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## Neutrophil to CD4 + lymphocyte ratio as a potential biomarker in predicting virus negative conversion time in COVID-19

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### ARTICLE INFO

#### Keywords:

COVID-19  
SARS-CoV-2  
Neutrophil to CD4 + lymphocyte ratio  
Negative conversion time

### ABSTRACT

**Background:** Since December 2019, novel coronavirus (SARS-CoV-2)-infected pneumonia (COVID-19) occurred in Wuhan, and rapidly spread throughout China. Our study aimed to evaluate the robustness of neutrophil to CD4 + lymphocyte ratio (NCD4LR) in predicting the negative conversion time (NCT) of SARS-CoV-2 in COVID-19 patients.

**Methods:** Univariate and multivariate analysis were conducted to evaluate the independency of NCD4LR in predicting NCT. Receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) were used to assess the diagnostic accuracy.

**Results:** Compared with low NCD4LR patients, patients with high NCD4LR had an older age; higher incidence of fever, fatigue, chest distress/breath shortness, severer disease assessment on admission; higher levels of inflammatory indicators; low levels of lymphocyte subsets, and a longer NCT. Multivariate analysis also identified NCD4LR as an independent risk factor for delayed NCT. ROC analysis showed that NCD4LR had a better performance than neutrophil to lymphocyte ratio in predicting the virus negative conversion within 2 weeks (AUC = 0.772), 3 weeks (AUC = 0.710), 4 weeks (AUC = 0.728), or 5 weeks (AUC = 0.815).

**Conclusion:** This study suggests that NCD4LR is a potential and useful biomarker for predicting the virus negative conversion time in COVID-19 patients. Furthermore, due to the NCD4LR value is easily calculated, it can be widely used as a clinical biomarker for disease progression and clinical outcomes in COVID-19 patients.

### 1. Introduction

Since December 2019, an outbreak of pneumonia of unknown cause occurred in Wuhan, and rapidly spread throughout China [1]. The pathogen was confirmed to be a distinct clade from the  $\beta$ -coronaviruses, which was officially named SARS-CoV-2, with the disease termed COVID-19. Currently, no antiviral drug demonstrated definite effects, and the main therapeutic strategy focused on symptomatic support. During hospitalization, the negative conversion of SARS-CoV-2 was essential in the discharge criteria [2]. Therefore, it was necessary to identify factors associated with the negative conversion time (NCT) in COVID-19, which could contribute to the disease progression and clinical outcomes.

Several studies focused on the effects of impaired immunity in the deterioration of COVID-19. Our recent study found that total

lymphocytes, CD4 + lymphocytes, CD8 + lymphocytes, B cells, and natural killer (NK) cells decreased in COVID-19 patients, and severe cases had a lower level than mild cases [3]. Qin *et al* also indicated the dysregulation of lymphocyte subsets might be highly associated with the development of COVID-19. Monitoring neutrophil to lymphocyte ratio (NLR) and lymphocyte subsets played a role in the diagnosis and treatment of COVID-19 [4]. Importantly, CD4 + lymphocytes responded more significantly to virus surveillance than CD8 + lymphocytes [4]. However, it was still unknown on the relationship between NCT and neutrophil to CD4 + lymphocyte ratio (NCD4LR) in patients with COVID-19. In this study, we aimed to clarify the characteristics of COVID-19 patients with higher NCD4LR and evaluate the robustness of NCD4LR in predicting NCT.

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## 2. Methods

### 2.1. Study design and participants

All consecutive patients with diagnosed COVID-19, who admitted to Zhongnan Hospital of Wuhan University from January 15 to March 2, were enrolled. Informed consent was obtained from each enrolled patient. This retrospective study was approved by the ethics committee of Zhongnan Hospital of Wuhan University (No. 2020011).

### 2.2. Definitions

The virus nucleic acid detection kit was confirmed COVID-19 patients through detecting the RNA of SARS-CoV-2 in throat swab samples using based on the manufacturer's protocol (Shanghai BioGerm Medical Biotechnology Co.,Ltd). Then, all patients were admitted and isolated for treatment within one week after symptom onset. During the hospitalization, each patient had a swab virus test every other day. NCT of SARS-CoV-2 was defined as the interval between symptom onset and the first of two consecutive negative virus tests.

In the severity assessment on admission, general illness was defined as mild clinical symptoms and there was no pneumonia phenotype on CT imaging. serious illness was defined if satisfying at least one of the following items: (i) breathing rate  $\geq 30$ /min; (ii) pulse oximeter oxygen saturation ( $SpO_2$ )  $\leq 93\%$  at rest; (iii) ration of partial pressure of arterial oxygen ( $PaO_2$ ) to the fraction of inspired oxygen ( $FiO_2$ )  $\leq 300$  mmHg (1 mmHg = 0.133 kPa). Critical illness was defined if satisfying at least one of the following items: (i) respiratory failure occurred and received mechanical ventilation; (ii) shock; (iii) combined with failure of other organs and received care in the intensive care unit (ICU) [2].

### 2.3. Flow cytometry

Samples of EDTA anticoagulated peripheral blood (2 mL) were collected from patients with COVID-19 before initial treatment. All samples were tested within 6 h of being obtained. Briefly, multi-color flow cytometry was used to measure the CD3 + /CD4 + /CD8 + T-cell, CD19 + B-cell, and CD16 + CD56 + NK-cell counts (cells/ $\mu$ L) by human monoclonal anti-CD3-fluorescein isothiocyanate (FITC), anti-CD4-phycoerythrin (PE), anti-CD8-allophycocyanin (APC), anti-CD19-PE, anti-CD16-APC, and anti-CD56-PE antibodies (BD Multitest) according to the manufacturer's instructions. The cells were analyzed on a BD FACS Canto II flow cytometry system (BD Biosciences).

### 2.4. Data collection

A COVID-19 case report form was designed to document primary data regarding demographic, clinical, and laboratory characteristics from electronic medical records. The following information was extracted from each patient: gender, age, medical history, chief complaints and severity assessment on admission, laboratory findings, and NCT.

### 2.5. Statistical analysis

Categorical variables were presented as n (%). Continuous measurements were presented as mean (with standard deviation, SD) if they were normally distributed or median and interquartile range (IQR) if they were not. Nonparametric comparative test for continuous data and  $\chi^2$  test or Fisher's exact test for categorical data were used to compare variables between groups.  $P < 0.05$  was considered statistically significant. To identify the independent risk factors associated with NCT, univariate and multivariate logistic regression models were applied. The significant variables ( $P < 0.05$ ) in the univariate analysis were selected and put into the multivariate logistic regression model by SPSS

**Table 1**

Characteristics of neutrophil to CD4 + lymphocyte ratio in patients with COVID-19 pneumonia.

Variable (unit, normal range)	Total (n = 95)	$\leq 0.012533$ (n = 48)	$> 0.012533$ (n = 47)	P value
Female, No. (%)	51 (53.6)	34 (70.8)	17 (36.2)	0.001
Age, mean $\pm$ SD	55 $\pm$ 16	50 $\pm$ 15	60 $\pm$ 16	0.006
Comorbidities, No. (%)				
Cardiovascular/ cerebrovascular	28 (29.4)	11 (22.9)	17 (36.2)	0.157
Respiratory	6 (6.3)	1 (2.1)	5 (10.6)	0.111
Endocrine	23 (24.2)	10 (20.8)	13 (27.6)	0.437
Nervous	3 (3.1)	1 (2.1)	2 (4.2)	0.617
Urinary	1 (1.1)	0 (0)	1 (2.1)	0.495
Hepatic	8 (8.4)	4 (8.3)	4 (8.5)	1.000
Symptoms and signs, No. (%)				
Fever	64 (67.3)	27 (56.2)	37 (78.7)	0.020
Fatigue	32 (33.6)	8 (16.7)	24 (51.1)	$P < 0.001$
Headache	2 (2.1)	2 (4.2)	0 (0)	0.495
Cough	36 (37.8)	14 (29.2)	22 (46.8)	0.076
Chest distress/breath shortness	36 (37.8)	8 (16.7)	28 (59.6)	$P < 0.001$
Anorexia	2 (2.1)	0 (0)	2 (4.2)	0.242
Diarrhea	1 (1.1)	1 (2.1)	0 (0)	1.000
Severity assessment on admission, No. (%)				
General	56 (58.9)	40 (83.3)	16 (34.0)	$P < 0.001$
Serious	15 (15.8)	6 (12.5)	9 (19.1)	
Critical	24 (25.2)	2 (4.2)	22 (46.8)	
Hospitalized treatment, No. (%)				
Corticosteroid	58 (61.1)	19 (39.6)	39 (82.9)	$P < 0.001$
Mechanical ventilation	23 (24.2)	2 (4.2)	21 (44.7)	$P < 0.001$
Virus negative conversion time, median (IQR)	19 (11–27)	13 (9–21)	24 (18–33)	$P < 0.001$

**Abbreviation:** COVID-19, coronavirus disease 2019; No., number; SD, standard deviation; IQR, interquartile range.

Statistics version 25.0 software. The “pROC” R package was used to conduct receiver operating characteristic (ROC) curve analysis to evaluate the diagnostic accuracy.

## 3. Results

### 3.1. Baseline characteristics of included patients

A total of 95 COVID-19 patients were included in this study (Table 1). The mean age was 55  $\pm$  16 years with 51 female patients (53.6%). The most common comorbidity was in the cardiovascular/cerebrovascular system (29.4%) and endocrine system (24.2%). The most common chief complaints were fever (67.4%), cough (37.8%), chest distress/breath shortness (37.8%), and fatigue (33.6%). On admission, 56 (58.9%), 15 (15.8%), and 24 (25.2%) patients were classified into general, serious, and critical illness, respectively. During the hospitalization, 58 patients (61.1%) received corticosteroid, and 23 (24.2%) with mechanical ventilation. Additionally, the median NCT was 19 days (IQR, 11–27).

### 3.2. Univariate analysis of factors associated with NCD4LR

All patients were divided into two groups according to the median NCD4LR (0.012533). Compared with low NCD4LR patients, those with a higher NCD4LR had an older age ( $P = 0.006$ ), higher incidence of fever ( $P = 0.02$ ), fatigue ( $P < 0.001$ ), chest distress/breath shortness ( $P < 0.001$ ), and severer disease assessment on admission ( $P < 0.001$ ). Moreover, higher NCD4LR patients were more likely to receive corticosteroid ( $P < 0.001$ ) and mechanical ventilation

**Table 2**  
Laboratory findings of neutrophil to CD4+ lymphocyte ratio in patients with COVID-19.

Variable (unit, normal range)	Total (n = 95)	≤ 0.012533 (n = 48)	> 0.012533 (n = 47)	P value
<b>Blood cytology</b>				
Leukocytes (3.5 ~ 9.5 × 10 <sup>9</sup> /L), median (IQR)	5.66 (3.59–8.93)	3.99 (3.10–5.63)	9.24 (5.76–10.70)	P < 0.001
Neutrophils (1.8 ~ 6.3 × 10 <sup>9</sup> /L), median (IQR)	3.81 (2.47–7.21)	2.51 (1.63–3.36)	8.11 (4.62–9.62)	P < 0.001
Platelets (125 ~ 350 × 10 <sup>9</sup> /L), median (IQR)	189 (134–230)	186 (156–225)	203 (120–267)	0.454
Monocytes (0.1 ~ 0.6 × 10 <sup>9</sup> /L), mean ± SD	0.52 ± 1.01	0.38 ± 0.16	0.66 ± 1.41	0.101
Lymphocytes (1.1 ~ 3.2 × 10 <sup>9</sup> /L), median (IQR)	0.77 (0.52–1.07)	1.04 (0.77–1.46)	0.58 (0.32–0.77)	P < 0.001
CD3+ (805 ~ 4459/μL)	477 (299–781)	774 (572–1095)	324 (195–455)	P < 0.001
CD3 + CD4+ (345 ~ 2350/μL)	290 (153–518)	513 (304–625)	180 (109–274)	P < 0.001
CD3 + CD8+ (345 ~ 2350/μL)	189 (114–330)	312 (197–423)	123 (71–179)	P < 0.001
CD4/CD8 ratio (0.96 ~ 2.05)	1.52 (1.05–2.29)	1.50 (1.11–2.04)	1.54 (1.04–2.51)	0.929
CD19+ (240 ~ 1317/μL)	118 (49–187)	149 (74–225)	81 (31–153)	0.005
CD16 + CD56+ (210 ~ 1514/μL)	126 (49–208)	173 (100–250)	57 (22–164)	P < 0.001
<b>Blood inflammatory indicators, median (IQR)</b>				
CRP (0 ~ 10 mg/L)	21.7 (5.8–76.3)	11.4 (3.1–35.3)	69.8 (16.6–119.3)	P < 0.001
ESR (0 ~ 15 mm/h)	26.5 (10.0–46.7)	18.5 (8.0–32.0)	46.5 (31.0–78.5)	P < 0.001
<b>Blood biochemistry</b>				
ALT (9 ~ 50 U/L), median (IQR)	24 (18–38)	22 (16–32)	25 (20–50)	0.269
AST (15 ~ 40 U/L), median (IQR)	27 (18–39)	27 (18–35)	29 (19–52)	0.053
ALB (40 ~ 55 g/L), mean ± SD	34.9 ± 5.8	37.5 ± 4.8	32.1 ± 5.5	P < 0.001
GLB (20 ~ 30 g/L), median (IQR)	29.2 (26.5–32.0)	28.5 (26.0–30.6)	30.8 (27.7–34.9)	P < 0.001
GGT (8 ~ 57 U/L), median (IQR)	29 (18–49)	23 (16–38)	36.5 (23–67)	0.007
ALP (30 ~ 120 U/L), mean ± SD	75 ± 26	72 ± 24	78 ± 27	0.361
Creatinine kinase (< 171 U/L), mean ± SD	62 ± 18	56 ± 14	68 ± 21	0.006
LDH (125–243 U/L), median (IQR)	209 (176–289)	192 (154–252)	275 (206–450)	0.001
CKMB (0 ~ 6.6 ng/mL), median (IQR)	70 (43–133)	70 (47–109)	84 (36–207)	0.387

**Abbreviation:** COVID-19, coronavirus disease 2019; No., number; SD, standard deviation; IQR, interquartile range; AST, aspartate aminotransferase; ALB, albumin; GLB, globulin; GGT, glutamyltranspetidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CKMB, MB isoenzyme of creatine kinase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ALT, alanine aminotransferase.

(P < 0.001). Importantly, the higher NCD4LR group showed a relatively longer NCT (P < 0.001) (Table 1).

In addition, laboratory findings showed that patients with high NCD4LR had higher levels of leukocytes (P < 0.001), neutrophils (P < 0.001), lymphocytes (P < 0.001), glutamyltranspetidase (GGT, P = 0.007), globulin (GLB, P < 0.001), creatinine kinase (P = 0.006), lactate dehydrogenase (LDH, P = 0.001), C-reactive protein (CRP, P < 0.001) and erythrocyte sedimentation rate (ESR, P < 0.001), and a lower level of albumin (ALB, P < 0.001). For lymphocyte subsets, lower levels of CD3+ (P < 0.001), CD3 + CD4+ (P < 0.001), CD3 + CD8+ (P < 0.001), CD19+ (P = 0.005) and CD16 + CD56+ (P < 0.001) lymphocytes were observed in the higher NCR4LR group (Table 2).

### 3.3. Multivariate analysis and ROC analysis

24 significant variables in the univariate analysis were selected to perform the multivariate analysis to identify independent risk factors associated with NCT. The results showed only chest distress/breath shortness (OR, 4.011; 95%CI, 1.333 to 9.455) and NCD4LR (OR, 2.575; 95%CI, 1.276 to 3.468) were positively correlated with NCT (Table 3).

To further evaluate the diagnostic accuracy of NCD4LR, ROC analysis was performed and NLR was selected as the control ratio. We found that NCD4LR had a better performance than NLR in predicting the negative conversion within 2 weeks (AUC = 0.772), 3 weeks (AUC = 0.710), 4 weeks (AUC = 0.728) and 5 weeks (AUC = 0.815) (Fig. 1). Additionally, NK cells were reportedly decreased in COVID-19 patients [3,5]. Therefore, the CD4/CD56 ratio was used to compare with NCD4LR, and the results also found that NCD4LR had a better predictive effect on negative conversion (Fig. S1). Moreover, we applied the stratified analysis for further data mining. For general patients, NCD4LR had a better performance in early (within 2 weeks) effective prediction of negative conversion (AUC = 0.774, Fig. 2a). For critical patients, NCD4LR performed better in predicting the negative conversion within 5 weeks (AUC = 0.833, Fig. 2b). For severe patients, NCD4LR also showed a good power in predicting the negative

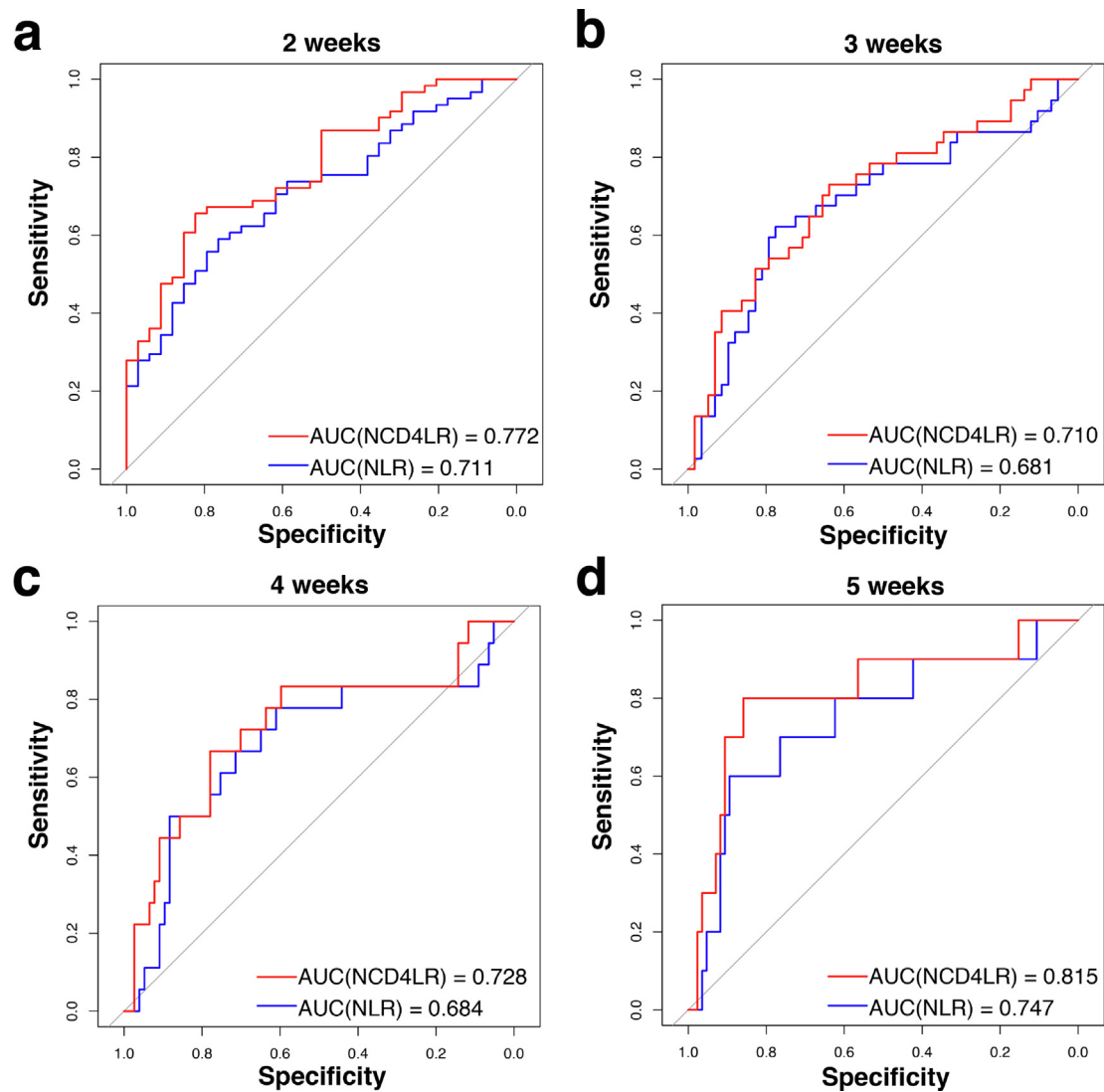
**Table 3**

Multivariate analysis of risk factors associated with negative conversion time of SARS-CoV-2 from symptom onset in COVID-19 patients.

	P value	OR (95% CI)
Female	0.936	0.896 (0.060 ~ 13.324)
Age	0.087	7.305 (0.749 ~ 71.283)
Fever	0.778	1.457 (0.106 ~ 19.977)
Fatigue	0.923	0.834 (0.021 ~ 33.843)
Chest distress/breath shortness	0.011	4.011 (1.333 ~ 9.455)
Corticosteroid	0.257	4.011 (0.363 ~ 44.366)
Mechanical ventilation	0.210	0.014 (0.001 ~ 10.898)
Severity assessment on admission	0.366	3.681 (0.219 ~ 61.950)
Leukocytes	0.511	2.501 (0.163 ~ 38.365)
Neutrophils	0.692	0.453 (0.009 ~ 22.895)
Lymphocytes	0.070	13.773 (0.807 ~ 234.951)
CD3 + lymphocytes	0.280	0.155 (0.005 ~ 4.543)
CD3 + CD4+ lymphocytes	0.199	0.106 (0.003 ~ 3.246)
CD3 + CD8+ lymphocytes	0.230	6.760 (0.299 ~ 153.019)
CD19 + lymphocytes	0.109	4.709 (0.708 ~ 31.304)
CD16 + CD56 + lymphocytes	0.097	0.152 (0.016 ~ 1.408)
CRP	0.305	0.237 (0.015 ~ 3.694)
ESR	0.837	0.765 (0.080 ~ 9.889)
ALB	0.191	5.501 (0.427 ~ 70.941)
GLB	0.543	0.533 (0.070 ~ 4.050)
GGT	0.144	10.082 (0.455 ~ 223.502)
Creatinine kinase	0.886	0.842 (0.080 ~ 8.840)
LDH	0.557	0.465 (0.036 ~ 6.004)
NCD4LR	0.029	2.575 (1.276 ~ 3.468)

COVID-19, coronavirus disease 2019; OR, odds ratio; CI, confidence interval; NCD4LR, neutrophil to CD4+ lymphocyte ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; GLB, globulin; GGT, glutamyltranspetidase; LDH, lactate dehydrogenase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

conversion within 5 weeks (AUC = 0.835), whereas the prediction performance was relatively poor within 3 (AUC = 0.658) or 4 weeks (AUC = 0.639) (Fig. 2c). Thus, the predictive power of NCD4LR for negative conversion within 2 or 5 weeks was superior to that within 3 or 4 weeks.



**Fig. 1.** ROC curve analysis of NCD4LR and NLR in predicting the negative conversion of SARS-CoV-2 within 2 (a), 3 (b), 4 (c), or 5 (d) weeks from symptom onset. ROC, receiver operating characteristic; AUC, area under ROC curve; NCD4LR, neutrophil to CD4+ lymphocyte ratio; NLR, neutrophil to lymphocyte ratio.

#### 4. Discussion

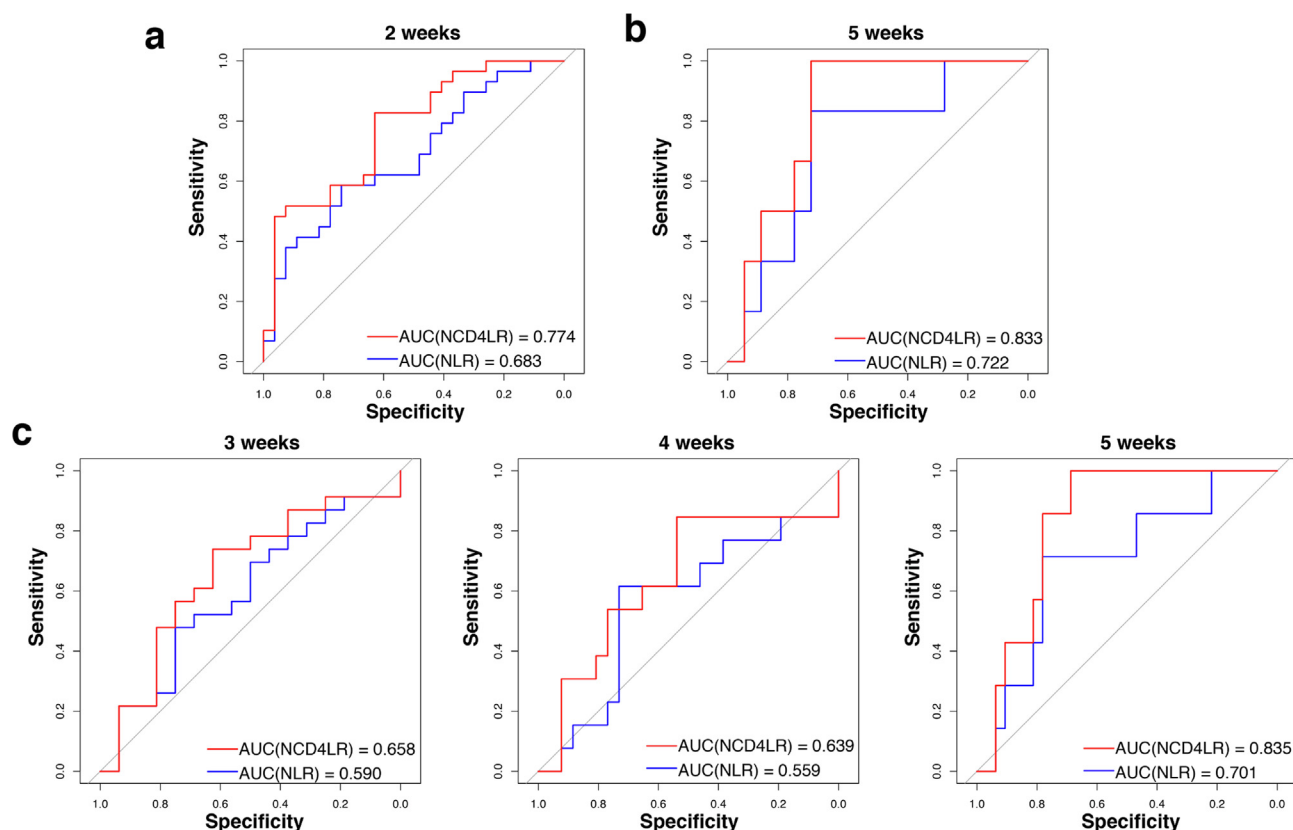
In this study, we reported that NCD4LR played a valuable role in predicting NCT of patients with COVID-19. In addition, we observed that high NCD4LR patients were mostly elderly male with higher incidence of fever, fatigue, chest distress/breath shortness, severer disease assessment on admission and higher levels of inflammatory indicators. To the best of our knowledge, it is the first study to explore the relationship between NCD4LR and NCT in COVID-19 patients.

Currently, the therapeutic strategies of COVID-19 were mainly symptomatic and supportive treatment. The discharged criteria for COVID-19 patients were not only no abnormalities in clinical symptoms or computed tomography imaging, but also two consecutive negative results of nucleic acid test [2]. However, no published studies reported that any clinical indicators were highly associated with NCT in COVID-19 patients. Our data found that the high NCD4LR patients had a longer median NCT than that in the low NCD4LR group (24 vs. 13 days), indicating worse immune function and prolonged virus clearance. Therefore, the progression and prognosis of COVID-19 could be assessed by calculating NCD4LR value.

Moreover, recent studies reported that NLR was a promising biomarker to efficiently diagnose and predict the clinical outcomes of COVID-19 patients and was an independent risk factor for mortality in

COVID-19 hospitalized patients [6,7]. It is well-known that neutrophils and lymphocytes are important components of the innate immune system. Neutrophils secreted large amounts of cytokines and chemokines to regulate immune responses, such as antiviral defense, hematopoietic action, angiogenesis, or fibrogenesis [8]. In patients with severe viral infections, neutrophil levels were significantly elevated, inducing a cytokine/chemokine storm, which ultimately lead to lung injury and acute respiratory distress syndrome [9]. Several studies even suggested that neutrophils should be used as a target for the treatment of severe influenza pneumonia [10]. In addition, approximately 80% of SARS patients had lymphopenia compared with healthy controls [11]. Patients with Middle East Respiratory Syndrome (MERS) also showed lymphopenia, albeit to a lesser extent than in patients with SARS [12]. Therefore, NLR was calculated as a useful indicator of inflammation during multiple diseases, such as viral infection, liver disease, and acute respiratory distress syndrome [13,14]. However, we found that NCD4LR value was significantly more accurate than NLR value in the prediction of NCT in patients with COVID-19 (Fig. 1 and Fig. 2). CD4+ lymphocytes, also known as helper T cells, played a crucial role in the detection and transmission of antigen information during viral infection. Channappanavar *et al* reported that approximately 90%~100% of SARS patients showed a marked decrease of CD4+ lymphocytes [11]. Cui *et al* also found the incidence of CD4+ lymphocytes decreased in





**Fig. 2.** Stratified analysis based on the severity of the patients' condition. **(a)** ROC curve analysis of NCD4LR and NLR in predicting the negative conversion within 2 weeks for general patients. **(b)** ROC curve analysis of NCD4LR and NLR in predicting the negative conversion within 5 weeks for critical patients. **(c)** For severe patients, ROC curve analysis of NCD4LR and NLR in predicting the negative conversion within 3, 4, or 5 weeks. ROC, receiver operating characteristic; AUC, area under ROC curve; NCD4LR, neutrophil to CD4 + lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; NCT, negative conversion time.

100% of SARS patients, whereas CD8 + lymphocytes decreased in 87%, B cells decreased in 76%, and NK cells decrease in 55% [15]. Therefore, CD4 + lymphocytes may be the most affected cell subpopulation in lymphopenia, which also explains that NCD4LR has better predictive power than NLR.

In addition, little is known about the relationship between NCD4LR and clinical characteristics in patients with COVID-19. Thus, we divided patients into two groups based on the median NCD4LR (0.012533) as the cutoff point. Our data found that high NCD4LR patients were mostly elderly male, which was consistent with previously studies [16]. In clinical manifestations, fever was the most common symptoms, followed by fatigue, chest distress and shortness of breath and cough. The prevalence of fever (67.3%) in COVID-19 patients was relatively low in this study compared with SARS or MERS. Guan *et al* found that only 43.8% of patients had fever on admission, while the prevalence of fever in SARS or MERS reached almost 100% [12,16,17]. Moreover, in the higher NCD4LR group, the incidence of fever was only 78.7%. Similarly, the incidence of cough was also not high, only 37.8%. These data suggested that we should pay attention to those patients without fever and cough to avoid a missed diagnosis. In addition, the incidence of chest distress/breath shortness and fatigue and levels of inflammation indicators (e.g. CRP, ESR, and LDH) were increased significantly in the high NCD4LR group [18,19]. This suggested that immune function of the high NCD4LR patients might be severely impacted. This view was supported by the significantly higher proportion of severe and critical patients on admission in the high NCD4LR group. Importantly, the CD4 + lymphocyte count was significantly decreased in the high NCD4LR patients than that in the low NCD4LR patients. This was consistent with the opinion of Qin *et al*, and further confirmed that CD4 + lymphocytes played an irreplaceable function in the immune regulation of COVID-19 patients.

There are several limitations in our research. First, this was a retrospective single-center study. A multi-center study may be better to test the accuracy of NCD4LR in predicting NCT. Second, only included inpatients for a certain period, the sample size is not large enough, and selection bias may occur. Third, NCD4LR value is collected and calculated on admission, not on the day of the onset of symptoms. Most patients took antipyretics before admission, which may affect the value of NCD4LR. Finally, we did not explore the dynamics of NCD4LR at different time points during the disease.

In conclusion, our study suggests that NCD4LR is a potential biomarker for predicting the virus negative conversion time in COVID-19 patients. A high NCD4LR value on admission indicates that patients are in poor conditions and are more likely to induce a longer NCT. Moreover, since NCD4LR are easily calculated from routine blood draws, it can be used widely as a simple and useful biomarker for disease progression and clinical outcomes in COVID-19 patients.

#### CRedit authorship contribution statement

**Haizhou Wang:** Conceptualization, Writing - original draft. **Yongxi Zhang:** Methodology, Writing - original draft. **Pingzheng Mo:** Methodology, Validation. **Jing Liu:** Writing - original draft, Validation. **Hongling Wang:** Supervision. **Fan Wang:** Writing - review & editing, Funding acquisition. **Qiu Zhao:** Conceptualization, Writing - review & editing, Supervision.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

### Acknowledgement

This study was supported by the Program of Excellent Doctoral (Postdoctoral) of Zhongnan Hospital of Wuhan University (Grant No. ZNYB2019003). We thank the patients, the nurses and physicians who provided care for the patients, and the investigators at Zhongnan Hospital of Wuhan University.

### Data sharing

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

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2020.106683>.

### References

- [1] Cumulative number of reported cases of 2019 novel coronavirus pneumonia. Beijing: National health commission of the People's Republic of China, 2020[EB/OL]. [2020-03-20]. <http://www.nhc.gov.cn/xcs/yqfkdt/202003/0fc43d6804b04a4595a2eadd846c0a6e.shtml>.
- [2] National Health Commission of China. New coronavirus pneumonia prevention and control program (7th edn). Mar 4, 2020. <http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml?spm=C73544894212.P59511941341.0.0>.
- [3] F. Wang, J. Nie, H. Wang, Q. Zhao, Y. Xiong, L. Deng, S. Song, Z. Ma, P. Mo, Y. Zhang, Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia, *J. Infect. Dis.* 221 (11) (2020) 1762–1769.
- [4] C. Qin, L. Zhou, Z. Hu, S. Zhang, S. Yang, Y. Tao, C. Xie, K. Ma, K. Shang, W. Wang, D.S. Tian, Dysregulation of immune response in patients with COVID-19 in Wuhan, China, *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* (2020).
- [5] E.J. Giamarellos-Bourboulis, M.G. Netea, N. Rovina, K. Akinosoglou, A. Antoniadou, N. Antonakos, G. Damosaki, T. Gkavogianni, M.E. Adami, P. Katsaounou, M. Ntaganou, M. Kyriakopoulou, G. Dimopoulos, I. Koutsodimitropoulos, D. Velissaris, P. Koufargyris, A. Karageorgos, K. Katrini, V. Lekakis, M. Lupse, A. Kotsaki, G. Renieris, D. Theodoulou, V. Panou, E. Koukaki, N. Koulouris, C. Gogos, A. Koutsoukou, Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure, *Cell host, & microbe* (2020).
- [6] Y. Liu, X. Du, J. Chen, Y. Jin, L. Peng, H.H.X. Wang, M. Luo, L. Chen, Y. Zhao, Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19, *J. Infect.* (2020).
- [7] A.P. Yang, J.P. Liu, W.Q. Tao, H.M. Li, The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients, *Int. Immunopharmacol.* 84 (2020) 106504.
- [8] C. Tecchio, A. Micheletti, M.A. Cassatella, Neutrophil-derived cytokines: facts beyond expression, *Front. Immunol.* 5 (2014) 508.
- [9] Y. Zhang, P. Zou, H. Gao, M. Yang, P. Yi, J. Gan, Y. Shen, W. Wang, W. Zhang, J. Li, P. Liu, L. Li, Neutrophil-lymphocyte ratio as an early new marker in AIV-H7N9-infected patients: a retrospective study, *Ther. Clin. Risk Manag.* 15 (2019) 911–919.
- [10] T. Narasaraju, A. Harshini, Neutrophils as Possible Therapeutic Targets in Severe Influenza Pneumonia, *J. Infect. Pulmonary Dis.* 2 (2) (2016).
- [11] R. Channappanavar, J. Zhao, S. Perlman, T cell-mediated immune response to respiratory coronaviruses, *Immunol. Res.* 59 (1–3) (2014) 118–128.
- [12] A. Assiri, J.A. Al-Tawfiq, A.A. Al-Rabeeah, F.A. Al-Rabiah, S. Al-Hajjar, A. Al-Barrak, H. Flemban, W.N. Al-Nassir, H.H. Balkhy, R.F. Al-Hakeem, H.Q. Makhdoom, A.I. Zumla, Z.A. Memish, Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study, *Lancet. Infect. Dis.* 13 (9) (2013) 752–761.
- [13] W. Li, X. Ai, Y. Ni, Z. Ye, Z. Liang, The Association Between the Neutrophil-to-Lymphocyte Ratio and Mortality in Patients With Acute Respiratory Distress Syndrome A Retrospective Cohort Study, *Shock (Augusta, Ga.)* 51 (2) (2019) 161–167.
- [14] Z. Fan, C. EnQiang, D.L. Yao, Y. LiBo, L. Hong, B. Lang, F. Ping, T. Hong, Neutrophil-lymphocyte ratio predicts short term mortality in patients with hepatitis B virus-related acute-on-chronic liver failure treated with an artificial liver support system, *PLoS ONE* 12 (4) (2017) e0175332.
- [15] W. Cui, Y. Fan, W. Wu, F. Zhang, J.Y. Wang, A.P. Ni, Expression of lymphocytes and lymphocyte subsets in patients with severe acute respiratory syndrome, *Clin. Infect. Dis: Off. Publ. Infect. Dis. Soc. Am.* 37 (6) (2003) 857–859.
- [16] W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, L. Liu, H. Shan, C.L. Lei, D.S.C. Hui, B. Du, L.J. Li, G. Zeng, K.Y. Yuen, R.C. Chen, C.L. Tang, T. Wang, P.Y. Chen, J. Xiang, S.Y. Li, J.L. Wang, Z.J. Liang, Y.X. Peng, L. Wei, Y. Liu, Y.H. Hu, P. Peng, J.M. Wang, J.Y. Liu, Z. Chen, G. Li, Z.J. Zheng, S.Q. Qiu, J. Luo, C.J. Ye, S.Y. Zhu, N.S. Zhong, Clinical Characteristics of Coronavirus Disease 2019 in China, *New Engl. J. Med.* 382 (18) (2020) 1708–1720.
- [17] Y. Yin, R.G. Wunderink, Mers, SARS and other coronaviruses as causes of pneumonia, *Respirology (Carlton, Vic.)* 23 (2) (2018) 130–137.
- [18] M. Drent, N.A. Cobben, R.F. Henderson, E.F. Wouters, M. van Diejen-Visser, Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation, *Eur. Respirat. J.* 9 (8) (1996) 1736–1742.
- [19] P. Mo, Y. Xing, Y. Xiao, L. Deng, Q. Zhao, H. Wang, Y. Xiong, Z. Cheng, S. Gao, K. Liang, M. Luo, T. Chen, S. Song, Z. Ma, X. Chen, R. Zheng, Q. Cao, F. Wang, Y. Zhang, Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China, *Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am.* (2020).