Letter to the Editor

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Neutrophil-to-lymphocyte ratio predicts the clearance of SARS-CoV-2 RNA in mild COVID-19 patients – a retrospective analysis from Dongxihu Fangcang Hospital in Wuhan, China

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To the Editor,

An outbreak of an unknown infectious pneumonia has recently occurred in Wuhan, China [1]. The pathogen of the disease was quickly identified as a novel coronavirus (SARS-CoV-2, severe acute respiratory syndrome coronavirus 2), and the disease was named coronavirus infection disease-19 (COVID-19) [2]. According to the New Coronavirus Pneumonia Diagnosis Program (5th edition) published by the National Health Commission of China [3], clinical manifestations consist of four categories: mild, moderate, severe, and critical. To address the social spread of the virus, Dongxihu Fangcang Hospital, which aims to provide centralized treatment and isolation of COVID-19, was built for patients who were confirmed to have the mild type of COVID-19. Hospital equipment usually consists of a series of compartments with different medical or technical support functions, with the ability to deliver early treatment. The mild type patients of COVID-19 can recover shortly after appropriate clinical intervention. In addition, virus exclusion after standard treatment is an important factor in determining the prognosis of COVID-19.

Routine blood test was inspected among patients in the Dongxihu Fangcang Hospital. This is the most readily available, efficient, and economic method of examination. Blood analysis examined lymphocytes (LYM), platelets (PLT), the neutrophil-to-lymphocyte ratio (NLR), the lymphocyte-to-monocyte ratio (LMR), the platelet-to-neutrophil ratio (PNR), and the platelet-to-lymphocyte ratio (PLR). These blood cell counts and ratios are valuable in understanding the systemic inflammatory response. Researches have shown that NLR has a certain value in evaluating in-hospital mortality, poor clinical outcomes, and severity of COVID-19 [4, 5]. However, it has been rarely reported if these laboratory indicators were correlated with viral expression in COVID19 patients.

The aim of this study is to retrospectively analyze clinical data of mild COVID-19 patients treated in the Dongxihu Fangcang Hospital. This study was conducted in order to identify a simple and effective indicator to assess the correlation between viral exclusion in mild COVID-19 patients and simple laboratory indicators. In this way, it may be possible to perform diagnostic risk stratification and graded treatment of COVID-19 patients.

All cases were taken from mild COVID-19 patients in the Dongxihu Fangcang Hospital, which is one of the designated hospitals for the COVID-19 issued by local authorities. When the situation in the epidemic area is stable, the Fangcang hospital was closed on March 8, 2020. The patients with negative nucleic acid will be discharged, and the patients with suspected or positive nucleic acid will be transferred to other hospitals for further treatment. By the time of hospital closure, the median length of stay was 22 days. Nucleic acid test results of the patients before admission were all positive for SARS-CoV-2. A total of 289 samples were included in this study. Excluding patients

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with missing medical history and data omission, 182 patients were finally included in this retrospective analysis. All cases were diagnosed, classified, and treated according to the notice on the issuance of a program for the diagnosis and treatment of novel coronavirus (SARS-CoV-2) infected pneumonia (trial 7th edition), published by the National Health Commission of China [6].

Cases were grouped according to the results of the most recent nucleic acid test. Patients whose two times nucleic acid test results were negative were included in the clearance group. Those whose nucleic acid test results were suspected or positive were included in the persistence group.

Through retrospective analysis of routine blood test indicators in patients with mild COVID-19, this study showed that LYM, PNR, and NLR were effective indicators of SARS-CoV-2 RNA clearance by the 15th day. PLT, LYM, NLR and LMR were effective indicators of SARS-CoV-2 RNA clearance by the time of hospital closure (median length of stay was 23 vs. 19.5 days, clearance group vs. persistence group) (Table1). LMR was an independent risk factor for the non-negative nucleic acid test results by the time of hospital closure. In addition, it was found that NLR had a high ability to predict SARS-CoV-2 RNA clearance both by the 15th day and the time of hospital closure (Figures 1, 2).

Studies have shown that certain hematological parameters (e. g. WBC, lymphopenia and CRP) are associated with the severity of COVID-19 [7]. However, the relationship between these indicators and clearance of SARS-CoV-2 RNA has been rarely reported. In this analysis, SARS-CoV-2 RNA clearance was analyzed in accordance to whether SARS-CoV-2 was excluded within a specific time. Routine blood tests were used to diagnose and predict SARS-CoV-2 RNA clearance. Recently, increased attention has been given to the inflammation index expressed by the ratio of granulocytes, lymphocytes and monocytes. These ratios include NLR, PNR, and LMR. Inflammation may also have a certain influence on virus exclusion and prognosis of patients. The NLR and the LMR are potential novel biomarkers of the baseline inflammatory response. A systemic inflammation marker, LMR, is widely recognized as a prognostic indicator of various malignancies [8]. In this study, the goal was to evaluate the transition of mild COVID with LMR and NLR. The results were satisfactory. LYM and the LMR increased with the increase of lymphocyte count, and the NLR decreased with the increase of lymphocyte count. In the outcome of the persistence group with the nucleic acid results, both the LYM and the LMR indices were higher than those in the clearance group, while the NLR was lower than

Table 1: Comparison of laboratory indicators between the clearance and persistence group.

Indicator	Clearance group	Persistence group	t	p-Value
By 15th day				
Patients, n	67	23		
WBC, 10^9/L	5.64 ± 1.45	5.72 ± 1.33	−0.243	0.810
PLT, 10^9/L	216.78 ± 57.98	242.65 ± 57.53	−1.85	0.068
NEU, 10^9/L	3.22 ± 1.20	2.99 ± 0.97	0.816	0.417
LYM, 10^9/L	1.74 ± 0.53	2.04 ± 0.59	−2.211	0.030[a]
MON, 10^9/L	0.47 ± 0.15	0.52 ± 0.26	−1.111	0.269
EOS, 10^9/L	0.16 ± 0.15	0.17 ± 0.13	−0.134	0.894
BASO, 10^9/L	0.039 ± 0.023	0.042 ± 0.021	−0.435	0.665
NLR	2.02 ± 0.97	1.55 ± 0.54	2.193	0.031[a]
PLR	135.91 ± 57.87	128.12 ± 44.88	0.587	0.559
LMR	3.90 ± 1.28	4.50 ± 1.74	−1.761	0.082
PNR	73.61 ± 27.37	87.53 ± 27.73	−2.084	0.044[a]
By the time of hospital closure				
Patients, n	150	32		
WBC, 10^9/L	5.80 ± 1.41	5.94 ± 1.48	−0.479	0.634
PLT, 10^9/L	226.69 ± 59.78	250.06 ± 58.47	−2.045	0.047[a]
NEU, 10^9/L	3.27 ± 1.14	3.12 ± 1.14	0.675	0.5
LYM, 10^9/L	1.82 ± 0.53	2.12 ± 0.62	−2.894	0.004[a]
MON, 10^9/L	0.50 ± 0.17	0.47 ± 0.15	0.950	0.343
EOS, 10^9/L	0.17 ± 0.14	0.19 ± 0.14	−0.754	0.452
BASO, 10^9/L	0.044 ± 0.024	0.041 ± 0.021	0.602	0.548
NLR	1.94 ± 0.85	1.58 ± 0.77	2.215	0.028[a]
PLR	134.21 ± 51.16	126.63 ± 43.44	0.780	0.437
LMR	3.91 ± 1.25	4.85 ± 1.54	−3.717	0.0001[a]
PNR	80.26 ± 60.29	85.33 ± 24.11	−0.467	0.641

The p-value of analytes showing a statistically significant difference (<0.05) is marked by 'a'.

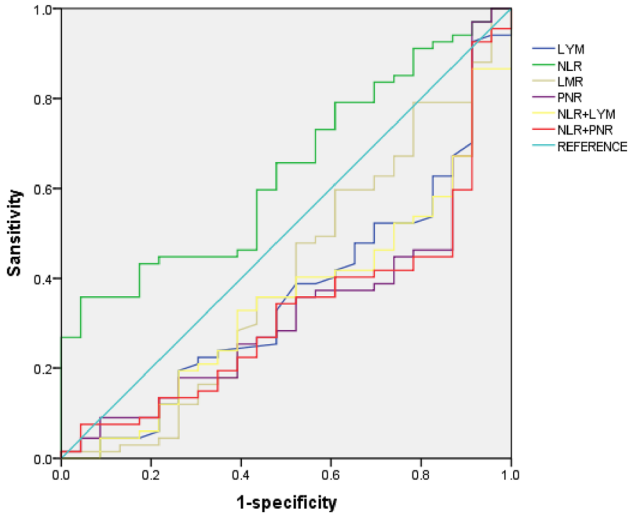

Figure 1: Receiver operating curve analysis used to identify patients with negative PCR results by the 15th day.

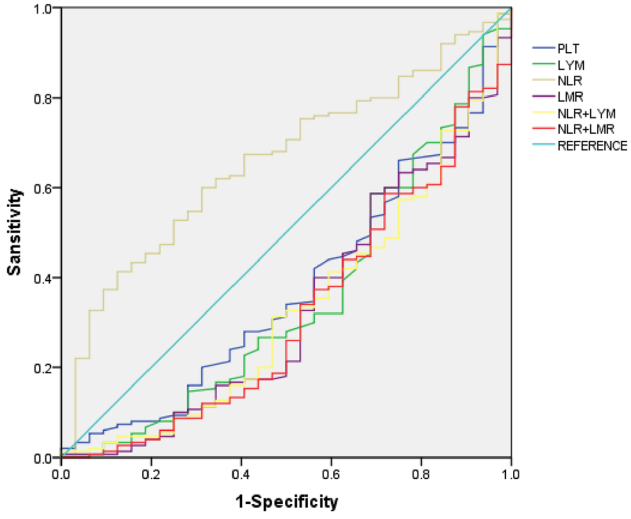

Figure 2: Receiver operating curve analysis used to identify patients with negative PCR results by the time of hospital closure.

the clearance group, indicating that high lymphocyte count may lead to the non-negative outcome of the nucleic acid results. Lymphocytopenia was presented in 83.2% of the patients on admission [9]. Therefore, it is speculated that Lymphocytopenia was not detrimental in mild COVID-19 patients. A decrease in the LMR proved beneficial to the exclusion of the virus in mild COVID-19 patients. A LMR>7.77 proved to be a new independent risk factor the clearance of SARS-CoV-2 RNA in mild COVID-19 patients.

The NLR, a well-known marker of systemic inflammation and infection, has been studied as a predictor of bacterial infection. It also plays a role in assessing the severity of COVID-19. Several patients with COVID-19 had a rising neutrophil count and a falling lymphocyte count during the severe phase [7]. However, in this study, it was found that when the clinical data for mild COVID-19 patients was analyzed, the NLR value at specific time points was higher in the clearance group. Similarly, the LMR and LYM decline may be associated with poor prognosis of COVID-19 and increased in-hospital mortality. In mild COVID patients, a moderate inflammatory response is sufficient to clear the body of the viral pathogen. Therefore, a moderate increase in the NLR is conducive to the clearance of SARS-CoV-2 from the patient. However, excessive inflammatory response will cause immune loss, fatal cytokine production, systemic inflammatory response, multi-organ failure and a series of additional clinical manifestations [10, 11]. This leads to a more severe, critical condition of patients. Consistent with previous reports, persistent increase in the NLR was associated with patient severity, inpatient mortality, and poor prognosis.

By analyzing the blood routine parameters of hospitalized patients, the negative outcome of the nucleic acid results in mild COVID-19 patients is particularly important for graded treatment of patients. Patient outcome can be predicted by the NLR in order to achieve graded diagnosis and treatment, optimize the allocation of rescue resources, and to avoid the occurrence of overtreatment or under treatment. In addition, these predictors will enable risk stratification, guide interventional studies to target patients at enhanced risk of developing severe disease and optimize allocation of limited human and technical resources in the ongoing pandemic [12].

However, the clinical data in the present study came from a single center and the sample size was limited. Comparison between the clearance and persistence group was based on the specific treatment conditions in the hospital and might not be fully applicable to some patients who were treated using a different protocol. In addition, this was a retrospective study, and there was no uniform cut-off point at the time of routine blood tests. Having more detailed results of continuous blood monitoring of the routine indicators would greatly improve the prediction accuracy of COVID-19 outcome. In addition, it is important to consider the stage of COVID-19, which was not considered in this study, since the lymphocyte count may also change at different stages. Thus, these data should be interpreted with some caution due to the limitations of the study. The next stage, we will start to study the blood indicators of patients with severe COVID-19, so as to infer the value of a moderate increase/decrease in hematological indicators to determine whether it has an impact on the clearance of the virus or the deterioration of the patient's condition.

From what has been discussed above, analysis of LYM, NLR and LMR have clinical value in the diagnosis and

prediction of SARS-CoV-2 RNA clearance in mild COVID-19 patients. Of these, the NLR is the powerful potential indicator. Continuous monitoring of LMR and NLR indicators is recommended during the treatment of COVID-19 to predict virus exclusion in COVID-19 patients.

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