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Cell population data analysis for early diagnosis and prognosis of COVID-19: A Case-Control Study

Priyadarshini Kumaraswamy Rajeswaran [1*], Preethi Muthusamy Sundar [1], Prasanna Nedungadi Kumar [1], Karthikeyan Shanmugam [2]

1. Department of Pathology, PSG Institute of Medical Sciences and Research, Off Avinashi Road, Peelamedu, Coimbatore, Tamil, Nadu, India
2. Department of Community Medicine, PSG Institute of Medical Sciences and Research, Off Avinashi Road, Peelamedu, Coimbatore, Tamil, Nadu, India
* Correspondence: Priyadarshini Kumaraswamy Rajeswaran. Department of Pathology, PSG Institute of Medical Sciences and Research, Off Avinashi Road, Peelamedu, Coimbatore, Tamil, Nadu, India. Tel: +919600201766; Email: drpriyadarshini.kr@gmail.com

Abstract

Background: Several hematological indicators have been linked to the intensity and course of Coronavirus Disease of 2019 (COVID-19), including platelets, total white blood cell (WBC) count, lymphocytes, neutrophils (as well as the neutrophil-lymphocyte and platelet-lymphocyte ratios), and hemoglobin. The purpose of this study was to assess the utility of cell population data (CPD) of lymphocyte and monocyte parameters in the early diagnosis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection.

Methods: The baseline complete blood count examination was performed for 222 patients with positive results for COVID-19 (case group) and 161 patients with negative results for COVID-19 (control group). Lymphocyte and monocyte CPD were calculated in both groups. The independent t-test was used to compare the mean values between the two groups. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminating capacity of the individual parameters.

Results: The analysis revealed that Standard Deviations of Monocyte Volume (SDMV) and Standard Deviations of Lymphocyte Conductivity (SDLC) showed the highest significance in predicting SARS-CoV-2 infection. Moreover, SDMV had a sensitivity of 93.7% and SDLC had a sensitivity of 80.6% at cut-off values of 22.25 and 10.9, respectively. In the case group, 49 of the 222 patients treated in the intensive care units (ICUs) showed a higher SDMV compared with the remaining 173 patients who were asymptomatic, or mildly symptomatic (P-value <0.03).

Conclusion: Our study demonstrates that SDMV and SDLC can serve as reliable and cost-effective markers for early prediction of SARS-CoV-2 infection. Furthermore, SDMV shows potential as a prognostic biomarker. These findings highlight the potential utility of CPD parameters in COVID-19 diagnosis and prognosis.

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Introduction

Most patients with Coronavirus Disease of 2019 (COVID-19) are asymptomatic or have mild symptoms. Only 14% develop severe infection requiring intensive care admission (1). Identification of asymptomatic and mildly symptomatic individuals is essential for isolation in order to control the spread of the infection. In a hospital setting, clinicians face a major hurdle in identifying those infected with COVID-19 amongst patients with varied clinical disorders seeking medical attention.

The gold-standard investigation for diagnosing Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection is Reverse Transcription Polymerase Chain Reaction (RT-PCR). However, it has its own demerits such as high cost, need for technical expertise, and unavailability in many resource-poor settings. Therefore, there is increasing interest among researchers to find alternative, less expensive, quicker, and reliable methods for detection of this viral infection. In this regard, the analysis of cell population data (CPD) parameters, based on their demonstrated utility in other infections, offers a promising avenue for the early detection of SARS-CoV-2 infection.

The Beckman Coulter LH 780, a fully automated hematology analyzer, generates cell CPD based on Volume (V), Conductivity (C), and Scatter(S) principles to provide us with the white blood cell (WBC) differential counts within complete blood counts. The cell volume (V) is obtained by voltage impedance, conductivity (C) is measured by radiofrequency, which provides information about the internal structure, and information about cytoplasmic granularity and nuclear complexity is given by laser light scatter (S). The Beckman Coulter LH 780 analyzer has a separate screen that gives statistical information on each WBC population (mean and standard deviation [SD]) of their VCS parameters.

There are published papers regarding the utility of the CPD parameters in early detection of sepsis, viral fevers, leukemia, and lymphomas (2-12). To the best of our knowledge, only three studies in the literature have investigated the utility of CPD parameters in COVID-19 (13-17). These studies (13-17) have shown encouraging results of utilizing CPD parameters in terms of their ability to predict infection. However, a comprehensive analysis of these parameters and their association with different disease severities is still lacking. This study aimed to evaluate the utility of lymphocyte and monocyte CPD in the early prediction of COVID-19. The VCS parameters of lymphocytes and monocytes were compared between COVID-19-infected patients and patients who were negative for COVID-19. Similarly, the VCS parameters were analyzed as prognostic markers among patients with COVID-19 requiring intensive care units (ICUs).

Methods

This prospective, observational, case-control study was conducted from August 2020 to October 2020. In this study, 400 adult (Age>18 years of both genders) patients, including 222 patients (Case group) with positive RT-PCR results (TRUPCR SARS-CoV-2 Kit, 3B Black Bio-Biotech, Bhopal, India) and 178 patients (Control group) with negative RT-PCR results, were included. The TRUPCR kit has a sensitivity of 73% and a specificity of 100% (18).

The case group (n=222) consisted of patients who came with the symptoms of COVID-19 and on further testing by HRCT showed features of COVID-19 and/or RT-PCR were found to be positive for the same. The control group included patients with respiratory complaints suspicious of SARS-CoV-2 infection who subsequently tested negative, as well as asymptomatic patients in whom RT-PCR testing was performed as part of work-up for a planned invasive procedure. Of 178 control patients, 17 had laboratory-proven other viral/bacterial infections and hence were excluded from the control group. After exclusion, 161 patients with negative RT-PCR results were included in the control group.

Whole blood venous samples were collected in K2EDTA for baseline complete blood count (CBC) examination for all the patients. The blood sample was analyzed using the Beckman Coulter LH-780 hematology analyzer (Beckman Coulter, Brea, CA) within 4 hours of collection. In addition, CPD of lymphocytes and monocytes was documented, which is provided by the hematology analyzer as mean and standard deviation (SD) values. The VCS parameters were recorded by a pathologist from the Coulter screen without knowledge of the patient's COVID-19 status. The demographic information, such as age and gender, was also noted. The Institutional Human Ethics Committee approved this study (ref no: 20/171).

Statistical analysis

The data were expressed as mean ± SD. The independent t-test was used to compare the mean values between the two groups. All analyses, including Receiver Operating Characteristic (ROC) curve analysis, were performed with IBM SPSS software, version 23.0 (SPSS, Chicago, IL). A P-value of less than 0.05 was considered statistically significant.

Results

Population characteristics

There were 146 males and 76 females in the case group (n=222) and 90 males and 71 females in the control group (n=161). The mean age ± SD was 51.4 ± 17.1 years in the case group and 46.3 ± 19 years in the control group.

Clinical characteristics

The symptoms in the case group ranged from fever (84%), myalgia (58%), dry cough (62%), dyspnea (45%), diarrhea (27%), and anosmia (16%). Chest CT in 75% (168 of 222 cases) showed patchy ground glass opacity, suggestive of atypical viral pneumonia. Of the 222 COVID-positive cases, 49 cases were managed in the ICU.

Leucocytes and VCS parameters for early prediction of SARS-CoV-2 infection

The baseline WBC total count and absolute differential counts of the leucocytes were compared between the case and control (Table 1). On comparison, a significant decrease was observed in the absolute counts of the monocyte, eosinophil, and basophil populations in the COVID-19 positive patients in comparison to the control group. There were no significant differences in total leukocyte count and absolute counts of neutrophil and lymphocyte between the cases and controls. Thus, considerable eosinopenia and monocytopenia were observed in the WBC parameters in the COVID-19 cases. Although there was a substantial decrease in the absolute lymphocyte count in the patients with COVID-19, the variation was not statistically significant. Table 2 represents the VCS parameters of the WBC population. In this regard, it is observed that the Mean Lymphocyte Volume (MLV), Mean Lymphocyte Scatter (MLS) and Mean Monocyte Scatter (MMS) are significantly decreased in the COVID-19 cases compared to the controls. The SD values of Lymphocyte Volume (SDLV), Lymphocyte Conductivity (SDLC), Lymphocyte Scatter (SDLS), Monocyte Volume (SDMV), and Monocyte Conductivity (SDMC) were increased only in the COVID-19 population. The other VCS parameters, such as Mean values of Lymphocyte Conductivity (MLC), Monocyte Volume (MMV), Monocyte Conductivity (MMC) and SD of Monocyte Scatter (SDMS), showed no significant difference between the two groups.

Table 1. Comparison of total leucocyte and differential leucocyte counts between case and control groups

Parameter $\times 10^3/\mu l$	Case (n=222)	Control (n=161)	P-Value
	Mean ± SD	Mean ± SD	
Total leukocyte count	8.7±6.7	10±6.4	0.05
Absolute neutrophil count	6.3±5.8	7.1±5	0.17
Absolute lymphocyte count	1.6±3	2±4.4	0.35
Absolute monocyte count	0.5±0.3	0.6±0.4	0.02*
Absolute eosinophil count	0.09±0.2	0.18±0.2	0.00*
Absolute basophil count	0.008±0.03	0.02±0.06	0.00*

Table 2. Comparison of VCS parameters of lymphocytes and monocytes between the case and control groups

Parameter	Case (n=222)	Control (n=161)	P-Value	Cut-off	AUC	95% CI	Sensitivity %	Specificity %
	Mean ± SD	Mean ± SD						
MLV	82.2 ±5.5	84.3±6.2	0.00 *	81.95	0.38	0.326-0.440	49.5	28
SDLV	16.1±2.1	15.5±2.7	0.02 *	15.03	0.607	0.548-0.665	69.4	52
MLC	116.6±6.9	117.1±3.5	0.39	115.5	0.394	0.337-0.451	49.5	31
SDLC	13.8±4	12.2±2.8	0.00 *	10.9	0.634	0.577-0.690	80.6	60
MLS	67.3±7.6	70.6±8.1	0.00 *	68.85	0.373	0.316-0.430	59	40
SDLS	19.3±3.7	18.2±3.3	0.03 *	16.94	0.609	0.551-0.666	79.3	40.4
MMV	172.3±9.8	169.5±10.3	0.06	167.85	0.601	0.543-0.658	66.7	50
SDMV	24.3±1.3	20.7±4.3	0.00 *	22.25	0.716	0.599-0.738	93.7	67
MMC	123.9±3.7	124.3±4	0.33	123.95	0.450	0.392-0.508	50	42.5
SDMC	5.4±1.2	5.1±1.1	0.01*	4.81	0.604	0.546-0.662	70	46.9
MMS	88.7±5.8	91.8±5.1	0.00 *	90.85	0.346	0.291-0.401	39.2	40
SDMS	9.99±1.68	9.89±1.60	0.68	10.02	0.515	0.457-0.573	50.5	51

Abbreviations: VCS, Volume, Conductivity, and Scatter; MLV, Mean Lymphocyte Volume; SDLV, Standard Deviation of Lymphocyte Volume; MLC, Mean Lymphocyte Conductivity; SDLC, Standard Deviation of Lymphocyte Conductivity; MLS, Mean Lymphocyte Scatter; SDLS, Standard Deviation of Lymphocyte Scatter; MMV, Mean Monocyte Volume; SDMV, Standard Deviation of Monocyte Volume; MMC, Mean Monocyte Conductivity; SDMC, Standard Deviation of Monocyte Conductivity; MMS, Mean Monocyte Scatter; SDMS, Standard Deviation of Monocyte Scatter.

The sensitivity and specificity of the VCS parameters were calculated at a desirable cut-off value for using them as early predictors of SARS-CoV-2 infection. The ROC curve of SDMV showed an AUC of 0.71 (Figure 1). SDMV demonstrated a sensitivity of 93.7% and specificity of 67% at a cut-off value of 22.25. Among the lymphocyte VCS parameters, SDLC at a cut-off value of 10.9 (AUC: 0.63) demonstrated a sensitivity of 80.6% and a specificity of 60% (Figure 2). The sensitivity and specificity of SDMV and SDLC were compared with the gold-standard RT-PCR for detection of COVID-19 (Table 3). Also, Of the 222 COVID-19 cases, 49 required ICU care. Since SDMV and SDLC levels were significantly elevated in these patients, we evaluated their effectiveness in predicting the need for ICU admission. The patients with COVID-19 who received treatment in the ICUs were compared to the other patients in the case group based on their SDMV and SDLC scores (Table 4). It is interesting to note that we also observed a significant rise in SDMV in the patients with COVID-19 receiving ICU care.

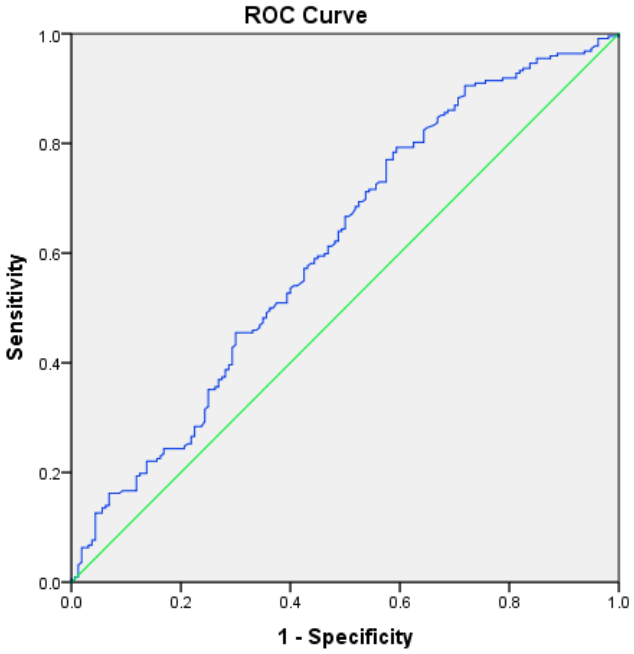

Figure 1. ROC curve analysis for SDMV performance in differentiating patients with COVID-19 from other patients. SDMV has a sensitivity of 93.7% and a specificity of 67% at a cut-off value of 22.35

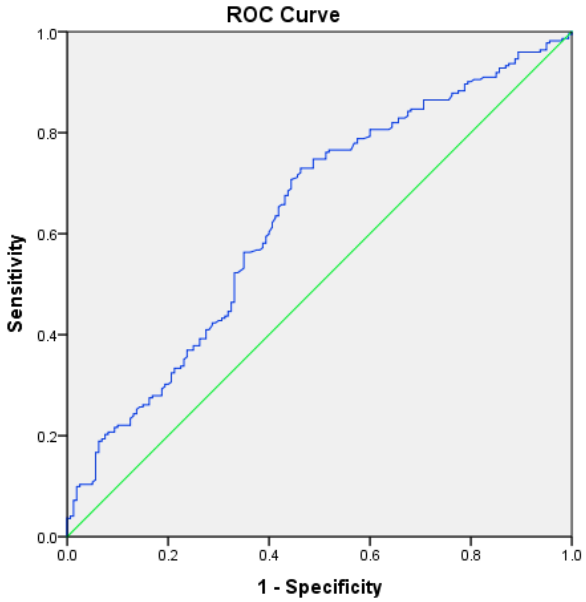

Figure 2. ROC curve analysis for SDLC performance in differentiating patients with COVID-19 from other patients. At a cut-off value of 10.9, SDLC has a sensitivity of 80.6% and a specificity of 60%

Table 3. Comparison of the sensitivity and specificity of SDMV and SDLC with the gold-standard RT-PCR

Parameter/Test method	Sensitivity (%)	Specificity (%)	95% CI
SDMV	93.7	67	0.599-0.738
SDLC	80.6	60	0.577-0.690
RT-PCR (18)	73	100	0.528–0.829

Table 4. Comparison of SDMV and SDLC values in the patients with COVID-19 managed in the ICUs with others in the case group

Parameter	Hospitalized patients with COVID-19 treated in the ICU (Mean ±SD) (n= 49)	Non-ICU hospitalized patients with COVID-19 (Mean ±SD) (n= 173)	P-Value
SDMV	25 ± 1.2	22 ± 1.8	0.03*
SDLC	15.4 ± 0.8	14.8 ± 1.1	0.60

Abbreviations: SDMV, Standard Deviation of Monocyte Volume; SDLC, Standard Deviation of Lymphocyte Conductivity; ICU, Intensive Care Unit.

Discussion

In practice, it is necessary to have trustworthy and readily available COVID-19 biomarkers to determine whether to immediately segregate a patient and start targeted treatments while awaiting confirmation test results. The WBC and CPD values that are readily available were shown in this study to be useful as predictive and diagnostic in early COVID-19. The average ages of patients in both groups were 51.4 ± 17.1 and 46.3 ± 19 years, respectively. In both groups, there was a greater proportion of men than women. These results also agreed with earlier studies that found a larger percentage of individuals over fifty years of age and a majority of men (19,20).

In recently published studies, the role of lymphocytes and monocytes in the immune mechanisms against SARS-CoV-2 infection has been demonstrated (18-20). In our study, we found a significant decrease in monocytes and eosinophils as reported in the literature (21-24). There were no significant changes in absolute neutrophil and lymphocyte counts, in contrast to the studies in the literature where neutrophilia and lymphopenia were observed (22,24). The decrease in monocyte and eosinophil counts can probably be attributed to increased apoptosis, which occurs during the pro-inflammatory cytokine storm in COVID-19 (24-27). Sehanobish et al. noted that eosinopenia may serve as an early diagnostic marker in patients with COVID-19 (28). The present study did not find decreased WBC, neutrophil, or lymphocyte numbers in the patients with COVID-19, similar to other studies reported previously (21-25).

The Beckman Coulter analyzer utilizes cellular population data as a tool for differential determination. They correlate with a morphological examination of leukocyte subtypes and several studies have shown that a diagnosis of bacterial, viral, or parasite illnesses may benefit from their investigation (13-17). Of the lymphocyte CPD, SDLV, SDLC, and SDLS were significantly increased. This can be attributed to the presence of reactive lymphocytes and size variation observed in those cells. MLV and MLS were significantly decreased. This decrease is due to the reduced laser light scatter in reactive lymphocytes. In our study, SDLC had a sensitivity of 80.6% and a specificity of 60% at a cut-off value of 10.9. This finding was not reported in other similar studies in the literature (12-16).

Size variation in monocytes was observed as an increase in SDMV. A similar finding has been reported in the literature (12-17). In our study, SDMV had a 93.7% sensitivity and 67% specificity at a cut-off value of 22.25, which was comparable to other studies (Table 5) (13,15). The specificity observed in our study and in previously published studies is low (13,15,16). This could be because our control group was made up of patients with other underlying illnesses, and these parameters may also be altered in other diseases (2-4). Recent reports indicate the mechanism of infection with SARS-CoV-2 can explain the dysregulated expression of cytokines, primarily interleukin-6 (IL-6) and IL-10, erroneous increase of pathological monocytes and upregulation of genes involved in their cell death (apoptosis) pathway, which explain the CPD

changes observed at disease onset in severe as compared to mild forms of COVID-19 (29,30). Hence, we observe SDMV in the patients with COVID-19.

Table 5. Comparison of SDMV in the present study with other studies

Study	Cut-off value	Sensitivity	Specificity	AUC	Sample size of COVID-19-positive cases
The present study	> 22.25	93.7%	67%	0.71	222
Ognibene et al. (13)	> 20	98%	65%	0.91	41
Zeng et al. (15)	> 20.11	82.26%	72.58%	0.85	93

The current study suggests the use of baseline WBC counts and VCS characteristics, which may enhance diagnostic characterization and help distinguish between severe and non-severe presentations. Our findings highlight the necessity of additional work to create precise admission algorithms based on the VCS parameters of WBCs in order to better support the management of patients with COVID-19. This study had some limitations. One of the limitations of this study is that the control population included patients without a diagnosis of COVID-19. Some of these patients might have had other viral diseases, which could alter the cell population data. Another limitation is the relatively small sample size.

Conclusion

The current study suggests the use of baseline WBC counts and VCS characteristics, which may enhance diagnostic accuracy and help distinguish between severe and non-severe presentations. Our findings highlight the necessity of additional work to create precise admission algorithms based on the VCS parameters of WBCs in order to better support the management of patients with COVID-19.

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Funding sources

Not applicable.

Ethical statement

The Institutional Human Ethics Committee approved this study (Ref no: 20/171).

Conflicts of interest

Not applicable.

Author contributions

PKR and PMS supervised the study, collected data and wrote the manuscript. PNK supervised the project and revised the manuscript. KS analyzed data, performed statistical analysis, and wrote the manuscript. The final manuscript was read and approved by all the authors.

Data availability statement

The data that support the findings of this study are not publicly available due to ethical and confidentiality restrictions, but may be available from the corresponding author upon reasonable request and subject to institutional approval.

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