.5 ± 1.6^{A,M,D,L}	12.3 ± 3.3*	11.2 ± 2.2^{D,L}	13.8 ± 3.9 ^{T}	13.6 ± 3.8	10.8 ± 1.2^{L}	12.1 ± 1.9
NLMALS-SD	13.4 ± 2.4^{A,M,D,L}	14.3 ± 3.1*	14.2 ± 2.8 ^{T,L}	14.5 ± 3.5 ^{T}	15.1 ± 3.2	12.3 ± 1.5^{L}	13.9 ± 3
NLALS-SD	28.8 ± 4.1^{A,M,D,L}	31.3 ± 4.9*	31.3 ± 5.2 ^{T,L}	30.9 ± 3.7^{L,T}	33.1 ± 6.1	27.8 ± 2.6^{L}	30.7 ± 6.2
NAL2-SD	10.6 ± 1.9^{A,M,D,L}	13.8 ± 3.9*	13.9 ± 4 ^{T,L}	13.1±3^{L,T}	15.9 ± 4.9	11.4±2^{L}	13.8 ± 4.5
Lymphocyte parameters							
LV-SD	14.8 ± 2.3^{A,M,D,L,T}	22.3 ± 5.2*	23.4±8^{D,L,T,R}	21.4 ± 4	21.9 ± 7.7	19.7 ± 3.9	17.6 ± 3.8
LC-SD	8.4 ± 1.9^{A,M,D,L,T}	12.0 ± 6.9*	12.7 ± 6.1^{D,T}	11.1 ± 9.1^{L,T}	13.4 ± 7.3	7.1 ± 1.4^{L}	10.5 ± 4.5
LMALS-SD	16.7 ± 1.5^{A,M,D,L}	19.2 ± 3.8*	19.1 ± 3.6 ^{T}	19.0 ± 3.2 ^{T}	20.8 ± 5.6	16.7 ± 1.1^{L}	16.8 ± 1.3
LUMALS-SD	20.4 ± 2.3^{A,M,D,L}	23.2 ± 4.9*	23.3 ± 4.8 ^{T}	23.1 ± 4.3 ^{T}	24.4 ± 6.4	20.1 ± 1.8^{L}	20.3 ± 1.9
LLMALS-SD	19.2 ± 1.4^{A,M,D,L}	21.8 ± 4.1*	22.5 ± 4.5 ^{T}	21.2 ± 3.3 ^{T}	22.1 ± 4.1	18.3 ± 2.7^{L}	19.7 ± 1.8
LLALS-SD	10.2 ± 1.1^{A,M,D,L,T,R}	14.1 ± 3.2*	15.±3^{D,T,R}	12.8±3^{L}	14.8 ± 3.7	11.9 ± 1.8^{L}	11.7 ± 1.7^{L}
LAL2-SD	11.5 ± 1.8^{A,M,DL}	14.7 ± 4.8*	16.3±6^{D,L,T,R}	12.9 ± 1.9^{L}	13.9 ± 2.6	12.3 ± 2.4^{L}	11.3 ± 0.9^{L}
Monocyte parameters							
MV-SD	18.9 ± 2.9^{A,M,D,L,T,R}	27.5 ± 4.5*	26.1 ± 3.9^{D,L,T}	29.7 ± 4.1 ^{T}	28.8 ± 4.7	22.9±3^{L}	27.8 ± 8.7
MC-SD	5.7 ± 2.1^{A,M,D,L,T}	9.1 ± 8.2*	8.8 ± 7.7^{D}	7.9 ± 4.3 ^{T}	13.1 ± 13.2	6.2 ± 1.3	16.2 ± 22.5
MMALS-SD	12.1 ± 1.6^{A,D,L}	13.0 ± 2.5*	12.4 ± 2.2^{D,L}	13.9±2^{L,T}	13.9 ± 3.7	12.1 ± 1.7	13.4 ± 3.5
MUMALS-SD	12.7 ± 2.1^{A,M,D,L}	15.0 ± 4*	13.3 ± 2.6^{D,L}	17.7 ± 4.1^{L,T}	15.4 ± 4.7	12.9 ± 2.7^{L}	14.5 ± 3
MLMALS-SD	16 ± 2.2 ^{M}	15.8 ± 3*	15.4 ± 3.1^{D,L}	16.2 ± 2.7	16.6 ± 3.3	15.5 ± 1.7	16.6 ± 2.6
MLALS-SD	26.6±4^{A,D,L,T}	28.6 ± 4.8*	27.5 ± 4.3^{D,L,T}	28.8 ± 4.1^{L,T}	31.3 ± 6.5	31.8 ± 3.6	27.6 ± 4.4
MAL2-SD	14.1 ± 2.7^{A,M,D,L}	19.4 ± 8.2*	18.3 ± 7.7^{D,L,R}	21.3 ± 9.1 ^{T}	22.0 ± 7.8	15.1±3^{L}	22.0 ± 12.9
Eosinophil parameters							
EV-SD	16.5 ± 2.9^{D,T}	16.5 ± 6.3*	16.3 ± 5 ^{T}	17.3 ± 7.3 ^{T}	17.7 ± 7	10.9 ± 8	12.8 ± 6.2
EC-SD	6.1 ± 5.3 ^{M,D,L,T}	6.5 ± 8*	7.1 ± 8.8 ^{T}	6.3 ± 7.7 ^{T}	6.0 ± 6.9	2.3 ± 1.7	4.3 ± 1.4
EMALS-SD	8.8 ± 1.4 ^{M,D,T}	8.4 ± 3.9*	8.8 ± 3.8^{D,T}	7.6 ± 3.7^{L}	9.5 ± 3.8	5.5 ± 3.9	8.5 ± 5.5
EUMALS-SD	9.9 ± 2.2 ^{M,D,L,T}	9.6 ± 4.9*	9.9 ± 4.8^{D,L,T}	8.6 ± 4.2^{L,T}	11.9 ± 5.6	5.6 ± 4.2	10.2 ± 4.8
ELMALS-SD	10.8±2^{A,M,D,T}	9.7 ± 4.3	10.0 ± 4.22	9.4 ± 4.6^{L}	10.1 ± 3.2	6.8 ± 4.8	9.8 ± 5.8
ELALS-SD	42.1 ± 4.9^{A,M,L,T}	38.2 ± 12.7*	38.1 ± 11.7^{D}	39.9 ± 13.5 ^{T}	38.1 ± 11.1	26.8 ± 19.1	37.8 ± 8.3
EAL2-SD	9.7 ± 2.9^{L,T,R}	9.9 ± 4.3*	9.7±4^{L,T}	9.9±4^{L,T,R}	11.6 ± 4.6	6.1 ± 4.5	15.3 ± 4.9

Prefix for various VCS parameters of neutrophils (N), lymphocytes (L), monocytes (M) and eosinophils (E) is used. The mean conductivity of neutrophils, lymphocytes, monocytes and eosinophils was higher in the control group compared to the specific infectious groups except for high eosinophil conductivity in typhoid cases (Tables 2 and 3).

The results in individual infections are discussed subsequently.

3.3.1 | Malaria

The WBC histogram typically showed a sharp pre-threshold peak/peak at the threshold (known as curve take-off point at 35fL) near the LD that did not touch the baseline. This curve take-off peak at the threshold is due to un-lysed RBCs that are infected with parasites which enters the WBC counting chamber. A shift of T1 and T2 to right with widening of F1 and F2 was observed indicating increased mean volumes of both lymphocyte and monocyte population when compared to the control group. There was also obliteration of T2 trough (Figure 1B) (MLV: Control- 85.7fL; malaria- 91.7fL; MMV: Control- 167.5fL; malaria-191.4fL).

The DLC scatterplots in malaria showed an abnormally increased level of nucleated RBCs. The low rotated LALS/AL2 scatter in the nucleated RBC (NRBC) plot (2D and 3D plots) showed the presence of unusual signal with round to upright cluster of spindle-shaped cells indicating parasitized RBCs (Figures 2B, 3C and D).

Malaria cases had a lower Hb, TLC and PC with lymphocytosis, monocytosis and eosinopenia compared to other groups. PCT was

significantly higher in malaria. There was a significant cytopenia (low Hb and TLC) with severe degree of thrombocytopenia, observed in malaria as compared to dengue, typhoid fever, leptospirosis and rickettsial infections. Monocytosis without neutropenia was commonly seen in these malarial patients.

The mean volume of neutrophil, monocyte and lymphocyte was increased in all five infections but the increase was significant in malaria compared to dengue fever [(MNV-143.2, MLV-85.7, MMV-167.5, MEV-154.3 in control group) (MNV-152.5, MLV-91.7, MMV-191.4, MEV-159 in malaria group) and (MNV was 147.2, MLV-90.9, MMV-183.8, MEV-157.2 in dengue group)].

3.3.2 | Dengue fever

The WBC histogram showed a shift of T1 and T2 to right with F1 and F2 widening indicating increased mean lymphocyte and monocyte volumes when compared to the control group (Figure 1C) (MLV: Control- 85.7fL; dengue- 90.9fL; MMV: Control- 167.5fL; dengue-183.8fL). The DLC scatterplots in dengue cases showed the lymphocyte dots extending into the variant lymphocyte window and monocyte dots were seen extending into the blast window (Figure 2C). The low rotated LALS/AL2 scatter was similar to that seen in healthy control (Figure 3E and F).

Dengue cases had thrombocytopenia, presence of reactive lymphocytes, monocytosis and neutropenia. Plasmacytoid and monocytoid reactive lymphocytes were almost always seen in every case of dengue.

It was noted that among the infections and control groups, dengue cases showed significantly higher values in most of the leucocyte light scatter data such as NMALS, NUMALS and NLMALS including SD of these VCS parameters, LLMAS, MLAMS, EMALS, EUMALS, ELALS and EAL2.

3.3.3 | Typhoid, leptospirosis and rickettsia

WBC histograms in typhoid fever, leptospirosis and rickettsia showed mild shift of T1 to the right with rest of the histogram curves comparable with that of healthy control (Figure 1D) (MLV: Control-85.74fL; leptospirosis- 92.04fL; typhoid fever- 92.46fL, rickettsial infection- 89fL). There was no differences in the DLC scatterplots (Figure 2D) and low rotated LALS/AL2 scatter (Figure 3E and F) in cases of leptospirosis, typhoid and rickettsial infections. In patients with leptospirosis, we observed lower Hb/ HCT, high normal TLC and lymphopenia compared to other groups.

When compared, the changes in VCS parameters were negligible in cases of typhoid fever (results almost similar to control group) than in other infections (Tables 2 and 3). However, the conductivity and scatter related data of eosinophils in typhoid fever were strikingly higher compared to control and other infectious groups (Tables 2 and 3).

4 | DISCUSSION

Leucocytes have a pivotal role in inflammation and exhibit definite changes in their morphology as well as in their cellular characters in infections.[12]

VCS technology has revolutionised the way laboratory medicine is practised giving quicker but yet accurate results.[15] This technology has provided a novel way to examine the various leucocyte response in diverse diseases and speculate the possible intracellular milieu of leucocytes. We have earlier published the data on utility of VCS parameters by LH780 haematology analyser in acute bacterial infection. This study convincingly showed that there are significant changes of VCS parameters in patients with acute bacterial infections as compared to the control group. The parameters such as MNV and MMV are sensitive to be used as rapid diagnostic indicators in acute bacterial infections.[15] Taking cue from the previous study, we embarked upon examining the utility of VCS parameters in differentiating the most common infective febrile illnesses in our geographic, namely malaria, dengue fever, leptospirosis, typhoid fever and rickettsial infection.

In malarial cases with a low parasite load, peripheral smear findings like neutrophilia, reduced lymphocytes and eosinophils, monocytosis, pigment in monocytes and/or neutrophils and presence of morphologically detectable plasmacytoid lymphocytes and cytopenia may point to haemoparasitemia.[16] Morphological changes of lymphocytes and monocytes are almost always observed in all cases of dengue with associated increase in their absolute numbers. Other clues in dengue include leucopenia, thrombocytopenia, monocytosis, presence of atypical lymphocytes with reversal of neutrophil/lymphocyte ratio.[17] In leptospirosis, there will be the presence of reactive lymphocytes in a background of neutrophilic leucocytosis/toxic granulation in neutrophils.[18] Enteric fever cases show leucopenia, neutropenia with relative lymphocytosis.[19] Rickettsia infects endothelial cells, neutrophils, monocytes, macrophages, dendritic cells and lymphocytes resulting in increased volume of neutrophils, lymphocytes and monocytes during the febrile episodes. Morphologically most cases of rickettsial infection have cytopenia with toxic changes in neutrophils and presence of large granular lymphocytes.[20] We have observed similar morphological clues in our study. The data related to these morphological clues were not statistically analysed because it was not in the scope of the current study objectives.

The utility of haematological parameters in malaria, dengue and other acute febrile illnesses have been studied in the past. Motchan et al conducted a 6-year retrospective analysis of malaria cases and found malaria-positive cases presents with reduced levels of Hb, RBC count, HCT, TLC, platelet count and PCT whereas RDW, MPV and PDW were elevated significantly paralleled to comparative group.[21] Nishimura et al documented lower TLC and platelet count in malaria and dengue patients with higher MPV, PDW and MCHC.[22] Viswanathan *et al* has recorded the role of platelet indices in combination with CBC and clinical symptoms

can aid in the diagnosis and treatment of tropical acute febrile illnesses.[23] Anabire *et al* did not find any specific changes in the haematological indices in patients with typhoid fever as compared to malaria patients.[19] We observed lowest Hb, TLC and platelet count and a significantly higher PCT in malaria compared to other groups.

We observed that malaria cases showed maximum increase in the MMV (191.4fL) with increased MNV (152.5fL) and MLV (91.7fL). Neutrophil and lymphocyte conductivity was strikingly unaffected in typhoid fever compared to other infection groups; however, we noted increased leucocyte volumes in patients with enteric fever. The volume and conductivity of neutrophils and lymphocytes (MNV, MLV, MNC and MLC) were reduced and increased neutrophilic scatter parameters were seen in dengue fever compared to other groups. Briggs *et al* performed a study on malaria cases and found that monocytes are increased in number. VCS technology identified the presence of activated monocytes in patients with malaria as monocyte volume and scatter were increased. In their study, they also observed decreased platelet count, an increase in volumes of neutrophils, lymphocytes and monocytes, increased scatter parameter of monocytes and also documented reduced neutrophil scatter and lymphocyte conductivity.[24] These findings were also seen in the present study as well as study conducted by Kalra et al.[12] Mathews et al analysed the cell population data in malaria cases using instrument LH750 Beckman Coulter and found significant difference in the SD volume of monocytes and lymphocytes in malaria.[25]

Kalra *et al* in their pilot study analysed the VCS parameters between healthy controls (800) and cases of acute febrile illnesses (200 cases – 50 cases of malaria, dengue, scrub typhus and typhoid each). They observed that TLC was significantly low in patients with malaria when compared to other groups. They also observed monocytosis with neutropenia in dengue patients, increased TLC in scrub typhus patients when compared to the other groups. Significantly higher NNV, MNV and MLV was seen in malaria-positive cases along with strikingly different lymphocyte scatter.[12] We observed a strikingly increased mean volume of neutrophils, lymphocytes and monocytes in malaria patients and lowest variations of VCS parameters in patients with typhoid fever.

The VCS parameters of controls were comparable with the study conducted by Kalra *et al*, but were different from those reported by Sharma et al.[12,26] In Sharma et al' s study, febrile controls were used whereas in both present and in the study by Kalra et al healthy controls were considered,[12,26] hence the difference. According to Tang et al, the VCS parameters do not fluctuate much in healthy people[27] and this evidence forms the basis for using these parameters in various clinical settings.

The limitation of the present study was the small number of cases of leptospirosis, enteric fever and rickettsial diseases. We did not observe much changes in these three infections as compared to those seen in dengue and malaria cases. Larger studies performed on these infections might give a better insight into the specific changes in VCS parameters.

This study shows that there are specific changes in VCS parameters that can be observed in cases of acute infectious febrile illnesses. These changes coupled along with clinical features and the morphological changes in peripheral smear (triple assessment) can aid in selecting specific tests for diagnosis. One should note that the additional information on VCS parameters are gathered without any additional cost and are unknowingly overlooked. Most of the laboratories with good work load have now shifted to a five-part differential auto-analyser platform and it can be additionally best used in evaluating the febrile cases. A presumptive yet mostly accurate diagnosis can be offered based on this triple assessment. Further, these changes can be transformed into specific test flags making VCS analysis a highly cost-effective tool in all endemic areas of tropical countries.

In summary, there was significant difference in the volume, conductivity and scatter of neutrophils, monocytes and lymphocytes between the five groups and the values are found to be maximum in malaria. MNV, MLV and MMV were increased significantly in malaria and dengue fever compared to leptospirosis, typhoid and rickettsial infections. Dengue cases showed significantly higher values in most of the leucocyte light scatter data. VCS parameters were the least altered in cases of typhoid fever except a strikingly high conductivity and scatter of eosinophils. Cell population data generated by VCS technology in automated machines such as Coulter ® DXH800 haematology analyser is a unique method for early and accurate diagnosis of specific infectious diseases.

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CONFLICT OF INTEREST

We, the authors, declare that there are no financial, personal or professional interests that could be construed to have influenced the work. There is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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