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ORIGINAL RESEARCH

Prognostic Value of Peripheral Blood nCD64 Index, mHLA-DR, and CD14$^+$monocyte Percentage in Different Infection Status in COVID-19 Patients

Mingli Zhu[1,*], Jianghao Cheng[1,2,*], Lingyan He[1,*], Chaodan Li[1], Bin Shi[1], Meitong Jin[1], Jianhua Yu[3], Jinsong Huang[3]

[1]Open Laboratory, Hangzhou Xixi Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China; [2]Laboratory Medicine, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, People's Republic of China; [3]Department of Infectious Diseases, Hangzhou Xixi Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jianhua Yu; Jinsong Huang, Email yujhmc@126.com; huangjinsongyz@126.com

Objective: To explore the value of the neutrophil CD64 (nCD64) index, monocytic HLA-DR (mHLA-DR), and the percentage of CD14$^+$ monocytes in the prognosis of Coronavirus disease 2019 (COVID-19).

Methods: Fifty-seven COVID-19 patients from December 2022 to November 2023 were divided into two groups: non-severe group (mild or moderate cases) and severe group (severe or critical cases). Among them, 17 patients were deceased. Flow cytometry was employed to detect the levels of nCD64 index, mHLA-DR, and CD14$^+$monocyte percentage in blood. The receiver operating characteristic (ROC) curve was used to evaluate the predictive value of mHLA-DR, nCD64 index, and CD14$^+$ monocyte percentage for COVID-19 prognosis.

Results: The expression levels of mHLA-DR and the percentage of CD14$^+$ monocytes in non-severe COVID-19 patients were significantly higher than those in severe and deceased patients ($P < 0.05$). In contrast, the nCD64 index in non-severe patients was significantly lower than that in severe and deceased patients ($P< 0.05$). The expression level of mHLA-DR and the percentage of CD14$^+$ monocytes in deceased patients were significantly lower than those in surviving patients ($P<0.001$), while the nCD64 index level in surviving patients was significantly lower than that in deceased patients ($P<0.001$). The area under the curve (AUC) for the nCD64 index, mHLA-DR and the percentage of CD14$^+$ monocytes were 0.850, 0.779 and 0.871, respectively. Patients with CD14$^+$ monocyte percentage > 4.9%, mHLA-DR value >16720.5, and nCD64 index <5.87 had a good prognosis value ($P < 0.05$).

Conclusion: The nCD64 index, mHLA-DR level, and percentage of CD14$^+$ monocytes may serve as biomarkers for predicting clinical outcomes in COVID-19 patients.

Keywords: nCD64 index, mHLA-DR, CD14$^+$ monocyte percentage, COVID-19

Introduction

According to the international definition of sepsis associated with organ dysfunction, severe Coronavirus disease 2019 (COVID-19) with acute respiratory distress syndrome (ARDS) should be considered as viral sepsis.[1] Reports have indicated that various viruses, including Rhinovirus, Influenza virus, Adenovirus, Respiratory syncytial virus, Herpes simplex virus, and Dengue virus, can lead to sepsis.[2] Similarly, coronaviruses such as MERS-CoV, SARS-CoV, and SARS-CoV-2 are also capable of causing sepsis. Previous studies have indicated that sepsis was the most frequently observed complication, followed by respiratory failure, ARDS, heart failure, and septic shock, 59-65% of COVID-19 patients have sepsis concurrently, and 100% of the deceased COVID-19 patients have sepsis.[3,4] This suggests that mortality in COVID-19 patients due to SARS-CoV-2 infection can be attributed to sepsis. Biomarkers used for sepsis may be useful for early identification of COVID-19 patients at risk of progressing to severe disease.

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CD64 is a high-affinity immunoglobulin receptor (FcγRI) primarily expressed on macrophages, monocytes, and eosinophils, with minimal expression on lymphocytes and resting neutrophils.[5] When stimulated by an inflammatory response or pro-inflammatory factors such as IFN-γ and G-CSF, its expression can increase tenfold within 4–6 hours, playing a crucial role in the early anti-infective response.[6] Studies have shown that the nCD64 index performs well in diagnosing and monitoring sepsis in critically ill patients.[7] Additionally, the nCD64 index holds significant value in tuberculosis diagnosis and treatment, with its expression levels helping to distinguish latent tuberculosis from active pulmonary tuberculosis.[8]

Classical monocytes ($CD14^+$, $CD16^-$) account for approximately 80–85% of the total monocyte population. They can migrate to sites of inflammation and differentiate into macrophages and phagocytes, playing a crucial role in the immune control of infections through their anti-inflammatory or pro-inflammatory activities.[9] This activity is reflected in the expression of surface HLA-DR. mHLA-DR is an immune status marker; a decrease in its expression indicates that the body is in a state of immune suppression, which is associated with excessive antigen stimulation and a reduction in pro-inflammatory monocyte numbers.[1] Current research has identified mHLA-DR as a biomarker for assessing immunosuppressive diseases such as sepsis.[10] Furthermore, downregulation of mHLA-DR has also been reported in non-survivors of severe community-acquired pneumonia admitted to the ICU.[11]

To date, there have been few studies reporting on the application of sepsis-related biomarkers in COVID-19. Therefore, the aim of our study is to assess, through flow cytometry analysis, whether a combination of the nCD64 index, mHLA-DR, and the percentage of $CD14^+$ monocytes can better define the immune dysregulation status of COVID-19 patients with varying disease severity, and to explore its prognostic value for COVID-19, thereby aiding clinicians in making earlier decisions.

Materials and Methods

Study Population

Blood samples from 57 COVID-19 patients admitted to Hangzhou Xixi Hospital, affiliated to Zhejiang Chinese Medical University, from December 2022 to November 2023 were collected. Each patient was hospitalized and underwent RT-PCR testing (Sansure Biotech Inc. China) with nasopharyngeal swabs, confirming all patients were infected with SARS-CoV-2. Human immunodeficiency virus (HIV) patients or other diseases related to immune system disorders were excluded. Based on clinical diagnosis by physicians and the severity of the patients' conditions, COVID-19 patients were classified into non-severe group (mild or moderate) and severe group (severe or critical). General demographic characteristics and survival outcomes of the patients were collected through the hospital's electronic medical record system and follow-up tracking.[12] This study was approved by the ethics committee of Hangzhou Xixi Hospital, affiliated to Zhejiang Chinese Medical University (approval number: [2022] No. 12) and was conducted in accordance with the Declaration of Helsinki. Furthermore, the confidentiality of all patient data was guaranteed.

Clinical Classification and Diagnostic Criteria

The clinical classification and diagnostic criteria for COVID-19 were implemented in accordance with Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Edition 9, https://www.gov.cn/zhengce/zhengceku/2022-03/15/5679257/files/49854a49c7004f4ea9e622f3f2c568d8.pdf. and Edition 8) issued by the National Health Commission of the People's Republic of China.[13] A diagnosis was made based on a comprehensive analysis of the epidemiological history, clinical manifestations and laboratory tests etc. A positive nucleic acid test for the novel coronavirus was the primary criterion for diagnosis. The non-severe group (n = 33) consisted of patients with mild-to-moderate COVID-19, while those with severe or critical types were included in the severe group (n = 24). Mild type was defined as one where the patients exhibited only mild clinical symptoms and no pneumonia manifestations on imaging. Patients with clinical manifestations and pneumonia manifestations on imaging were classified as moderate type. Patients with any of the following characteristics at the time of admission were considered to have severe COVID-19: (1) shortness of breath with a respiratory rate (RR) ≥ 30 breaths per minute, (2) oxygen saturation ≤ 93%, (3) arterial partial pressure of oxygen (PaO2) to fraction of inspired oxygen (FiO2) ratio of ≤ 300 mmHg (1 mmHg = 0.133 kPa), or (4) progressive clinical symptoms with a significant increase in lung lesions > 50% within 24 to 48 hours on imaging. Patients meeting any of the

following criteria were classified as critical type: developing respiratory failure requiring mechanical ventilation; experiencing shock; or having concurrent organ failure requiring ICU monitoring and treatment.

Detection of CD64 Expression in Neutrophils

Ethylene diamine tetra-acetic acid (EDTA-) anticoagulated blood was collected, stored at 4–8 °C and processed within 4 h after withdrawal. 20 μL of CD64 PE reagent (Becton Dickinson Company, USA) and 100 μL of sample were added to a polystyrene tube, and were gently vortex to mixed and incubated in the dark at room temperature (20°C to 25°C) for 15 to 30 minutes. 2 mL of FACS™ lysing solution (Becton Dickinson Company, USA) was added, gently mixed and incubated in the dark at room temperature for 10 minutes, then was centrifuged at 300×g for 5 minutes and the supernatant was discarded. 2 to 3 mL of PBS buffer was added and was centrifuged at 200×g for 5 minutes, then the supernatant was removed. 0.5 mL of 1% paraformaldehyde solution was added and mixed thoroughly, then was performed detection and analysis using BD FACSCanto flow cytometer (Becton Dickinson Company, USA). The formula for calculating the nCD64 index is: nCD64 index (adult) = $(CD64MFI_{NEO}/CD64MFI_{LY})/(CD64MFI_{MO}/CD64MFI_{NEO})$ (MFI-mean fluorescence intensity, NEO-neutrophils, LY-lymphocyte, MO-monocyte). The CD64 neutrophil index (CD64 index) can serve as a sensitive biomarker for infection and inflammation. The lowest reference value from the lab is < 1.0.

Detection of CD14 Positive Cells within the Monocyte Population and Monocytic HLA-DR Expression

20 μL of CD14-FITC and 5 μL of HLA-DR-APC reagents (Becton Dickinson Company, USA) and 100 μL of sample were added to a polystyrene tube, gently mixed and incubated in the dark at room temperature (20°C to 25°C) for 15 to 30 minutes. 2 mL of FACS™ lysing solution were added, gently vortex to mixing and incubating in the dark at room temperature for 10 minutes. The following steps are the same as described above. The cut-off values for CD14 and monocytic HLA-DR expression are 16% and 90% respectively. Low mHLA-DR levels lead to reduced antigen presentation and decreased acquired immune activation.

SARS-Cov-2 Viral Load Detection

The total RNA in nasopharyngeal swab samples was extracted. Yarui MA-6000 real-time fluorescent quantitative PCR instrument was used to perform RNA amplification according to the kit instructions. The amplification parameters were as follows: reverse transcription at 50°C for 3 minutes; predenaturation at 95°C for 5 seconds; denaturation at 95°C for 5 seconds; the fluorescence was annealed, extended and detected at 60°C for 16s, 41 cycles. Results: FAM and ROX channels correspond to ORFlab gene and N gene of SARS-CoV-2, respectively. Positive: Both channels meet the Cycle threshold (Ct) of the sample to be tested ≤40, and the amplification curve is S-shaped. Negative: Ct results of 2 channels >40 or no detection. Suspicious: One channel results Ct≤40, the other channel 35<Ct≤40.

Statistical Analysis

Data were analyzed using SPSS statistical software version 27.0.1. Categorical data were expressed as numbers and percentages [n(%)], and comparisons were made using the χ^2-test. For skewed continuous data, the median and interquartile range M (Q1, Q3) were used. The efficacy of each parameter was evaluated using receiver operating characteristic (ROC) curves. Survival functions at critical cut-off points obtained from the ROC curve were analyzed using the Kaplan-Meier method. Multivariate Cox regressions were used to identify the variables associated with the risk of death and assessed by crude hazard ratio (HR) and adjusted HR (aHR) with their 95% confidence intervals (95% CI). A *P*-value of less than 0.05 was considered statistically significant.

Results

Baseline Characteristics of the Patients

This study included 57 COVID-19 patients admitted to Hangzhou Xixi Hospital, affiliated to Zhejiang Chinese Medical University, between December 2022 and November 2023. As shown in Table 1, 33 patients (57.9%) were in the non-severe

Table 1 Baseline Characteristics of COVID-19 Patients

Baseline Characteristics	Non-Severe Group (n=33)	Severe Group (n=24)	Total (n=57)	*P*-value
Gender (Male/Female)	15/18	16/8	31/26	0.178
Age (years)	73.0(60.0–81.5)	83.5(76.3–86.0)	77.0(63.0–85.0)	0.001
Weight (Kg)	63.6±9.2	57.0±12.9	62.8±9.6	0.686
Height (m)	1.63±0.08	1.64±0.11	1.63±0.08	0.981
Smoking History (n, %)	5(15.2)	7(29.2)	12(21.1)	0.324
Underlying Conditions (n, %)	21(63.6)	15(62.5)	36(63.2)	>0.999
Diabetes (n, %)	6(18.2)	6(25.0)	12(21.1)	0.743
Coronary Heart Disease (n, %)	6(18.2)	4(16.7)	10(17.5)	>0.999
Hypertension (n, %)	17(51.5)	11(45.8)	28(49.1)	0.790
Outcome Events (n, %)				
Improved	32(97.0)	8(33.3)	40(70.2)	<0.001
Death	1(3.0)	16(66.7)	17(29.8)	

Note: *P* <0.05 was statistically significant.
Abbreviation: COVID-19: Coronavirus disease 2019.

group, consisting of 15 males and 18 females, while 24 patients were in the severe group, with 16 males and 8 females. A total of 17 patients died, 16 of whom were from the severe group. The median age of all patients was 73 years, with a range from 25 to 90 years. The age of patients in the non-severe group was significantly lower than that of the severe group (P<0.001). Common underlying conditions included diabetes (n=12, 21.1%), coronary heart disease (n=10, 17.5%), and hypertension (n=28, 49.1%), with 12 patients (21.1%) having a history of smoking.

Overview of nCD64, mHLA-DR, and CD14$^+$ Monocyte Percentages in COVID-19 Patients with Different Disease Severity

To compare the differences in immune status among COVID-19 patients with varying disease severity and to evaluate the efficacy of sepsis-related markers in differentiating between clinical classifications of COVID-19, we analyzed the nCD64 index, mHLA-DR levels, and the percentage of CD14$^+$ monocytes across different clinical types of COVID-19 patients. The results showed that the expression level of mHLA-DR in non-severe COVID-19 patients was significantly higher than that in severe and deceased patients (18109.8 ± 6295.0 vs 10774.9 ± 5267.3, P<0.001; 18,109.8 ± 6295.0 vs 10460.8 ± 4898.8, P<0.001). The expression level of mHLA-DR in deceased patients was significantly lower than that in surviving patients (10774.9 ± 5267.3 vs 17121.0 ± 6602.0, P < 0.001). Additionally, the nCD64 index level in non-severe COVID-19 patients was also significantly lower than that in severe and deceased patients [0.95 (0.28–2.45) vs 14.60 (5.30–31.01), P<0.001; 0.95 (0.28–2.45) vs 14.66 (6.66–30.83), P<0.001]. The nCD64 index level of the surviving patients was significantly lower than that of the deceased patients [1.09 (0.42–3.35) vs 14.66 (6.66–30.83), P < 0.001]. Finally, the percentage of CD14$^+$ monocytes in non-severe COVID-19 patients was significantly higher than that in severe and deceased patients (9.2 ± 3.6 vs 4.9 ± 2.6, P<0.001; 9.2 ± 3.6 vs 4.1 ± 2.2, P<0.001). The percentage of CD14$^+$ monocytes in surviving patients was significantly higher than that in deceased patients (8.7 ± 3.5 vs 4.1 ± 2.2, P < 0.001), as illustrated in Figure 1.

mHLA-DR, nCD64, and CD14$^+$ Monocyte Percentages as Predictors of Clinical Outcomes in COVID-19 Patients

We analyzed the predictive value of various sepsis biomarkers for the prognosis of COVID-19 patients. ROC analysis revealed that mHLA-DR, nCD64 index, and CD14$^+$ monocyte percentage were important in distinguishing clinical outcomes among COVID-19 patients. The results showed that CD14$^+$ monocyte percentage had the largest AUC (0.871), with a sensitivity of 87.5% and specificity of 76.5%. Next in significance were the nCD64 index (AUC=0.850) and mHLA-DR (AUC=0.779). Subsequently, Kaplan-Meier analysis was used to test the survival function of critical

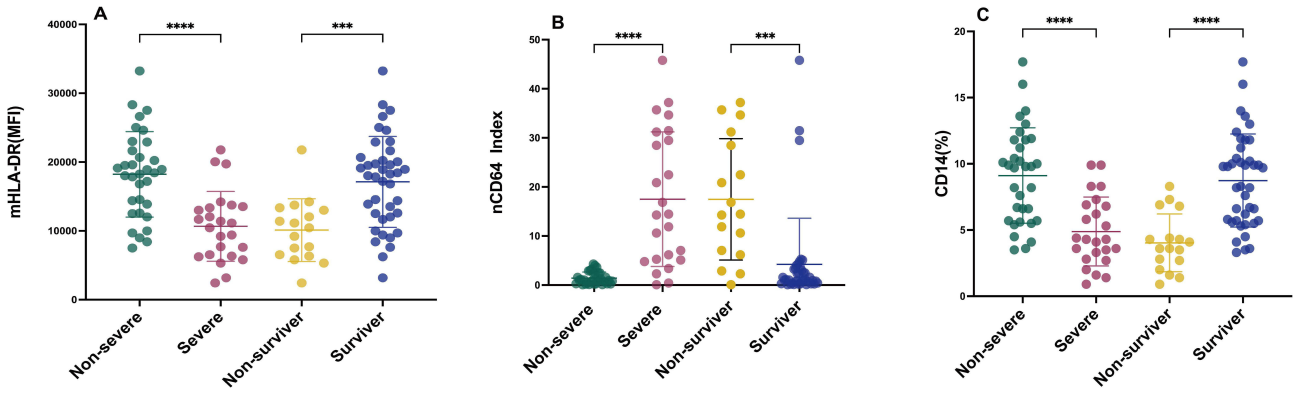

Figure 1 Levels of mHLA-DR, nCD64 Index, and percentage of CD14$^+$ monocytes in COVID-19 patients with different disease severity.
Notes: COVID-19 patients were divided into two pairs and four groups: non-severe and severe, surviver and non-surviver. The levels of mHLA-DR (**A**), nCD64 index (**B**), and percentage of CD14$^+$ monocytes (**C**) were analyzed. Median values with ranges are provided. ***$P < 0.001$; ****$P < 0.0001$.
Abbreviations: mHLA-DR, monocytic HLA-DR; MFI, mean fluorescence intensity; nCD64, neutrophil CD64.

threshold points obtained from the ROC curve. CD14$^+$ monocyte percentage and mHLA-DR values below 4.9% and 16720.5, respectively, and nCD64 values above 5.87 showed a significant association with mortality (P<0.001). Patients with CD14$^+$ monocyte percentage >4.9%, mHLA-DR >16720.5, and nCD64 <5.87 had a favorable prognosis, with the majority recovering and being discharged. Refer to Figure 2 for details. According to multivariate regression analysis, reduced CD14$^+$ monocytes and smoking were independent predictors of COVID-19 survival (aHR=0.491, 95% CI: 0.301–0.801, P=0.004 and aHR=3.388, 95% CI: 1.089–10.538, P=0.035, respectively) (Table 2).

Correlation Analysis of nCD64, mHLA-DR, CD14+ Monocyte Percentage and Viral Load

We investigated the correlation between the nCD64, mHLA-DR, percentage of CD14$^+$ monocytes and SARS-CoV-2 viral load. As shown in Figure 3, the level of nCD64 index was negatively correlated with the circulation threshold of SARS-

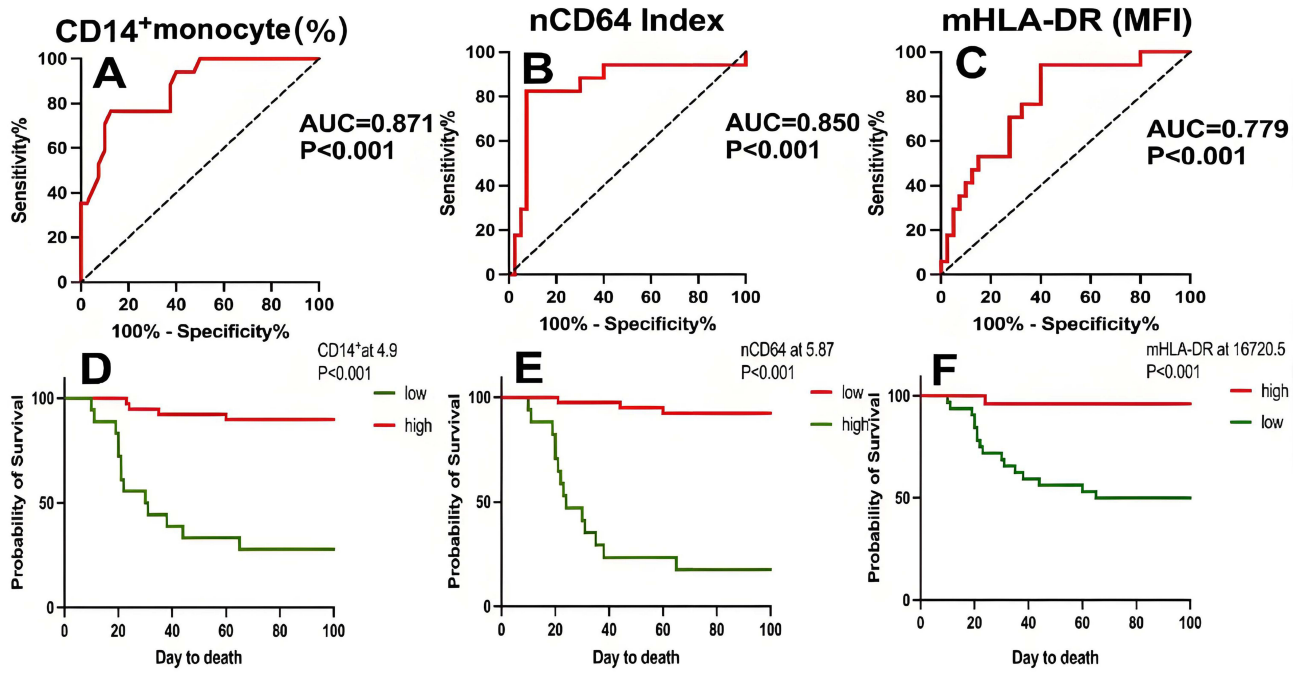

Figure 2 ROC and Kaplan-Meier curves for CD14$^+$ monocyte percentage, nCD64, and mHLA-DR (**A**–**C**) ROC curves illustrating the performance of CD14$^+$ monocyte percentage, nCD64 index, and mHLA-DR in predicting adverse clinical outcomes in COVID-19 patients. (**D**–**F**) Kaplan-Meier survival function analysis using the threshold values obtained from the ROC curves of CD14$^+$ monocyte percentage, nCD64 index, and mHLA-DR.
Abbreviations: mHLA-DR, monocytic HLA-DR; MFI, mean fluorescence intensity; nCD64, neutrophil CD64.

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Table 2 Multivariate Regression Analysis of Risk Factors for Mortality in Patients with COVID-19

Variables	HR	95% CI	*p*-value	aHR	95%CI	*p*-value
Smoking	3.581	1.190–10.773	0.023	3.388	1.089–10.538	0.035
mHLA-DR (MFI)	1.0	1.0.–1.0	0.026	1.0	1.0–1.0	0.194
$CD14^+$ monocytes	0.487	0.322–0.736	<0.001	0.491	0.301–0.801	0.004
nCD64	1.016	0.981–1.051	0.374			
Age	1.007	0.941–1.077	0.838			
Gender	1.236	0.383–3.999	0.724			
Height	1.034	0.926–1.154	0.554			
Weight	1.042	0.951–1.141	0.382			
Diabetes	1.073	0.364–3.162	0.899			
Coronary Heart Disease	1.973	0.545–7.141	0.30			
Hypertension	1.389	0.498–3.872	0.53			

Abbreviations: COVID-19, Coronavirus disease 2019; HR, hazard ratios; CI, confidence intervals; aHR, adjusted hazard ratios; mHLA-DR, monocytic HLA-DR; MFI, mean fluorescence intensity; nCD64, neutrophil CD64.

CoV-2 (r=−0.3020, *P*=0.0224). However, there was no correlation between the expression level of mHLA-DR, the percentage of $CD14^+$ monocytes and the circulating threshold of SARS-CoV-2 (r=−0.0898, *P*=0.5066; r=−0.0890, *P*=0.9294). The results showed that the higher the nCD64 index of patients, the lower the SARS-CoV-2 circulation threshold, that is, the higher the viral load, and the increase of the nCD64 index of patients may be caused by the high viral load of patients with SARS-CoV-2.

The Differential Efficacy of nCD64 and Common Inflammatory Indicators in COVID-19 Patients with Different Disease Severity

To further evaluate whether the differential value of septicaemia related indicators in COVID-19 patients with different clinical types was superior to other common inflammatory indicators, and whether the combined diagnostic value is improved compared with a single indicator, we included neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and serum amyloid A (SAA) in the comparative study. ROC analysis was shown in Table 3. It was found that nCD64, NLR, PLR and SAA had important differential value in differentiating COVID-19 patients with different clinical types (*P*<0.05). The combined diagnosis model was established by binary logistic regression analysis, and the results showed that the AUC of nCD64 was 0.910, which was larger than that of NLR (0.809), PLR (0.702) and SAA (0.889). An expression level of nCD64 of 4.13 is the optimal threshold for identifying patients with different clinical subtypes of COVID-19 (sensitivity =83.3%, specificity =96.9%). The efficacy of nCD64 combined with a single common inflammatory indicator has been improved in the identification of COVID-19

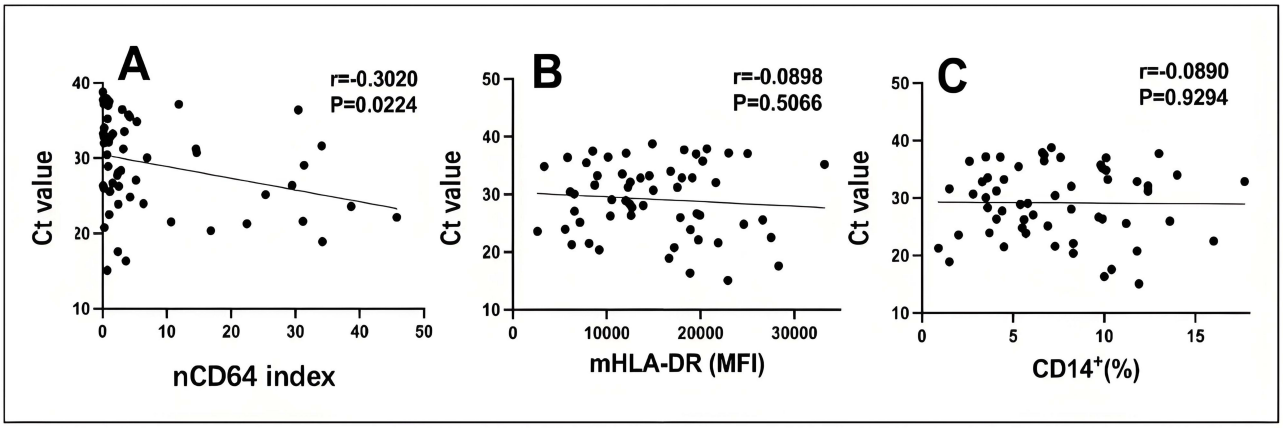

Figure 3 Correlation between nCD64 index (**A**), mHLA-DR (**B**), $CD14^+$ monocytes (**C**) and SARS-CoV-2 viral load.
Abbreviations: mHLA-DR, monocytic HLA-DR; MFI, mean fluorescence intensity; nCD64, neutrophil CD64; Ct, Cycle threshold.

Table 3 ROC Analysis Results of Common Inflammatory Indicators Used to Distinguish Patients with Different Clinical Subtypes of COVID-19

Marker	AUC	Sensitivity	Specificity	95% CI	*P* value
nCD64	0.910	83.3%	96.9%	0.811–1.000	<0.001
NLR	0.809	83.3%	78.1%	0.691–0.927	<0.001
PLR	0.702	66.7%	81.2%	0.550–0.854	0.01
SAA (mg/L)	0.889	75.0%	90.6%	0.804–0.974	<0.001
nCD64 vs NLR	0.949	91.7%	96.9%	0.881–1.000	<0.001
nCD64 vs PLR	0.939	87.5%	96.9%	0.869–1.000	<0.001
nCD64 vs SAA	0.938	87.5%	96.9%	0.864–1.000	<0.001
Combined	0.953	87.5%	100%	0.892–1.000	<0.001

Note: *P*<0.05 was statistically significant.
Abbreviations: ROC, receiver operating characteristic; COVID-19, Coronavirus disease 2019; AUC, area under the curve; CI, confidence intervals; nCD64, neutrophil CD64; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; SAA, serum amyloid A.

patients with different clinical types. The AUC of nCD64 combined with three common inflammatory indicators for COVID-19 patients with different clinical types was the highest (0.953), and the sensitivity and specificity were 87.5% and 100%, respectively. According to the above analysis, the differential value of nCD64 in COVID-19 patients with different clinical types was superior to other common inflammatory indicators. The AUC of the combined detection of nCD64 and PLR, NLR or SAA was 0.939, 0.949 and 0.938 respectively, compared with that of the single detection. In addition, the four combined tests of PLR, NLR, SAA and nCD64 could greatly improve the differential value of COVID-19 patients with different clinical types (Figure 4).

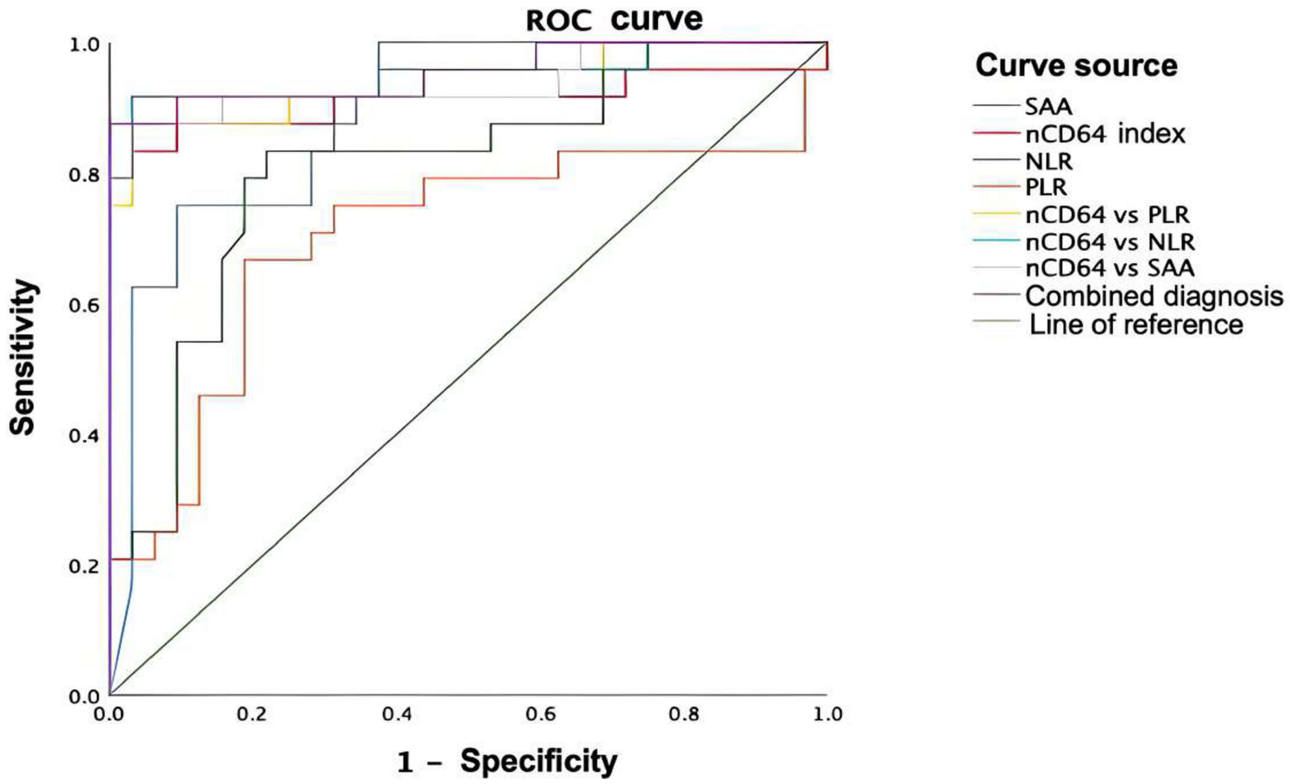

Figure 4 ROC curves for COVID-19 patients with different disease severity.
Notes: Performance of the ROC curve of SAA, NLR, PLR, nCD64, nCD64 combined with SAA, nCD64 combined with NLR, nCD64 combined with PLR and the combined detection of the four indicators in differentiating patients with different clinical subtypes of COVID-19.
Abbreviations: ROC, receiver operating characteristic; nCD64, neutrophil CD64; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; SAA, serum amyloid A.

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Discussion

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection.[14] Reports indicate that viral sepsis can be triggered by various viruses, including influenza virus, rhinovirus, respiratory syncytial virus, adenovirus, dengue virus, and coronaviruses.[15] Most COVID-19 patients exhibit lymphocytopenia accompanied by a severe inflammatory response, resembling changes observed in sepsis.[3] One study found that 65% of COVID-19 patients were observed with sepsis concurrently, while another indicated that 100% of COVID-19-related deaths involved sepsis.[4] These findings suggest that severe COVID-19 meets the criteria for classification as viral sepsis. Therefore, biomarkers used for sepsis hold significant value for the early identification of COVID-19 patients at risk of poor outcomes.

In this study, we explored the value of sepsis-related biomarkers such as the nCD64 index, mHLA-DR, and monocyte percentage in the differential diagnosis and prognostic assessment of COVID-19. We found that the mHLA-DR levels and $CD14^+$ monocyte percentages were significantly lower in severe COVID-19 patients and those who died compared to non-severe COVID-19 patients, while the nCD64 index was significantly higher ($P<0.05$). Thus, elevated nCD64 and reduced mHLA-DR and monocyte percentages were significantly associated with the severity of the COVID-19 disease course. The effectiveness of the nCD64 index in differentiating severe COVID-19 patients from non-severe ones was significantly higher than that of common inflammatory indicators such as NLR, PLR, and SAA. Moreover, the combined detection of the nCD64 index and common inflammatory indicators could greatly enhance the discriminatory value for patients with different clinical types of COVID-19. Additionally, we found that the level of the nCD64 index was negatively correlated with the cycle threshold of SARS-CoV-2, while there was no correlation between mHLA-DR and the percentage of monocytes and the cycle threshold of SARS-CoV-2. Therefore, we speculated that the complex interaction between pro-inflammatory and anti-inflammatory mechanisms in COVID-19 patients may lead to prolonged immunosuppression, which may be reflected by the low expression of mHLA-DR and the decreased percentage of monocytes.

The CD64 molecule is an IgG Fc fragment receptor I belonging to the immunoglobulin superfamily.[16] In healthy individuals, neutrophils express very low levels of CD64. When the body is suffered with an infectious disease, the nCD64 index of neutrophils increases rapidly. CD64 mRNA in neutrophils begins to be expressed 1 to 3 hours after infection, and the upregulation of CD64 on the cell surface can be detected after 3 to 6 hours, which has the ability to detect infection early. However, it will continue to rise due to the persistence of infection, so it can be used as an early reference indicator of infection.[17] The nCD64 index, widely discussed as an infection marker, has primarily been studied in the context of sepsis, demonstrating high sensitivity and specificity as a diagnostic and prognostic marker for critically ill patients with sepsis.[18,19] Additionally, the nCD64 index has been evaluated as a prognostic biomarker for mortality in acute exacerbations of chronic obstructive pulmonary disease (COPD).[20] Moreover, the nCD64 index can provide a reliable basis for the antibiotic treatment plan of patients with chronic obstructive pulmonary disease complicated with pulmonary infection.[21] In addition, the nCD64 index is also of great significance in the diagnosis and treatment of tuberculosis and the evaluation of anti-tuberculosis effects, and can be used as a monitoring indicator for treatment.[22] In the rapid diagnosis of neonatal pneumonia, compared with PCT, the nCD64 index has higher sensitivity and specificity.[23] Studies have shown that CD64 has important diagnostic value in systemic inflammatory response and is more reliable than traditional white blood cells, CRP, and PCT in the early diagnosis and treatment of sepsis. In systemic inflammatory response, the nCD64 index has been proven to have important diagnostic value for sepsis and also has the potential to accurately distinguish between bacterial and viral infections, and is expected to become an ideal marker for differentiating viral and bacterial infections.[24] Therefore, the nCD64 index may serve as a specific indicator related to the severity of inflammatory responses in various diseases.

As a systemic inflammatory disease, the severity of COVID-19 is closely associated with immune pathological conditions that induce widespread inflammation. However, the application of the nCD64 index in COVID-19 has not been fully explored. Our study found that in severe and fatal COVID-19 patients, CD64 was significantly overexpressed due to systemic inflammation, resulting in a notable increase in nCD64 levels compared to non-severe COVID-19 patients. Furthermore, the nCD64 index demonstrated significant value in distinguishing different clinical outcomes among COVID-19 patients, with an AUC reaching 0.85. An nCD64 index >5.87 was strongly correlated with mortality risk, indicating that elevated nCD64 is an effective marker for identifying COVID-19 patients at high risk of death. Our findings are consistent with those of Hoffmann et al, who compared CD64 expression levels on neutrophils between SARS-CoV-2-positive and -negative patients,

but did not correlate CD64 expression with disease severity.[25] Additionally, Yeh et al showed that the nCD64 index is more effective than general inflammatory markers in assessing the severity of sepsis,[26] but its utility in evaluating the severity of COVID-19 has not been thoroughly investigated. This suggests that the nCD64 index may provide an advantage in assessing the severity of COVID-19, potentially serving as an effective early diagnostic indicator, which could aid in implementing timely interventions to improve patient outcomes and conserve healthcare resources.

Recent studies have indicated that immunosuppression is a major contributor to adverse clinical outcomes in COVID-19 patients.[27] Monocyte HLA-DR serves as a marker reflecting the immune status, related to effective antigen presentation. Continuous antigen stimulation and immune exhaustion lead to a decrease in HLA-DR expression on monocytes.[28] Some studies have found that trauma patients with decreased HLA-DR expression on monocytes have a higher proportion of concurrent infections and sepsis.[29] Lekkou et al found that the HLA-DR of monocytes in the deceased group with severe community-acquired infection and sepsis was significantly lower than that in the surviving group at 3, 10, 13, and 17 days after admission. Therefore, HLA-DR can be used as an early and continuous monitoring indicator for prognosis judgment.[30] Research has shown that mHLA-DR is a reliable predictor of mortality in severe sepsis and has important applications in immune monitoring post-kidney transplantation.[31,32] Comparisons between COVID-19 patients and healthy subjects have revealed reduced mHLA-DR expression in COVID-19 patients.[33] In our study, we represented mHLA-DR expression levels using median fluorescence intensity (MFI) and found that severe and fatal COVID-19 patients had significantly downregulated mHLA-DR levels compared to the non-severe patients. This underscores the graded immune suppression in COVID-19 patients, with more severe immunosuppression observed in those with severe COVID-19. Additionally, we found a significant correlation between mHLA-DR expression levels and mortality; COVID-19 patients with mHLA-DR values <16720.5 had poor prognoses. This finding aligns with the study by Arunachalam et al, which documented reduced mHLA-DR expression in severe COVID-19 patients and suggested that this decline may be associated with high expression of the S100A12 gene encoding EN-RAGE.[34] Furthermore, other studies have shown that high IL-6 levels can downregulate mHLA-DR expression via the STAT3 signalling pathway, and a high IL-6/mHLA-DR ratio strongly predicts adverse clinical outcomes in COVID-19 patients.[33]

$CD14^+$ monocytes play a crucial role in acute bacterial infections,[35] as they can secrete cytokines and chemokines in response to pathogen stimulation, migrating to the site of infection to exert their anti-infective functions.[36] Meanwhile, $CD14^+$ monocytes also play a significant role in chronic diseases such as atherosclerosis. Under the stimulation of chronic inflammation, they are recruited to the intima, accumulate lipids and cholesterol derivatives, and then differentiate into foam cells, forming early plaques in the intima and leading to atherosclerosis.[37] Moreover, $CD14^+$ monocytes are essential in initiating and coordinating the host response to viral infections,[38] such as those caused by influenza virus, respiratory syncytial virus, or hantavirus. They achieve this by secreting cytokines and chemokines and gathering at the infection site or lymph nodes to effectively control the viral infection. Research has shown that $CD14^+$ monocytes are significantly reduced in sepsis patients, with the expression of ARHGEF10L mRNA in monocytes negatively correlating with disease severity and patient survival.[39] Gao et al reported that tissue factor expression on $CD14^+$ monocytes is a new prognostic marker for elderly sepsis patients.[40] In our study, we found that the percentage of $CD14^+$ monocytes in non-severe or survival COVID-19 patients was significantly higher than in severe and fatal COVID-19 patients. Furthermore, a percentage below 4.9% was significantly associated with mortality, and the $CD14^+$ monocyte percentage showed important predictive value for different clinical outcomes in COVID-19 patients (AUC=0.871). In the multivariate analysis, reduced $CD14^+$ monocytes were identified as a risk factor for a worse survival. Our findings are similar to those of Gatti et al, who also included the measurement of CD11b expression on monocyte populations and noted that the decrease in monocytes might be related to the recruitment of inflammatory lung tissue.[41] However, another study found a higher percentage of $CD14^+$ monocytes in severe COVID-19 patients,[42] which contradicts our results; this discrepancy may be attributed to the smaller sample size of COVID-19 patients in that study. Channappanavar et al observed an increase in pulmonary macrophages in severe COVID-19 patients, suggesting that the reduction in peripheral blood monocyte percentage in severe cases may be related to an increase in lung monocyte-macrophage populations.[43]

In summary, our study indicates that the immune function status is significantly impaired in severe COVID-19 patients, primarily evidenced by a reduction in the percentage of $CD14^+$ monocytes and mHLA-DR expression, along with an increase in the nCD64 index. Additionally, the nCD64 index, mHLA-DR levels, and $CD14^+$ monocyte percentage could serve as potential biomarkers for predicting adverse clinical outcomes in COVID-19 patients and may assist in differentiating between various types of COVID-19 cases.

We acknowledge that this study has some limitations. Firstly, the number of COVID-19 patients included in this study is limited, necessitating larger-scale research to further validate our findings. Secondly, we did not explore the underlying mechanisms related to the changes in CD64, HLA-DR, and monocytes in the context of SARS-CoV-2 infection, which should be investigated in future studies. Despite these limitations, our research provides reliable data indicating that the nCD64 index, mHLA-DR expression levels, and $CD14^+$ monocyte percentage can be potential biomarkers for clinical classification and disease prognosis in COVID-19 patients.

Conclusion

In this study, we found that sepsis-related biomarkers, such as the nCD64 index, mHLA-DR levels, and $CD14^+$ monocyte percentage, hold significant value in the diagnosis and prognosis of COVID-19. The differences in these indicators reflect an immunosuppressive state in severe patients.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of Hangzhou Xixi Hospital, affiliated to Zhejiang Chinese Medical University (approval number: [2022] No. 12) and was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors have read and approved the manuscript.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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