Original Article

Predictive Nomogram for Severe COVID-19 and Identification of Mortality-Related Immune Features

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What is already known about this topic? Several clinical factors and predictive models have been studied to aid early identification of severe cases. A low level of lymphocyte in severe 2019 novel coronavirus disease (COVID-19) cases has been demonstrated.

What does the article add to our knowledge? The novel nomogram based on age, C-reactive protein (CRP), and D-dimer aided the early identification of severe cases of COVID-19 with high accuracy. Low levels of $CD45RO^{+}CD3^{+}$ T and natural killer (NK) cells correlated with increased mortality.

How does the study impact current management guidelines? The nomogram incorporating age, CRP, and D-dimer could aid early identification of severe COVID-19 cases. $CD45RO^{+}CD3^{+}$ T cells and NK cells could aid identification of prognosis in severe COVID-19 cases.

BACKGROUND: Patients with severe 2019 novel coronavirus disease (COVID-19) have a high mortality rate. The early identification of severe COVID-19 is of critical concern. In addition, the correlation between the immunological features and clinical outcomes in severe cases needs to be explored.
OBJECTIVE: To build a nomogram for identifying patients with severe COVID-19 and explore the immunological features correlating with fatal outcomes.
METHODS: We retrospectively enrolled 85 and 41 patients with COVID-19 in primary and validation cohorts, respectively. A predictive nomogram based on risk factors for severe COVID-19 was constructed using the primary cohort and evaluated internally and externally. In addition, in the validation cohort, immunological features in patients with severe COVID-19 were analyzed and correlated with disease outcomes.
RESULTS: The risk prediction nomogram incorporating age, C-reactive protein, and D-dimer for early identification of patients with severe COVID-19 showed favorable discrimination in both the primary (area under the curve [AUC] 0.807) and validation cohorts (AUC 0.902) and was well calibrated. Patients who died from COVID-19 showed lower abundance of peripheral $CD45RO^{+}CD3^{+}$ T cells and natural killer cells, but higher neutrophil counts than that in the patients who recovered ($P = .001$, $P = .009$, and $P = .009$, respectively). Moreover, the abundance of $CD45RO^{+}CD3^{+}$ T cells, neutrophil-to-lymphocyte ratio, and neutrophil-to-natural killer cell ratio were strong indicators of death in patients with severe COVID-19 (AUC 0.933 for all 3).
CONCLUSION: The novel nomogram aided the early identification of severe COVID-19 cases. In addition, the abundance of $CD45RO^{+}CD3^{+}$ T cells and neutrophil-to-lymphocyte and neutrophil-to-natural killer cell ratios may serve as useful prognostic predictors in severe patients. **© 2020 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).** **(J Allergy Clin Immunol Pract 2021;9:177-84)**

Key words: *COVID-19; Risk factors; Nomogram; Immunological feature; $CD45RO^{+}CD3^{+}$ T cells*

In December 2019, the 2019 novel coronavirus disease (COVID-19) emerged in Wuhan, Hubei Province, China, and

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Abbreviations used
ARDS- Acute respiratory distress syndrome
AUC- Area under the ROC curve
CI- Confidence interval
COVID-19- 2019 novel coronavirus disease
CRP- C-reactive protein
IQR- Quartile interval
NK- Natural killer
NLR- Neutrophil-to-lymphocyte ratio
NNKR- Neutrophil-to-NK cell ratio
OR- Odds ratio
ROC- Receiver operator characteristic
SARS-CoV- Severe acute respiratory syndrome coronavirus

rapidly became a global viral pandemic drawing international concern.[1,2] Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes respiratory and intestinal symptoms similar to other coronaviruses, such as SARS-CoV and Middle East respiratory syndrome coronavirus.[3] In severe cases, shortness of breath rapidly develops into acute respiratory distress syndrome (ARDS) combined with multiple organ dysfunction syndrome, which increases the risk of mortality.[4] Therefore, identifying possible predictors for severe COVID-19 outcomes is of critical concern. Importantly, the immune status of patients with COVID-19 closely correlates with viral clearance and disease recovery.[5] Several studies have reported diminished T lymphocytes, including $CD4^+$ and $CD8^+$ T cells, and elevated circulating cytokines in patients with severe COVID-19 compared with the levels in nonsevere cases.[6,7] However, only a few studies have explored the relationship between immune markers and clinical outcomes in severe patients. Thus, the aim of this study is to identify risk factors for the severity of COVID-19-associated pneumonia and build a predictive model for the early identification of patients with severe COVID-19. Moreover, correlations between immunological features and fatal outcomes in severe cases were explored.

METHODS

Patients and study design

We carried out a retrospective study to build a predictive nomogram for severe COVID-19. In one COVID-19 treatment group of Wuhan Union Hospital, a total of 93 patients with COVID-19 were consecutively admitted from January 27 to March 16, 2020. Of these patients, 85 were selected to form the primary cohort according to the following inclusion criteria: (1) patients diagnosed with COVID-19; (2) patient who had the laboratory test results (including blood routine, coagulation function, liver function, kidney function, myocardial enzyme, C-reactive protein [CRP]) available within the first week after admission. In another COVID-19 treatment group of Wuhan Union Hospital, all 41 patients who were admitted from February 6 to March 16, 2020, formed the validation group and used to validate the nomogram. Before that, the 41 patients provided informed consent and were enrolled in the study for immune analysis. The follow-up endpoint for the 41 patients was recovery from illness or death, and time point for follow-up was set on the discharge day of the last patient. The diagnosis of COVID-19 was based on the Guidelines for Diagnosis and Treatment of Novel Coronavirus Pneumonia (6th version) released by National Health Commission of China. A confirmed case was defined as an individual with laboratory confirmation of SARS-CoV-2, which required positive results of SARS-CoV-2 RNA, irrespective of clinical signs and symptoms. All patients were divided into nonsevere and severe groups. For the diagnosis of severe COVID-19, at least 1 of the following criteria should be met: (1) shortness of breath with respiratory rate $\geq$30 times/minute; (2) arterial oxygen saturation (resting status) $\leq$93%; and (3) arterial blood oxygen partial pressure/fraction of inspiration O_2 $\leq$300 mm Hg. Those without these criteria were grouped as nonsevere cases. The discharge criteria of patients with COVID-19 were set as 3 consecutive (minimum 24-hour sampling interval) negative tests for viral nucleic acids from oropharyngeal swabs, SpO_2 $\geq$99% without oxygen inhalation, and complete remission of flu-like symptoms.

This study adhered to the principles of the Declaration of Helsinki. The study of immune analysis in patients with COVID-19 was approved on February 6, 2020, by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (#2020/0004). A written informed consent was obtained from patients on admission to Wuhan Union Hospital for permission of immune analysis. The informed consent for collecting general information and routine laboratory test results of all patients was waived by the Ethics Committee.

Data collection

For building a predictive nomogram for severe COVID-19, data were collected from the patients with COVID-19 in the primary and validation cohorts. We retrospectively reviewed the SARS-CoV-2 RNA diagnosis results and first clinical results collected within the first week of hospitalization. Clinical laboratory test included D-dimer, activated partial thromboplastin time, prothrombin time, albumin, aspartate aminotransferase, alanine transaminase, blood urea nitrogen, creatine kinase, serum creatinine, CRP, hemoglobin, platelet, white blood cell, neutrophil%, lymphocyte%, and monocyte%. Demographic data, baseline disease, date of illness onset, and treatment regiments were collected according to the electronic medical records.

Multiparametric flow cytometric analysis

Immune cell subsets were analyzed in 41 patients. Blood samples were collected during later stages of treatment, given by visible improved symptomatology or refractory/nonimproved ARDS and/or organ failure after a significant treatment period. Peripheral venous blood (2-4 mL) was collected in K3-EDTA anticoagulant tubes. Surface markers were used to identify different immune cell subsets, and gating strategy is shown in Figure E1 (available in this article's Online Repository at www.jaci-inpractice.org). All monoclonal antibodies used are listed in Table E1 (available in this article's Online Repository at www.jaci-inpractice.org). We incubated 100 μL of whole blood with antibodies for 15 minutes at room temperature followed by incubation with 2 mL of BD lysing buffer at room temperature for 10 minutes to lyse erythrocytes. After that, samples were washed once with phosphate-buffered saline, resuspended in 300 μL of phosphate-buffered saline, and stored at 4°C until acquirement. Sample acquirement was performed on a BD FACSCanto flow cytometer, and data were analyzed using the Diva software.

In addition, serum cytokines were analyzed from 21 patients. The whole blood was centrifuged at 300 × *g* for 15 minutes. The serum was extracted and stored at −20°C before test. Multiple serum cytokines (IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-1β, IL-17A, IL-12p70, TNF-α, IFN-α, and IFN-γ) were quantified using the

TABLE I. Baseline characteristics of the study cohorts

	Primary cohort (n = 85)	Validation cohort (n = 41)	*P* value
Age (range) (y)	61 (51-68)	60 (52-66)	.708
Gender, n (%)			.088
Female	30 (35.3)	20 (48.8)	
Male	55 (64.7)	21 (51.2)	
Basic disease, n (%)			
Hypertension	25 (29.4)	9 (22.0)	.377
Diabetes	12 (14.1)	7 (17.1)	.664
Cardiovascular disease	8 (9.4)	3 (7.3)	.754
Malignancy	6 (7.1)	1 (2.4)	.426
Disease type, n (%)			.849
Nonsevere	37 (43.5)	19 (46.3)	
Severe	48 (56.5)	22 (53.7)	

Human 12 Cytokine Kit (Jiangxi Saiji Bio-Tech, Jiangxi, China) following the manufacturer's manual.

Statistical analysis

Categorical variables were expressed as frequency and percentage, and significance was detected using the χ^2 or Fisher's exact test. The quantitative variables were expressed as mean ± standard deviation, and significance was evaluated using Student's *t*-test. Non-normally distributed variables were expressed in median and quartile intervals (IQR), and significance was determined using the Mann-Whitney *U* test. For building a nomogram based on the primary cohort, variables with $P < .05$ (univariate analysis) were selected as covariates for adjustment in the multivariate logistic regression model to estimate odds ratio (OR) with 95% confidence intervals (95% CIs). Afterward, a nomogram predicting severe COVID-19 was formulated based on the results of multivariate analysis using the package of *rms* in R version 3.6.3. The performance of the nomogram was validated internally (primary cohort) and externally (validation cohort) by discrimination (a relatively corrected concordance index) and calibration (calibration curves and Hosmer-Lemeshow calibration test), which were performed by bootstrapping with 1000 resamples. During the external validation of the nomogram, the total points of each patient in the validation cohort were calculated based on the established nomogram. The receiver operator characteristic (ROC) curves were performed to investigate predictive values. Cutoff points were identified following Youden's index of ROC curves. Sensitivity, specificity, and predictive values were calculated. $P < .05$ was considered statistically significant. SPSS 25.0 statistical software and R package were used for statistical analysis.

RESULTS

Clinical characteristics and outcomes of patients

There were no significant differences in age, gender, baseline disease, or disease severity between the patients included in the primary cohort and those excluded (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). In addition, no significant differences between the primary and the validation cohorts were observed concerning age, gender, baseline disease, and disease severity according to the discharge diagnosis (Table I). In the primary cohort, 37 (43.5%) patients were in the nonsevere group, and 48 (56.5%) were in the severe group. The median age of the severe group was higher than that of the nonsevere group (median age, 65.0 [IQR, 56.3-70.5] years vs 55.0 [IQR, 46.0-63.0] years; $P < .001$). Patients in the severe group were more likely to have hypertension than were those in the nonsevere group (18 [37.5%] vs 7 [18.9%]; $P = .062$). Compared with those in the nonsevere group, patients in the severe group had worse laboratory measurements, including elevated white blood cells, neutrophil%, D-dimer, aspartate aminotransferase, blood urea nitrogen, and CRP as well as decreased lymphocyte% and albumin (Table II).

Among the 85 patients in the primary cohort, 80 (37 in the nonsevere group and 43 in the severe group) were discharged after a median hospital stay of 29.5 (5-53) days (nonsevere: 19 [5-46] days; severe: 42 [11-53] days), and 5 patients within the severe group died after a median hospitalization of 20 (5-48) days. Among the 41 patients who underwent immune analysis, 34 (19 in the nonsevere group and 15 in the severe group) were discharged after recovery from COVID-19 with a median hospital stay of 23.6 (4-56) days (nonsevere: 17.8 [4-47] days; severe: 32.4 [11-56] days). The remaining 7 patients with severe disease died from ARDS, multiple organ dysfunction syndrome, and/or sepsis after a median hospitalization of 30.7 (7-55) days.

Nomogram establishment

In the primary cohort, univariable and multivariable analyses by the logistic regression model demonstrated that old age and high levels of D-dimer and CRP were independent risk factors for predicting COVID-19 severity, with ORs of 1.078 (95% CI: 1.015-1.114, $P = .014$), 1.394 (95% CI: 1.027-1.893, $P = .033$), and 1.023 (95% CI: 1.002-1.045, $P = .035$), respectively (Table III).

To provide a quantitative tool that predicts individual probability of developing a severe case of COVID-19, a novel prognostic nomogram that incorporated age, D-dimer level, and CRP level was established based on multivariable logistic analyses in the primary cohort (Figure 1). The probability of severe COVID-19 of each patient could be calculated by adding the scores for the 3 variables.

Nomogram validation

The nomogram was validated internally in the primary cohort and externally in the validation cohort. In the primary cohort, the area under the ROC curve (AUC) was 0.807 (95% CI: 0.715-0.900) for the age-D-dimer-CRP combination, which was superior to the AUC of any 1 of the 3 parameters (ranging from 0.635 to 0.726), with a sensitivity of 72.9% and specificity of 74.8%. In the validation cohort, the AUC was 0.900 (95%

TABLE II. Characteristics of patients in the primary cohort

	Primary cohort (n = 85)		
Characteristics	**Nonsevere (n = 37)**	**Severe (n = 48)**	***P* value**
Age (range) (y)	55.0 (46.0-63.0)	65.0 (56.3-70.5)	**<.001**
Gender, n (%)			.178
Male	21 (56.8)	34 (70.8)	
Female	16 (43.2)	14 (29.2)	
Hypertension, n (%)	7 (18.9)	18 (37.5)	.062
Diabetes, n (%)	5 (13.5)	7 (14.6)	.888
Cardiovascular disease, n (%)	3 (8.1)	5 (10.4)	.511
Malignancy, n (%)	3 (8.1)	3 (6.3)	.530
WBC (×109/L)	5.3 (4.4-6.3)	6.1 (4.5-8.1)	**.040**
HB (g/L)	128.0 (118.5-138.5)	129.0 (115.3-267.0)	.961
PLT (×109/L)	220.0 (153.0-257.0)	199.5 (124.5-267.0)	.410
NEU %	63.8 (55.9-75.0)	75.9 (64.1-84.3)	**.001**
LYM %	23.7 (16.7-31.8)	14.3 (0.1-24.5)	**.001**
Monocyte %	7.5 (6.2-9.5)	7.1 (4.2-9.1)	.107
D-dimer (mg/L)	0.4 (0.2-1.4)	0.7 (0.4-6.4)	**.034**
APTT (s)	38.3 (35.3-40.9)	36.3 (32.8-41.2)	.114
PT (s)	13.1 (12.6-13.4)	13.4 (12.5-14.6)	.216
ALB (g/L)	34.6 (4.8)	29.9 (4.7)	**<.001**
AST (U/L)	25 (18.0-32.5)	31.5 (22.0-43.5)	**.027**
ALT (U/L)	23.0 (19.0-47.5)	30 (20.0-48.0)	.435
CK (U/L)	54.0 (40.0-106.0)	91.0 (41-192.0)	.097
BUN (mmol/L)	4.2 (3.3-5.0)	5.5 (3.9-7.8)	**.004**
sCr (μmol/L)	69.4 (58.7-80.7)	74.6 (65.7-89.9)	.119
CRP (mg/L)	6.8 (2.2-31.4)	40 (10.9-87.3)	**.001**

Bold indicates statistical significance ($P < .05$).

ALB, Albumin; *ALT*, alanine transaminase; *APTT*, activated partial thromboplastin time; *AST*, aspartate aminotransferase; *BUN*, blood urea nitrogen; *CK*, creatine kinase; *CRP*, C-reactive protein; *HB*, hemoglobin; *LYM*, lymphocyte; *NEU*, neutrophil; *PLT*, platelet; *PT*, prothrombin time; *sCr*, serum creatinine; *WBC*, white blood cell.

CI: 0.800-1.00) with a sensitivity of 86.4% and specificity of 89.5% (Figure 2, *A* and *B*). The C-index for the prediction nomogram was 0.807 (95% CI: 0.716-0.899) for the primary cohort and 0.900 (95% CI: 0.800-0.999) for the validation cohort and calculated to be 0.791 and 0.869, respectively, via bootstrapping validation. A high C-index suggested good capacity for the identification of severe COVID-19. The calibration curves of the nomogram showed strong agreement between predicted probability and actual observation in both the primary and validation cohorts (Figure 2, *C* and *D*). The Hosmer-Lemeshow test yielded nonsignificant statistics ($P = .489$, .054) in both the primary and validation cohorts, suggesting that the calibration was accurate.

Immunological features of nonsevere and severe groups in the later stage of treatment

All 41 patients had their blood collected for immune cell analyses. As shown in Table IV, subsets of T cells ($CD4^+$, $CD8^+$, $CD45RA^+$, $CD45RO^+$, and Treg) and B cells were lower in the severe group than in the nonsevere group. However, natural killer (NK) cell and neutrophil counts showed no differences between severe and nonsevere cases. Cytokine profiles were available for 21 patients (8 nonsevere and 13 severe). There were no significant differences in cytokine levels between the groups, except for IFN-α, which was higher in the nonsevere group than that in the severe group ($P = .019$, see Table E3 in this article's Online Repository at www.jaci-inpractice.org).

Immunological features in patients with severe disease according to clinical outcome

Among the 41 patients who underwent immune analysis, 22 patients with severe disease were further divided into recovery (15 patients) and death groups (7 patients), according to their clinical outcome. As shown in Table IV, neutrophil counts were higher in the death group than that in the recovery group (9.6 vs 3.5×10^9/L, $P = .009$). Moreover, the death group showed lower counts of $CD45RO^+CD3^+$ T cells (including $CD45RO^+CD4^+$ and $CD45RO^+CD8^+$ T cells) and NK cells than the counts in the recovery group ($P = .001$, .009), whereas $CD45RA^+CD3^+$ T-cell subsets showed no significant differences between the 2 groups ($P = .581$). The proportions of T cells expressing activation markers (CD25, CD69, or HLA-DR) or exhaustion markers (CD279) were comparable between the recovery and death groups ($P = .273$, $P = .783$, $P = .490$, and $P = .837$, respectively). No obvious differences in Treg and B-cell counts were observed between the 2 groups ($P = .581$, $P = .680$). Cytokine analysis showed that IL-6 and IL-10 levels were significantly higher in the death group than those in the recovery group ($P = .006$, $P = .011$, see Table E3 in this article's Online Repository at www.jaci-inpractice.org). Considering the possible effect of corticosteroid therapy on immune cells, we also analyzed the differences between lymphocyte subsets in patients with severe cases of COVID-19 who had received or not received corticosteroid therapy. The results showed no significant differences in immune cells between these 2 treatment groups, except for NK cells, which

TABLE III. Univariable and multivariable analysis of severe COVID-19 by the logistic regression model in the primary cohort

Univariate and multivariate analysis of severe COVID-19 by Logistic regression model.	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.073 (1.027-1.120)	.002	1.078 (1.015-1.114)	.014
NEU %	1.054 (1.016-1.094)	.005		
LYM %	0.951 (0.912-0.992)	.021		
D-dimer	1.359 (1.069-1.726)	.012	1.394 (1.027-1.893)	.033
PT	1.560 (1.012-2.404)	.044		
ALB	0.810 (0.725-0.905)	<.001		
CK	1.007 (1.000-1.014)	.046		
CRP	1.021 (1.007-1.035)	.003	1.023 (1.002-1.045)	.035

ALB, Albumin; *CI*, confidence interval; *CK*, creatine kinase; *COVID-19*, 2019 novel coronavirus disease; *CRP*, C-reactive protein; *LYM*, lymphocyte; *NEU*, neutrophil; *OR*, odds ratio; *PT*, prothrombin time.

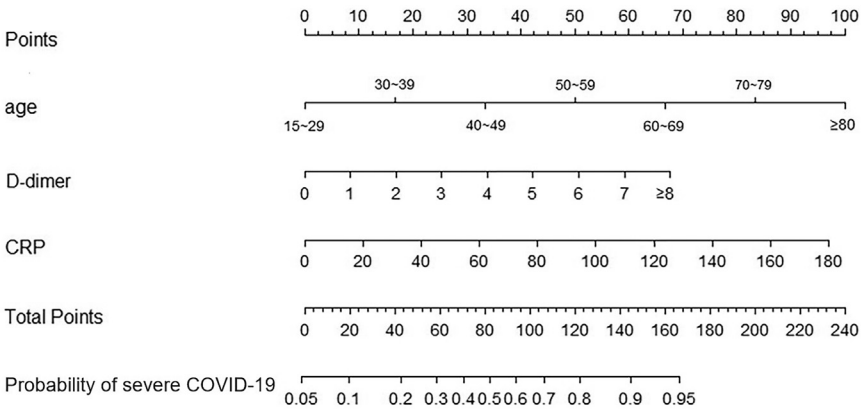

FIGURE 1. Developed prediction nomogram. To use this nomogram, the specific value for each patient should be located on each variable axis, and a line plotted upward to determine the points for each variable value. The sum of the points should be found on the "Total Points axis" and a perpendicular line drawn downward to determine the probability of severe COVID-19. *COVID-19*, 2019 novel coronavirus disease; *CRP*, C-reactive protein.

were lower in patients who received corticosteroid therapy (156.1 vs 251.5 × 10^6/L, $P = .048$).

To evaluate the predictive value of these immunological features toward a fatal outcome, ROC curves were drawn and AUC values calculated. We also included the ratio of neutrophils to different lymphocyte subsets as parameters. Our results identified $CD45RO^+CD3^+$ T cells, neutrophil-to-lymphocyte ratio (NLR), and neutrophil-to-NK cell ratio (NNKR) as likely prognostic predictors with high AUC values (0.933 for all 3). Simultaneously, the cutoff values were calculated from the ROC curves, with a value of 449.3 for $CD45RO^+CD3^+$ T cells (specificity: 86.7%, sensitivity: 100%), 5.0 for NLR (specificity: 93.3%, sensitivity: 85.7%), and 35.6 for NNKR (specificity: 86.7%, sensitivity: 100%; see Table E4 in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

In the first part of our study, old age and high levels of D-dimer and CRP were identified as independent risk factors for severe COVID-19. We further constructed a nomogram using these clinical factors for the sorting and monitoring of patients with COVID-19. The constructed nomogram, which was internally and externally validated, showed good potential for discrimination and calibration and can be used for identifying patients at a high risk of severe COVID-19.

Severe cases exhibit excessive immune responses that release a large number of inflammatory factors, causing organ injury and death in most extreme cases.[8] CRP, as an acute-phase reactive protein, reflects a hyperimmune inflammatory state, whereas elevated D-dimer levels are evidence of hypercoagulation and hyperfibrinolysis.[9] Elevated levels of D-dimer may correlate with pulmonary arterial injuries caused by SARS-CoV-2, which result in embolisms in the alveolar terminal capillaries.[10] Lastly, old age has been linked to cytokine dysregulation,[11] in that elderly patients with chronic diseases are more likely to show high inflammatory states after infections.[12] Several studies have proposed the impact of CRP and D-dimer in predicting COVID-19 severity, which is consistent with our results.[13,14] Considering that CRP and D-dimer levels are routinely measured in laboratory tests, this novel nomogram was a user-friendly method to identify patients at a high risk of severe COVID-19.

In the second part of our study, we observed significantly lower counts of $CD45RO^+CD3^+$ T and NK cells in the death group than those in the recovery group. Notably, we found that $CD45RO^+CD3^+$ T cells decreased stepwise in the nonsevere, severe-recovery, and severe-death groups. Possible causes of these

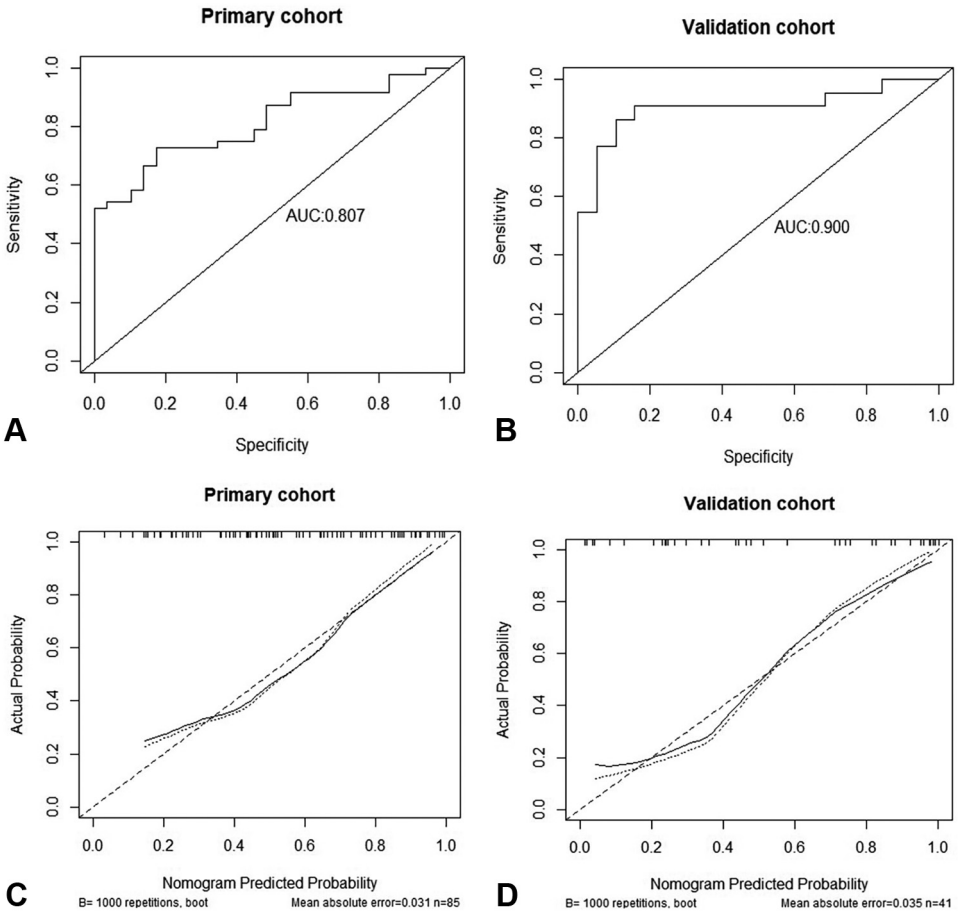

FIGURE 2. The receiver operating characteristic and calibration curves of the nomogram. The receiver operating characteristic and calibration curves for performance to distinguish individuals with severe COVID-19 from those with nonsevere COVID-19 in the (**A**, **C**) primary cohort and (**B**, **D**) validation cohort, respectively. *AUC*, Area under the curve; *COVID-19*, 2019 novel coronavirus disease.

decreased peripheral T-cell levels include direct damage from SARS-CoV-2, inhibited T-cell expansion, and increased apoptosis induced by autoimmune antibodies triggered by the virus.[15,16] The downward trend of $CD45RO^+CD3^+$ T cells in our study may reflect the persistence of the virus and progression of the illness.

CD45RO is a memory cell marker, and the $CD45RO^+CD3^+$ T-cell population represents the memory T-cell pool, which contains cells that can respond to recalled soluble antigens. CD45RO is also a marker expressed during T-cell activation and is accompanied by a loss of CD45RA marker.[17] In addition to the neutralizing antibodies produced by B cells, T-cell activation is also fundamental in generating an effective immune response against respiratory coronaviruses.[18] Studies on SARS-CoV-1 have identified that virus-specific T cells play an important role in SARS-CoV clearance and tend to have a central memory phenotype ($CD45RO^+CD27^+$).[19] In a murine model of SARS-CoV infection, virus-specific effector $CD4^+$ and $CD8^+$ T cells were poorly generated to control the virus. However, the adoptive transfer of those cells was able to control SARS-CoV infection.[20] On the basis of information gathered from SARS-CoV research, we suspect that low levels of total $CD45RO^+CD3^+$ T cells could imply a low level of activated or virus-specific T cells against SARS-CoV-2 in the peripheral blood, resulting in a weak immune response and persistent disease. Adoptive transfer of $CD45RO^+CD3^+$ T cells to control the virus may therefore represent a potential therapeutic strategy in severe cases showing refractory COVID-19 pneumonia.

NK cells also play a crucial role against coronaviruses.[21] The significantly lower NK cell counts in the death group than that in the recovery group reflected a compromised innate immune system. Because our results suggested that NK cells were affected by corticosteroid therapy, we believe that corticosteroids should be used with caution during the treatment of severe cases of COVID-19.

We observed higher levels of IFN-α in the nonsevere group than that in the severe group. Notably, recombinant IFN-α is an approved therapeutic agent that inhibits viral replication.[12] Our results may suggest that the high IFN-α production in the nonsevere group was conducive to disease management. IL-6 is a key inflammatory cytokine and has been identified as an independent risk factor for COVID-19 severity in previous studies.[22,23] The present study showed that IL-6 levels were significantly higher in the death group than those in the recovery group, which indicates a persistent high inflammatory state in the death group.

Liu et al[24] identified the neutrophil-to-$CD8^+$ T-cell ratio and NLR as powerful metrics for the early identification of severe COVID-19 cases. We compared immunological features among

TABLE IV. Immune cells subsets in nonsevere, severe, recovery, and death groups in patients with COVID-19 in the validation cohort

	Nonsevere (n = 19)	Severe (n = 22)	*P* value	Recovery group (n=15)	Death group (n = 7)	*P* value
Age	57 (37-59)	66 (60-75)	**<.001**	64 (60-74)	67 (63-79)	.541
Female	10 (52.6%)	10 (45.5%)	.758	6 (40.0%)	4 (57.1%)	.652
Lymphocytes	2320 (1790-2480)	1350 (985-1625)	**<.001**	1420 (1190-1910)	990 (610-1560)	.058
$CD3^+$T (total T) cells	1618.7 (1287.4-1974.3)	928.3 (701.0-1055.3)	**<.001**	974.4 (261.0)	618.0 (322.0)	**.012**
$CD45RA^+CD3^+$ T cells	568.9 (370.1-752.7)	297.3 (193.6-507.0)	**<.001**	287.3 (189.7-418.3)	459.6 (158.1-516.4)	.581
$CD45RO^+CD3^+$ T cells	866.4 (548.8-1142.6)	457.3 (353.4-672.1)	**<.001**	617.6 (457.3-726.2)	350.4 (265.3-371.7)	**.001**
$CD4^+$T (Th) cells	924.2 (727.7-1156.9)	689.6 (429.7-683.8)	**<.001**	592.2 (194.2)	504.9 (224.8)	.360
$CD45RA^+CD4^+$ T cells	300.9 (160.4-351.3)	144.3 (103.7-297.3)	**.021**	126.9 (89.2-228.7)	286.3 (126.3-355.8)	.210
$CD45RO^+CD4^+$ T cells	620.4 (425.8-854.4)	335.7 (289.6-454.1)	**.001**	436.0 (313.3-460.1)	295.4 (226.2-307.9)	**.007**
$CD8^+$T (Ts) cells	441.0 (319.6-733.2)	308.9 (240.7-363.4)	**<.001**	335.8 (115.0)	219.8 (72.2)	**.024**
$CD45RA^+CD8^+$ T cells	276.5 (157.7-357.1)	137.0 (98.9-208.2)	**<.001**	144.6 (70.5)	149.9 (75.9)	.874
$CD45RO^+CD8^+$ T cells	211.6 (138.2-277.4)	135.6 (63.4-208.4)	**.009**	182.6 (64.9)	109.0 (23.4)	**.002**
$CD4^+/CD8^+$ T cells	1.8 (1.4-2.5)	2.0 (1.4-2.5)	.661	1.9 (1.3-2.4)	2.5 (2.0-2.6)	.224
$CD25^+CD3^+/CD3^+$ T cells (%)	4.6 (2.9-7.3)	4.4 (3.4-6.4)	.567	4.2 (2.9-6.0)	5.7 (3.7-8.5)	.273
$CD69^+CD3^+/CD3^+$ T cells (%)	9.5 (4.7-16.8)	9.9 (3.5-20.8)	.794	11.2 (2.9-21.3)	9.9 (3.6-25.9)	.783
HLA-$DR^+CD3^+/CD3^+$ T cells (%)	10.5 (7.1-13.4)	11.6 (6.3-18.1)	.360	11.6 (5.9-18.3)	14.0 (9.6-15.3)	.490
$CD279^+CD3^+$T cells/$CD3^+$ T cells (%)	6.6 (3.8-18.5)	9.2 (4.5-20.1)	.676	12.0 (3.5-18.9)	7.5 (5.0-25.1)	.837
$CD4^+CD25^+CD127^{dim/-}$ (Treg)	34.6 (25.7-46.2)	23.6 (20.8-32.4)	**.041**	22.7 (20.2-32.6)	28.2 (21.6-37.0)	.680
Treg cells/$CD4^+$ T cells (%)	3.8 (3.3-5.2)	5.1 (3.5-5.9)	**.039**	5.1 (3.4-5.9)	5.5 (4.9-7.2)	.237
$CD19^+CD3^-$ (B cells)	239.5 (158.6-313.9)	126.5 (69.1-161.4)	**.010**	140.6 (62.8-159.9)	126.5 (75.4-427.6)	.581
$CD16^+CD56^+CD3^-$ (NK cells)	224.4 (144.5-360.1)	197.2 (88.7-251.5)	.619	224.0 (154.7-479.5)	100.9 (27.6-195.2)	**.009**
Neutrophils ($\times 10^9$/L)	4.2 (3.4-5.5)	4 (2.2-7.3)	.979	3.5 (2.0-4.2)	9.6 (7.0-14.2)	**.009**

Bold indicates statistical significance ($P < .05$).
COVID-19, 2019 novel coronavirus disease; *NK*, natural killer.
All data take $\times 10^6$/L as the unit, otherwise was noted.

severe cases who had different outcomes and found that $CD45RO^+CD3^+$ T cells, NLR, and NNKR were useful predictors of clinical outcomes.

To our knowledge, this is the first clinical study showing decreased $CD45RO^+CD3^+$ T and NK cells in patients with COVID-19, and their possible association with increased mortality in patients with severe COVID-19. Determining the alterations in $CD45RO^+CD3^+$ T and NK cell populations, clinicians may be able to predict poor prognoses of severe patients and follow potentially life-saving interventions.

However, this study has some limitations. First, the sample size enrolled in this study is relatively small. Prospective multicenter studies with larger sample sizes are needed to verify the findings. Second, we were not able to perform the immune cell analyses in the early stages of the illness. This restricted the dynamic monitoring of immune cells, which may provide more comprehensive information. Further studies and clinical practice are needed to disclose the immune responses in SARS-CoV-2 infection. Collectively, the new nomogram combining age, D-dimer level, and CRP level could aid in the early identification of severe COVID-19 cases. Furthermore, $CD45RO^+CD3^+$ T-cell subsets, NLR, and NNKR could aid identification of prognosis in severe COVID-19 cases. The adoptive transfer of $CD45RO^+CD3^+$ T or NK cells may serve as novel therapeutic strategies to control disease progression in patients with severe COVID-19.

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H. Wang, W. Chen, and Y. Hu conceptualized the study and conceived the project; M. Wang and H. Mei contributed with the blood samples collection; L. Cai, X. Zhou, M. Wang, H. Mei, and X. Zhao contributed with data collection; L. Cai, L. Ai, and S. Mu contributed with samples processing and cytometry analysis; L. Cai and X. Zhou analyzed the data and wrote the paper; H. Wang, W. Chen, and Y. Hu secured funding and supervised the work; and all the authors reviewed and approved the final manuscript.

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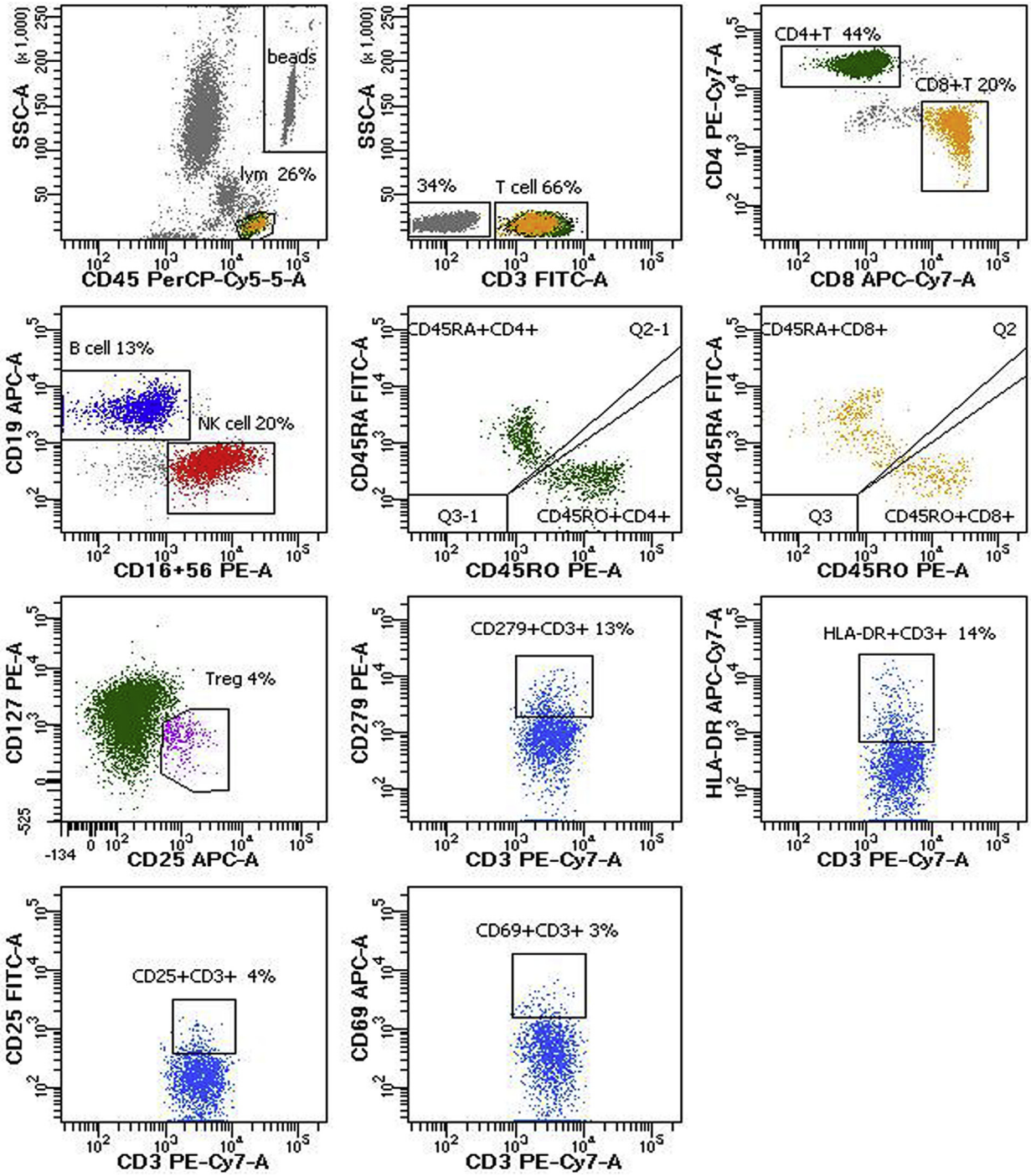

FIGURE E1. Gating strategy of lymphocyte subsets in a representative patient.

TABLE E1. List of antibodies used for flow cytometric analysis

Antibodies	Supplier
CD3FITC, CD3APC, CD3PE-Cy7, CD4PE-Cy7, CD8APC-Cy7, CD11cAPC, CD16PE, CD56PE, CD19APC, CD25FITC, CD25APC, CD45PerCP, CD45RA FITC, CD45ROPE, CD69APC, CD123PE, CD127PE, HLA-DRAPC-Cy7	BD
CD279PE	BioLegend

TABLE E2. Baseline characteristics of the included and excluded patients of the primary cohort

	Included patients (n = 85)	Excluded patients (n = 8)	*P* value
Age (y)	61 (20-87)	56 (41-72)	.167
Gender, n (%)			> .999
Female	30 (35.3)	3 (37.5)	
Male	55 (64.7)	5 (62.5)	
Basic disease, n (%)			
Hypertension	25 (29.4)	2 (25.0)	>. 999
Diabetes	12 (14.1)	2 (25.0)	.345
Cardiovascular disease	8 (9.4)	0 (0)	> .999
Malignancy	6 (7.1)	0 (0)	> .999
Disease type, n (%)			.728
Nonsevere	37 (43.5)	4 (50.0)	
Severe	48 (56.5)	4 (50.0)	

TABLE E3. Cytokine profiles in nonsevere severe, recovery, and death groups in patients with COVID-19

Cytokines	Normal range (pg/mL)	Nonsevere (n = 8)	Severe (n = 13)	*P* value	Recovery group (n = 9)	Death group (n = 4)	*P* value
IL-2	≤L-24	24.1 (21.6-25.7)	20.6 (18.7-23.9)	.054	19.6 (18.3-23.9)	21.2 (19.5-26.2)	.404
IL-4	≤L-49	9.7 (8.7-10.6)	8.6 (7.8-9.4)	.168	8.6 (7.3-9.4)	8.8 (8.0-10.3)	.916
IL-5	≤L-5	1.8 (1.7-2.1)	1.9 (1.4-2.2)	.972	1.6 (1.2-2.1)	2.2 (1.9-2.3)	.244
IL-6	≤L-64	14.0 (11.3-15.1)	36.4 (11.2-376.2)	.185	12.9 (10.7-47.9)	727.2 (230.4-1878.4)	**.006**
IL-8	≤L-84	11.1 (10.6-12.5)	17.5 (10.6-36.5)	.161	11.3 (10.2-31.2)	37.9 (20.2-373.4)	.076
IL-1β	≤L-1β	9.8 (9.0-10.6)	8.5 (7.5-10.3)	.205	8.0 (6.8-9.9)	9.4 (8.3-10.8)	.292
IL-17A	≤L-17	21.1 (17.2- 23.9)	17.8 (14.9-21.2)	.060	17.5 (13.0-20.3)	20.4 (16.7-21.2)	.284
IL-10	≤L-1	11.6 (10.4-14.0)	14.1 (9.6-15.1)	.860	11.3 (8.9-12.7)	16.8 (13.7-32.4)	**.011**
IFN-α	≤FN-	9.2 (8.8-10.1)	7.7 (7.2-8.9)	**.019**	7.9 (6.5-8.9)	7.6 (7.4-9.0)	.606
TNF-a	≤a.5	8.1 (7.4-9.1)	7.7 (7.4-8.4)	.645	8.0 (6.6-8.9)	7.9 (7.7-8.5)	.940
IL-12p70	≤L-1	10.4 (7.8-21.7)	9.7 (7.5-11.2)	.301	9.8 (6.1-11.2)	8.7 (7.7-10.9)	.918
IFN-γ	≤FN-γ	12.7 (10.9-15.1)	11.9 (9.8- 13.1)	.341	11.9 (10.3-13.1)	11.2 (9.8-14.1)	.883

Bold indicates statistical significance ($P < .05$).
COVID-19, 2019 novel coronavirus disease.

TABLE E4. ROC curve analysis of immune cells in patients with severe COVID-19

	AUC	*P* value	95% CI	Cutoff	Specificity (%)	Sensitivity (%)
Neutrophils	0.848	.010	0.643-1.0	6.7	86.7	85.7
NK cells	0.848	.010	0.686-1.0	197.3	73.3	100
$CD3^+$ T cells	0.819	.018	0.635-1.0	904.3	66.7	85.7
$CD45RO^+CD3^+$ T cells	0.933	.001	0.819-1.0	449.3	86.7	100
$CD45RO^+CD4^+$ T cells	0.857	.008	0.701-1.0	346.6	66.7	100
$CD8^+$ T cells	0.829	.015	0.658-1.0	312.5	60.0	100
$CD45RO^+CD8^+$ T cells	0.895	.003	0.754-1.0	122.6	73.3	100
NLR	0.933	.001	0.825-1.0	5.0	93.3	85.7
NCD3R	0.895	.003	0.74-1.0	6.9	93.3	85.7
NCD3ROR	0.895	.003	0.742-1.0	14.7	86.7	85.7
NCD4ROR	0.876	.005	0.68-1.0	21.6	93.3	85.7
NCD8ROR	0.905	.003	0.781-1.0	34.2	73.3	100
NNKR	0.933	.001	0.827-1.0	35.6	86.7	100

AUC, Area under the curve; *CI*, confidence interval; *COVID-19*, 2019 novel coronavirus disease; *NCD3R*, neutrophil-to-$CD3^+$ T-cell ratio; *NCD3ROR*, neutrophil-to-$CD45RO^+CD3^+$ T-cell ratio; *NCD4ROR*, neutrophil-to-$CD45RO^+CD4^+$ T-cell ratio; *NCD8ROR*, neutrophil-to-$CD45RO^+CD8^+$ T-cell ratio; *NK*, natural killer; *NLR*, neutrophil-to-lymphocyte ratio; *NNKR*, neutrophil-to-NK cell ratio; *ROC*, receiver operator characteristic.