Letter to the Editor

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Decreased "WBC*LYM" was observed in SARS-CoV-2-infected patients from a fever clinic in Wuhan

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To the Editor,

Since December 2019, a series of pneumonia cases caused by a novel coronavirus have been reported in Wuhan, Hubei Province, China. The coronavirus soon raised intense attention not only within China but also internationally, and was initially named 2019-nCoV by the World Health Organization (WHO) [1]. Shortly after that, the disease was renamed by the WHO as coronavirus disease 2019 (COVID-19) and the virus was renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group (CSG) [2, 3]. Up to March 1, 2020, COVID-2019 has caused tens of thousands of human infections and thousands of deaths in and out of China.

As a highly infectious disease, the early detection, isolation and treatment of COVID-2019 are of great importance. However, the initial symptoms of COVID-2019 are similar to other respiratory virus infections with cough, fever and muscle ache [4]. These clinical symptoms confounded early detection of infected cases, especially against a background of ongoing influenza and other respiratory viruses like respiratory syncytial virus and adenovirus. Reliable rapid tests and feasible differential diagnosis are crucial for clinicians in their first contact with suspected patients.

Several studies have taken advantage of calculated hematology parameters, such as neutrophil (NEU)-to-lymphocyte (LYM) ratio (NLR), LYM-to-monocyte ratio (LMR) and platelet-to-LYM ratio (PLR), in the diagnosis and prognosis of inflammatory response-related virus infection [5]. These parameters are not only readily available but also cost-effective. As a newly discovered virus, information regarding the hematology parameters of COVID-19 patient is limited [1, 4]. Although there have been studies showing the use of calculated hematology parameters to help with distinguishing disease severities and predict the prognosis for COVID-19 [6, 7], the application of these parameters in the diagnosis and differential diagnosis is none.

A retrospective study on complete blood count (CBC) with differential results of patients who presented to the fever clinic of Tongji Hospital with symptoms of COVID-19-like illness between February 1, 2020 and February 20, 2020 was performed through case reviewing. Inclusion criteria were fever with a body temperature above 37.3 °C, accompanied or not accompanied by cough, chest tightness, muscle ache, shortness of breath and diarrhea. Patients with hematopathy, cancer and sepsis were excluded. The SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) testing of throat swab was performed in the laboratory of Tongji Hospital. These patients with COVID-19-like symptoms were divided into two groups. Patients diagnosed with COVID-19 according to the WHO interim guidance and confirmed by RT-PCR testing were included in the SARS-CoV-2-positive patient group (SPPG). Patients with two or more consecutive negative RT-PCR test results were included in the SARS-CoV-2-negative patient group (SNPG). Patients with co-infection of SARS-CoV-2 and other respiratory viruses including influenza A/B, respiratory syncytial virus and adenovirus were also excluded in SPPG. Sysmex XN-9000 hematology analyzer was used to obtain the CBC with differential results for patients in each group.

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Table 1: Age, gender and complete blood count with differential results of SARS-COV-2-positive patient and SARS-CoV-2-negative patient groups with similar symptoms.

Parameters	Total	SARS-CoV-2-negative patient group	SARS-CoV-2-positive patient group	χ^2/t/Z	p-Value
n	225	131	94	–	–
Age, years	52.0 (36.0–62.5)	50.0 (36.0–57.0)	56.0 (39.7–68.0)[a]	−2.861	0.004
Males	107 (47.6%)	62 (47.3%)	45 (47.9%)	0.006	0.936
WBC, 10^9/L	6.00 (4.50–7.27)	6.34 (5.09–7.97)	5.07 (3.86–6.62)[a]	−4.318	0.000
<3.5[b]	19 (8.4%)	3 (2.3%)	16 (17.0%)[a]	13.459	0.000
>9.5[b]	22 (9.8%)	19 (14.5%)	3 (3.2%)[a]	6.692	0.010
NEU, 10^9/L	3.68 (2.71–5.18)	3.81 (2.89–5.60)	3.35 (2.28–4.89)[a]	−3.051	0.002
<1.8[b]	13 (5.8%)	3 (2.3%)	10 (10.6%)[a]	5.494	0.019
>6.3[b]	30 (13.3%)	21 (16.0%)	9 (9.6%)	1.426	0.232
LYM, 10^9/L	1.38 (0.99–1.87)	1.62 (1.22–2.02)	1.14 (0.86–1.58)[a]	−4.736	0.000
<1.1[b]	68 (30.2%)	25 (19.1%)	43 (45.7%)[a]	17.126	0.000
MON, 10^9/L	0.47 (0.33–0.64)	0.50 (0.38–0.69)	0.42 (0.31–0.59)[a]	−2.582	0.010
>0.6[b]	66 (29.3%)	44 (33.6%)	22 (23.4%)	2.277	0.131
RBC, 10^{12}/L	4.53 (4.22–4.87)	4.59 (4.25–4.93)	4.42 (4.19–4.80)	−1.463	0.143
Decreased[c]	26 (11.6%)	13 (9.9%)	13 (13.8%)	0.479	0.489
HGB, g/L	138.5 ± 16.3	139.5 ± 17.9	137.2 ± 13.8	−1.070	0.286
Decreased[c]	18 (8.0%)	10 (7.6%)	8 (8.5%)	0.000	0.997
HCT, %	40.62 ± 4.56	40.90 ± 4.90	40.23 ± 4.05	−1.094	0.275
Decreased[c]	32 (14.2%)	16 (12.2%)	16 (17.0%)	0.678	0.410
MCV, fL	90.07 ± 3.53	89.92 ± 3.36	90.27 ± 3.77	0.743	0.458
MCH, pg	30.6 (29.4–31.6)	30.5 (29.2–31.7)	30.6 (29.7–31.6)	−0.295	0.768
MCHC, g/L	339 (332–346)	339 (333–346)	340 (332–346)	−0.046	0.964
RDW-SD, fL	41.0 (39.0–42.7)	40.9 (39.0–42.6)	41.1 (39.1–43.2)	−1.024	0.306
PLT, 10^9/L	229 (177–279)	237 (190–288)	206 (157–268)[a]	−2.473	0.013
<125[b]	13 (5.8%)	5 (3.8%)	8 (8.5%)	1.445	0.229
>350[b]	19 (8.4%)	13 (9.9%)	6 (6.4%)	0.474	0.491
PDW, fL	13.2 (11.6–15.0)	13.3 (11.6–15.2)	13.2 (11.6–14.9)	−0.186	0.853
MPV, fL	11.07 ± 1.11	11.03 ± 1.12	11.13 ± 1.10	−0.695	0.488
PCT, L/L	0.25 (0.19–0.30)	0.26 (0.21–0.32)	0.23 (0.18–0.30)[a]	−2.381	0.017
WBC*LYM, 10^{18}/L[2]	8.25 (5.32–12.94)	9.96 (6.63–14.64)	6.04 (3.69–9.15)[a]	−5.843	0.000

n, number; WBC, white blood cell count; NEU, neutrophil; LYM, lymphocyte; MON, monocyte; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-SD, red blood cell distribution width standard deviation; PLT, platelet; PDW, platelet distribution width; MPV, mean platelet volume; PCT, thrombocytocrit; WBC*LYM, white blood cell count multiplied by lymphocyte count. Continuous variables were defined as mean ± standard deviation for Gaussian distribution data and median (interquartile range) for non-Gaussian distribution data; categorical variables were given as number and percentages; an unpaired t-test was used for normal distribution data; the Mann-Whitney U test was used for non-normal distribution data; chi-square (χ^2) test was used for the comparison of rates. [a]Compared with the SARS-CoV-2-negative patient group, $p < 0.05$. [b]All cut-off values adopted in Table 1 were from the reference ranges recommended in WS/T 405-2012 "Reference intervals for blood cell analysis" in China available from http://www.nhc.gov.cn/ewebeditor/uploadfile/2013/01/20130109171100186.pdf. [c]RBC decreased is defined as male <4.3×10^{12}/L or female <3.8×10^{12}/L; HGB decreased is defined as male <130 g/L or female <115 g/L; HCT decreased is defined as male <40.0% or female <35.0%.

CBC with differential results at the request of clinicians at the initial evaluations was recorded along with age and gender for each patient.

We used the Statistical Package for Social Sciences (SPSS) Version 15.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis and a p-value ≤0.05 was considered statistically significant. Compared with patients in SNPG, the white blood cell count (WBC), NEU, LYM, monocyte, platelet count and thrombocytocrit were significantly lower for patients in SPPG (Table 1). Thus, these six parameters were chosen as candidates. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic value of selected parameters. Among those parameters, WBC and LYM were recognized as they produced the largest two areas under the curve (AUC). In order to increase the diagnostic values, a combination parameter

of LYM and WBC, i.e. WBC*LYM (formula: WBC multiplied by LYM), was then calculated. As shown in Table 2, using WBC*LYM to distinguish SARS-CoV-2-positive from -negative patients produced the largest AUC ($p < 0.05$) among all parameters. The sensitivity (73.40%) and specificity (63.36%) for WBC*LYM are highest if 8.47 was used as the cut-off value (Table 2).

The SARS-CoV-2 RT-PCR testing of respiratory tract specimen was recommended by the WHO to confirm COVID-19 [8]. However, clinicians are usually unable to obtain the RT-PCR result in their first contact with suspected patients. Additionally, during the pandemic, the RT-PCR testing was often restricted. Serology for diagnostic purposes is recommended only when RT-PCR is not available [8]. Whereas it takes time for the immune system to produce antibodies, serology may be suitable for a retrospective analysis, but not for an early diagnosis. We undertook this study with the aim of exploring hematology parameters to help identify COVID-19 among patients presenting with similar symptoms while awaiting RT-PCR results. To the best of our knowledge, this is the first study on applying calculated hematology parameters to identify COVID-19 in suspected patients.

Lymphopenia has been previously reported by a series of studies on SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) infections as well as SARS-CoV-2 [1, 4, 9, 10]. It was also observed in our study with a proportion of 45.7% in SPPG. Insufficient T-cell priming, lack of virus-specific T cells and cytokine-induced T-cell apoptosis were the major reasons for the lymphopenia in SARS-CoV [9], while MERS-CoV was found to be able to infect T cells directly and induce T-cell apoptosis by extrinsic and intrinsic apoptosis pathways [10]. As for SARS-CoV-2, the mechanism is still unclear for now. Liu et al. analyzed the changes in LYM subsets in mild and severe COVID-19 cases, and found that the development of lymphopenia in severe patients was mainly related to the significantly decreased absolute counts of T cells, especially CD8+ T cells, but not to B cells and NK cells [6]. This may provide clues to the mechanism of lymphopenia in SARS-CoV-2 infection.

Increased NLR was reported to be related to severe COVID-19 and NLR was chosen as a useful prognostic factor for COVID-19 by studies before [6, 7]. However, the diagnostic value of NEU in COVID-19 was shown to be disappointing in this study (AUC: 0.619). Reasons for the poor diagnosis value for NEU in this study may be that the parameter may depend on the stage of the disease in which the CBC analysis is performed or on the type of population assessed. On the contrary, except for LYM, WBC seemed to have the best diagnostic value in the differential diagnosis of COVID-19 among all parameters. However, the AUC of WBC*LYM is only 0.729. This reminds us that hematology parameters can be affected by a lot of factors inside and outside the human bodies. When using these parameters, epidemiological history, clinical symptoms and computerized tomography scans should be combined together to make a reasonable decision. Nevertheless, as CBC with differential results is the most widely used laboratory test for patients with cold symptoms and it is readily available even in primary hospitals, this parameter can still provide clues for clinicians in their first contact with suspected patients without available SARS-CoV-2 RT-PCR results.

There are several limitations in this study. First, relatively few cases were enrolled in this study and they are

Table 2: Diagnostic values of WBC, NEU, LYM, MON, PLT, PCT and WBC*LYM for distinguishing SARS-CoV-2-positive patients from SARS-CoV-2-negative patients with similar symptoms.

Parameters	Cut-off value[a]	Sensitivity, %	Specificity, %	LR+	LR−	AUC (95% CI)	p-Value[b]
WBC, 10^9/L	≤5.07	51.06	77.10	2.23	0.63	0.669 (0.603–0.730)	0.023
NEU, 10^9/L	≤2.72	38.30	83.21	2.28	0.74	0.619 (0.552–0.683)	0.003
LYM, 10^9/L	≤1.20	55.32	75.57	2.26	0.59	0.685 (0.620–0.745)	0.031
MON, 10^9/L	≤0.4	47.87	72.52	1.74	0.72	0.601 (0.534–0.665)	0.001
PLT, 10^9/L	≤189	45.74	76.34	1.93	0.71	0.597 (0.529–0.661)	0.001
PCT, L/L	≤0.18	31.91	86.26	2.32	0.79	0.593 (0.526–0.658)	0.000
WBC*LYM, $10^{18}/L^2$	≤8.47	73.40	63.36	2.00	0.42	0.729 (0.665–0.785)	\

WBC, white blood cell count; NEU, neutrophil; LYM, lymphocyte; MON, monocyte; PLT, platelet; PCT, thrombocytocrit; WBC*LYM, white blood cell count multiplied by lymphocyte count; LR^+, positive likelihood ratio; LR^-, negative likelihood ratio; AUC (95% CI), area under the receiver operating characteristic curve (95% confidence interval). [a]The Youden index of receiver operating characteristic curve was the largest when this cut-off value was used. [b]Using the method recommended by Delong et al., the AUC of WBC*LYM was compared with other parameters, and $p < 0.05$ was considered statistically significant.

all patients from Wuhan, and large-scale multicenter clinical studies are required to corroborate this evidence. Second, there are no routine medical examinations available for healthy people due to COVID-19 outbreak, so no healthy controls are included in the study. Third, although we have excluded patients with co-infection of SARS-CoV-2 and other respiratory viruses including influenza A/B, respiratory syncytial virus and adenovirus in SPPG, confounding factors still exist and may produce a certain degree of deviation. Last, there is a probability of false-negative SARS-CoV-2 RT-PCR results depending on the reagent sensitivity and specimen sampling skills. Although the inclusive criteria are two or more consecutive negative results for SNPG, false negatives are still inevitable.

In summary, decreased WBC*LYM was observed in SARS-CoV-2-infected patients compared with SARS-CoV-2-negative patients with suspected symptoms in this study. WBC*LYM can be used as a supplementary parameter to help clinicians in their first contact with suspected patients awaiting SARS-CoV-2 RT-PCR results.

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