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Original Research

Lymphocyte cell population as a potential hematological index for early diagnosis of COVID-19

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Abstract: The pandemic diseases caused by SARS-CoV-2 are now threatening human health and survival. Early diagnosis and isolation of mild or asymptomatic COVID-19 patients is important to control the spread of SARS-CoV-2. In this study, we investigate the potential clinical utility of lymphocyte CPD for early diagnosis of COVID-19. To investigate the potential of lymphocyte cell population data (lymphocyte CPD) for use in early diagnosis of coronavirus disease 2019 (COVID-19). Lymphocyte CPD of healthy control (n = 51), common cold patients (n = 49) and mild COVID-19 patients (n = 126) were generated using hematology analyzer. The parameters were subjected to sensitivity and specificity analysis to determine their suitability as biomarkers for early diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Normality analysis showed that lymphocyte CPD followed a normal distribution. There were no significant differences in white blood cells (WBC) and lymphocyte (LY#) counts as well as the neutrophil-to-lymphocyte ratio (NLR) among the groups ($p > 0.05$). Lymphocyte volume standard deviation (LV-SD), lymphocyte conductivity standard deviation (LC-SD) and lymphocyte light scatter standard deviation (LS-SD) were significantly higher in the COVID-19 group than in common cold and control groups ($p < 0.05$). The corresponding mean lymphocyte light scattering (MLS) was significantly reduced in the COVID-19 group, relative to the common cold group, but was significantly increased, when compared with the control group ($p < 0.05$). Moreover, there was no significant difference in mean lymphocyte volume (MLV) between the COVID-19 group and the common cold or control group ($p > 0.05$), but it was significantly higher in the common cold group than in the control group ($p < 0.05$). At a cutoff value ≥ 16.38, LS-SD was more sensitive and specific than other lymphocyte CPD parameters. At a cutoff value ≥ 11.89, LC-SD achieved 84.4 % sensitivity, 87.5 % specificity, and an area under the curve (AUC) of 0.888. However, at a cutoff value ≥ 15.95, LS-SD reached 81.3 % sensitivity, 75 % specificity and an AUC of 0.876. These results suggest that lymphocyte CPD parameters have great diagnostic potential for SARS-CoV-2 infection and can be used for early diagnosis of the disease.

Key words: COVID-19; Diagnosis; Hematology analyzer; Lymphocyte cell population data; SARS-CoV-2 infection.

Introduction

Coronavirus disease 2019 (COVID-19) was a disease caused by a novel coronavirus. The virus formerly known as 2019-nCoV is now called SARS-CoV-2 (1, 2). Although most SARS-CoV-2 infected patients present mild symptoms, they have the potential of transmitting the disease, thereby making it difficult to effectively control the spread of COVID-19 (3). Therefore, early diagnosis of SARS-CoV-2 infection and isolation of mild or asymptomatic patients are crucial for effective control and management of the pandemic (4). At present, the diagnosis of COVID-19 depends mainly on clinical presentations, computerized tomography (CT) imaging of the chest, and routine blood and nucleic acid analyses (5). However, there is an urgent need for highly sensitive and effective biomarkers that can be used for early diagnosis of COVID-19 (4).

The new generation of the hematological analyzer has taken advantage of several technological innovations that have allowed for the expansion of the panel of potential information obtained from complete blood count (CBC). In particular, novel parameters of leukocyte count and differential are emerging as potentially useful markers in a number of human disorders (6). It was only recently that CPD characterizing different leukocyte populations became available These data have been employed for the diagnosis of plasmodium, bacterial and viral infections, as well as leukemia (7-9). This study aimed to investigate the potential of lymphocyte CPD for use in the early diagnosis of COVID-19.

Materials and Methods

Materials

Di-potassium EDTA anticoagulant bottles were purchased from Becton Dickinson (USA). An automated immunoassay analyzer was obtained from SEAC (Italy). Serological marker kits for rhinovirus, adenovirus, parainfluenza virus, respiratory syncytial virus, echovirus, cytomegalovirus, Coxsackie virus and Epstein-Barr virus were products of MSK Biology Technology Co. Ltd. (China). Fluorescence qRT-PCR machine was bought from Applied Biosystems (USA) and the pri-

mers were purchased from Shanghai ZJ Biotech. Co. Ltd. Hematology analyzer (Coulter LH750) was obtained from Beckman Coulter (USA).

General information on patients

A total of 226 patients were included in this study. They were randomly assigned to three groups: control group, common cold group and COVID-19 group. Patients (n = 51) who were negative for SARS-CoV-2 with negative viral serological results served as control. They comprised 31 males and 20 females aged 26 – 50 years (mean age = 38 ± 12 years). The common cold group (n = 49) consisted of 31 males and 18 females aged 31 – 41 years (mean age = 36 ± 10 years), while COVID-19 group (126 SARS-CoV-2 infected patients) comprised 31 males and 20 females aged 30 – 46 years (mean age = 38 ± 16 years). The common cold patients had positive viral serology results, but they were negative for SARS-CoV-2. The COVID-19 patients were diagnosed according to the diagnostic guidelines of the National Health Commission of China (seventh edition). They were positive for SARS-CoV-2 but negative for common cold serum antibodies. Amongst them were 4 asymptomatic carriers and 122 mild patients. The asymptomatic patients were closely monitored to prevent them from coming into contact with other patients. The mild symptoms were cough, headache, diarrhea, myalgia and fatigue. Patients' serological indices were determined using an automated immunoassay analyzer. Fluorescence quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used for the amplification of ORF1ab, N and E genes of the SARS-CoV-2 genome during the diagnosis of COVID-19 patients. The study protocol was approved by the Research Ethics Board of The Second Affiliated Hospital of Nantong University and was accepted by the legal guidance of the patients involved.

Generation of lymphocyte CPD

Peripheral venous blood was drawn from each patient into di-potassium EDTA anticoagulant bottles and stored at room temperature. Whole blood samples were analyzed within 4 h after collection, and lymphocyte CPD was optically and electronically generated using a hematology analyzer. The parameters determined were MLV, LV-SD, MLC, LC-SD, MLS, LS-SD, total WBC, neutrophil count (NE#), LY# and NLR.

Determination of sensitivity and specificity of lymphocyte CPD in the prediction of early SARS-CoV-2 infection

The sensitivity and specificity of lymphocyte CPD in the diagnosis of SARS-CoV-2 infection were determined at specific cutoff points. The larger the Youden index, the better the diagnostic performance of a particular parameter. The cut-off value, sensitivity and specificity corresponding to the largest Youden index were then recorded.

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was performed using SPSS (13.0). Groups were compared using Student's *t*-test. The Youden index was used to select an optimal threshold value (the cutoff point) for the diagnostic markers. Statistical significance was assumed at $p < 0.05$.

Results

Lymphocyte CPD performance characteristics

The coefficient of variation (% CV) within-run in common cold samples were 1.41 % (MLV = 91.57), 2.28 % (LV-SD = 15.22), 2.01 % (MLC = 113.42), 2.20 % (LC-SD = 11.16), 2.11 % (MLS = 86.13), and 2.23 % (LS-SD = 16.22). The % CV within-run in healthy control patient samples were 1.64 % (MLV = 87.75), 1.82 % (LV-SD = 14.28), 1.66 % (MLC = 121.60), 1.91 % (LC-SD = 9.67), 1.84 % (MLS = 70.25) and 1.87 % (LS-SD = 14.86).

Results of normality analysis

As shown in Table 1, normality analysis showed that the lymphocyte CPD followed a normal distribution.

Lymphocyte CPD of patients in the three groups

There were no significant differences in WBC and LY# counts as well as NLR among the groups ($p > 0.05$). Lymphocyte volume standard deviation (LV-SD), LC-SD and LS-SD were significantly higher in the COVID-19 group than in common cold and control groups ($p < 0.05$). The corresponding MLS was significantly reduced in the COVID-19 group, relative to the

Table 1. Results of analysis of normality.

Parameter	Group					
	Control		Common cold		COVID-19	
	z	*p*	*z*	*p*	*z*	*p*
WBC (x 10^9/L)	0.421	0.994	0.555	0.917	1.018	0.251
LY# (x 10^9/L)	0.592	0.875	0.626	0.828	0.658	0.780
NLR	0.585	0.883	0.552	0.920	0.805	0.536
MLV (fL)	0.570	0.901	0.715	0.687	0.721	0.581
LV-SD	0.705	0.701	0.620	0.837	0.778	0.581
MLC	0.498	0.966	0.892	0.404	0.634	0.816
LC-SD	0.572	0.899	0.748	0.630	1.140	0.148
MLS	0.573	0.898	0.986	0.285	0.810	0.528
LS-SD	0.613	0.846	0.963	0.312	0.595	0.871

Table 2. Lymphocyte CPD of patients in the three groups.

Parameter	Group			*f*	*p*
	Control (n = 51)	Common cold (n = 49)	COVID-19 (n = 126)		
WBC (× 10^9/L)	6.20 ± 1.32	6.64 ± 1.79	6.04 ± 2.27	0.66	0.52
LY.# (× 10^9/L)	1.97 ± 0.43	2.08 ± 0.55	2.03 ± 0.61	1.05	0.34
MO.# (× 10^9/L)	0.40 ± 0. 11	0.44 ± 0. 12	0.43 ± 0. 11	0.97	0.61
NLR	1.81 ± 0.55	2.04 ± 0.58	1.85 ± 0.60	0.24	0.77
MLV (fL)	88.26 ± 2.61$^{\#}$	91.33 ± 2.44	88.99 ± 6.34	5.65	0.01
LV-SD	14.41 ± 0.66**	15.18 ± 2.02**	17.76 ± 3.41	28.87	< 0.001
MLC	120.14 ± 2.14*	112.19 ± 8.54	116.61 ± 7.52	13.01	< 0.001
LC-SD	9.31 ± 1.17**	10.69 ± 1.43**	19.17 ± 8.63	34.02	< 0.001
MLS	69.36 ± 7.53**	85.15 ± 7.44**	80.10 ± 8.79	46.02	< 0.001
LS-SD	14.85 ± 0.89**	16.12 ± 1.56**	20.32 ± 4.67	48.17	< 0.001

$^{*}p < 0.05$ and $^{**}p < 0.01$, compared with COVID-19 group; $^{\#}p < 0.05$, compared with common cold group.

Table 3. Sensitivity and specificity of lymphocyte CPD in the prediction of early SARS-CoV-2 infection.

Parameter	AUC	Cutoff value	Sensitivity	Specificity
MLV (fL)	0.563	≥ 86.8	93.7	35.0
LV-SD	0.905	≥ 15.38	84.3	95.0
MLC	0.670	≤ 118.9	43.7	19.6
LC-SD	0.953	≥ 11.45	87.5	95.0
MLS	0.893	≥ 73.4	84.4	95.0
LS-SD	0.970	≥ 16.38	90.5	95.0

common cold group, but it was significantly increased when compared with the control group ($p < 0.05$). There was no significant difference in MLV between the COVID-19 group and the common cold or control group ($p > 0.05$), but it was significantly higher in the common cold group than in the control group ($p < 0.05$). These results are shown in Table 2.

Sensitivity and specificity of lymphocyte CPD in the prediction of early SARS-CoV-2 infection

At a cutoff value ≥ 16.38, LS-SD was more sensitive and specific than any of the other lymphocyte CPD parameters. The receiver operating characteristic (ROC) curve analysis showed that LS-SD had the highest AUC, relative to each of the other parameters. Similarly, LV-SD and LC-SD showed better diagnostic performance than any of the other parameters. These results are shown in Table 3 and Figure 1.

Sensitivity and specificity of lymphocyte CPD in the differentiation of SARS-CoV-2 infection from the common cold

The receiver-operating characteristic curve analysis showed that LC-SD had the highest AUC when compared to MLV, MLC, MLS, LV-SD and LS-SD. At a cutoff value ≥ 11.89, LC-SD reached 84.4 % sensitivity, 87.5 % specificity, and an AUC of 0.888. At a cutoff value ≥ 15.95, LS-SD reached 81.3 % sensitivity, 75 % specificity and an AUC of 0.876 (Table 4 and Figure 2).

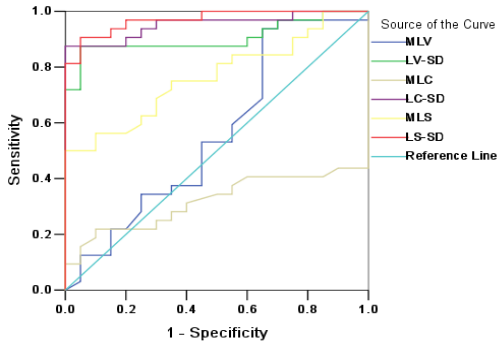

Figure 1. Receiver-operating characteristics curve analysis of lymphocyte CPD.

Discussion

Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2, is a new infectious disease that first emerged in Hubei province, China. Its early symptoms are similar to those of the common cold hence, the need for highly specific and effective biomarkers for early diagnosis of the disease. Although early diagnosis of COVID-19 is pivotal for improving prognosis, the diagnostic approach and monitoring of patients constitute major challenges.

Cell population data (CPD) has shown great promise in the diagnosis of viral infections (9). Viral infections result in lymphocyte activation, undifferentiated lymphocyte proliferation, and antibody or cytokine/lymphokine secretion (10). The immune defense against a viral infection is more dependent on T cells and less dependent on antibodies. Cytotoxic T cells are important in killing virus-infected cells. A natural killer (NK) cell is a type of lymphocyte and it is a component of

Table 4. Sensitivity and specificity of lymphocyte CPD in the differentiation of SARS-CoV-2 infection from the common cold.

Parameter	AUC	Cutoff value	Sensitivity	Specificity
MLV (fL)	0.740	≤ 90.8	87.3	62.4
LV-SD	0.787	≥ 15.39	87.4	68.6
MLC	0.687	≥ 109.27	87.3	62.6
LC-SD	0.888	≥ 11.89	84.4	87.5
MLS	0.680	≤ 87.21	90.1	62.5
LS-SD	0.876	≥ 15.95	81.3	75.0

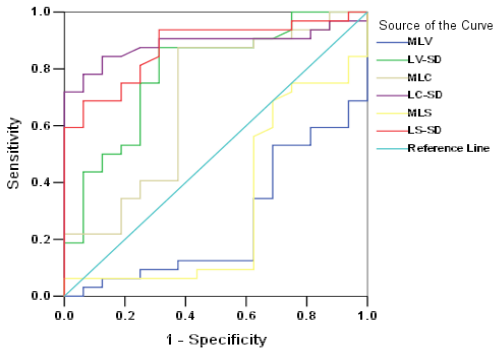

Figure 2. Receiver-operating characteristic curve analysis of lymphocyte CPD in the differentiation of SARS-CoV-2 infection from the common cold.

the innate immune system. These cells serve to contain viral infections while the adaptive immune response is generating antigen-specific cytotoxic T cells that can clear the infection. Studies have shown that WBC and lymphocyte counts in COVID-19 patients are either normal or mildly reduced (3). The new Coulter LH750 analyzer is capable of providing an expanded leukocyte differential count with as many as 22 CPD parameters that can be generated along with CBC. This study investigated the potential of lymphocyte CPD for use in early diagnosis of COVID-19. The results showed that LV-SD, LC-SD and LS-SD were significantly higher in the COVID-19 group than in the common cold group. Moreover, MLS was significantly lower in the COVID-19 group than in common cold patients, but it was significantly higher than that of the control group. These results suggest that the immune response induced by SARS-CoV-2 infection may be different from that induced by a common cold-related viral infection. Viral infections generally enhance MLV (9). In this study, there was no significant increase in MLV of COVID-19 patients, relative to the control group. Likely, NK cell counts are markedly reduced in COVID-19 patients (3). Natural killer (NK) cells comprise approximately 10 to 15 % of total peripheral blood lymphocytes, and they are usually larger in terms of cell volume or size (11, 12). The results of previous studies demonstrated that SARS-CoV infection-induced apoptosis in peripheral lymphocytes (13-15). Similarly, phylogenetic analysis showed that SARS-CoV-2 had 79 % similarity with SARS-CoV (16). Hence, SARS-CoV-2 may reduce lymphocyte count via the induction of apoptosis. In this study, it appears SARS-CoV-2 infection-induced morphological changes in immune cells were more complicated than those of viral infection due to the common cold. Activated lymphocytes usually have increased volume and more cytoplasmic granules (9). On the other hand, the loss of cell volume is an early and fundamental feature of programmed cell death or apoptosis (17). The results of sensitivity and specificity analysis indicate that the distribution width (SD values) of lymphocyte CPD may be better hematological parameters for differential diagnosis of SARS-CoV-2 infection.

The results of this study suggest that lymphocyte CPD parameters have great diagnostic potential for SARS-CoV-2 infection and can be used for early diagnosis of the disease.

Acknowledgements
None.

Conflicts of interest
There are no conflicts of interest in this study.

Author's contribution
All work was done by the author named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Xinling Li; Yihua Zhu, XingJian Cao, Yonghui Lu, Dongsheng Xu, Renfei Lu, Xinling Li collected and analysed the data; Yihua Zhu wrote the text and all authors have read and approved the text prior to publication.

Ethical approval
The study protocol was approved by the Research Ethics Board of The Second Affiliated Hospital of Nantong University.

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