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RESEARCH ARTICLE

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Serum biomarker panel for disease severity and prognosis in patients with COVID-19

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Abstract

Background: Coronavirus disease-2019 (COVID-19) has become a worldwide emergency and has had a severe impact on human health. Inflammatory factors have the potential to either enhance the efficiency of host immune responses or damage the host organs with immune overreaction in COVID-19. Therefore, there is an urgent need to investigate the functions of inflammatory factors and serum markers that participate in disease progression.

Methods: In total, 54 COVID-19 patients were enrolled in this study. Disease severity was evaluated by clinical evaluation, laboratory tests, and computed tomography (CT) scans. Data were collected at: admission, 3–5 days after admission, when severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA detection became negative, and composite endpoint.

Results: We found that the positive rate in sputum was three times higher than that in throat swabs. Higher levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer (D-D), interleukin-6 (IL-6) and neutrophil-to-lymphocyte ratio (NLR) or lower lymphocyte counts suggested more severe disease, and the levels of cytokines and serum markers were intrinsically correlated with disease progression. When SARS-CoV-2 RNA detection became negative, the receiver operating characteristic (ROC) curve demonstrated that LDH had the highest sensitivity independently, and four indicators (NLR, CRP, LDH, and D-D) when combined had the highest sensitivity in distinguishing critically ill patients from mild ones.

Conclusions: Monitoring dynamic changes in NLR, CRP, LDH, IL-6, and D-D levels, combined with CT imaging and viral RNA detection in sputum, could aid in severity evaluation and prognosis prediction and facilitate COVID-19 treatment.

KEYWORDS
biomarker panel, coronavirus disease, inflammatory markers, severe acute respiratory syndrome coronavirus 2

Jing Li and Mingyang Tang contributed equally to the manuscript.

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes coronavirus disease-2019 (COVID-19), has resulted in an ongoing pandemic.[1,2] Although most patients have a favorable prognosis, those with advanced age and those with chronic underlying diseases may have worse outcomes.[3] In critical cases of COVID-19, multiple organ dysfunction syndrome (MODS), which includes sepsis, septic shock, and failure of the renal and respiratory systems, progresses rapidly and is fatal.[2]

SARS-CoV-2 can use its spike 1 (S1) protein to bind to cells that express the surface receptor of angiotensin-converting enzyme 2 (ACE2).[4] Furthermore, transmembrane protease serine 2 (TMPRSS2) can cleave the ACE2 receptor and the S1 protein, thereby facilitating viral entry in to the cells.[5] Owing to active replication and release of the virus within the host cells, they undergo pyroptosis and release damage-associated molecular patterns (DAMPs). These DAMPs can trigger innate and adaptive immunity by recruiting monocytes, macrophages, and T cells to the site of infection.[6] Consequently, the released DAMPs and the initiated immune responses can switch B cells into specific plasma cells that secrete antigen-specific antibodies (e.g., IgM, IgA, and IgG) for SARS-CoV-2 neutralization.[6] In patients who recovered from COVID-19 pneumonia, the host immune interactions undergo many phases such as incubation, syndromic, and recovery periods, in which the virus initiates replication, reaches a peak at 5–6 days after symptom onset, and then gradually decreases, respectively.[6] Correspondingly, the host immune system initiates the release of multiple serum proteins or cytokines that is accompanied by disease progression.[7] Recent studies showed that inflammatory markers, such as neutrophil counts,[8] CRP,[9] cytokines,[10] and erythrocyte sedimentation rate,[11] are elevated in patients with COVID-19 and severe COVID-19 seems to be related to exacerbated immune response and events associated with a cytokine storm, referring to massive inflammatory activation in response to infection.[10] The cytokine storm is also considered the top reason for death among COVID-19 MODS patients.[12,13] Moreover, recurrent hospitalizations in patients with COVID-19 and frailty in elderly or diabetic patients during COVID-19 infection are suggested to be also related to increased inflammatory burden.[14–16] The biomarkers that involved in the immune-inflammatory and coagulation pathways, such as CRP, NLR, D-D, have been used to assess the disease severity and prognosis of multiple chronic, inflammatory or autoimmune diseases, such as irritable bowel disease,[17] diabetes mellitus,[18] and Hashimoto's disease.[19] Therefore, accurate monitoring of inflammatory factors plays an important role in the judgment of disease progression and the selection of treatment strategies for COVID-19 patients. Inflammatory factors can either enhance the efficiency of host immune responses or damage host organs with immune overreaction in COVID-19.[20]

Because the functions of inflammatory factors and serum markers that participate in disease progression are controversial, therefore warranting urgent exploration, this study aimed to determine the following aspects: (1) profiling the trends of inflammatory factors and serum markers between mild and severe cases and (2) assessing the specificity and sensitivity of COVID-19-associated inflammatory markers and their joint roles in severity evaluation that may further guide clinical treatment or prognosis prediction.

2 | METHODS

2.1 | Study design and participants

We retrospectively reviewed patient database and focused on the changing trends in cytokines and serum markers and their associations with the severity and prognosis of COVID-19. A total of 54 patients with a COVID-19 diagnosis were hospitalized in the First Affiliated Hospital of Bengbu Medical College from January 2020 to March 2020. Hospitalization duration was longer than 2 weeks for all patients, and each patient underwent severity assessment during disease progression, including clinical evaluation, laboratory tests, and computed tomography (CT) scans. Data were collected at: admission, 3–5 days after admission, when viral RNA detection became negative, and composite endpoint. The study was approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College (approval no. 2020KY067).

2.2 | Laboratory confirmation and treatment

We collected sputum and throat swab specimens from all patients at admission and used RT-PCR for the detection of RNA of SARS-CoV-2 (tested on an ABI 7500 system, USA). Viral RNAs were extracted using a commercial kit specific for SARS-CoV-2 (Da An Gene Co., Ltd). The specimens were considered positive if the cycle of threshold (Ct) value of the ORF-1ab and the N gene was not higher than 40 and negative if the Ct value was undetermined. Specimens with a Ct value between 40 and 42 of double genes or single gene were repeated and considered positive if the repeat results were the same as the initial result. If the repeat Ct values were undetermined, they were considered negative. These detections were started at the admission time point and were repeated every 24 h. Specifically, laboratory tests included routine blood tests (SYSMEX, XE-5000), which revealed the whole content of blood cells (e.g., red and white blood cell quantity and ratio, platelet [PLT] quantity, and neutrophil-to-lymphocyte ratio [NLR]) and serum biochemistry tests (measured using cobas 8000, Roche) (e.g., C-reactive protein [CRP] and lactate dehydrogenase [LDH]). The coagulation function (e.g., D-dimer [D-D]) was measured using CS5100 SYSMEX, and procalcitonin (PCT) was measured with a fluorescence immunochromatographic system (Wondfo, QT-200) and tests for other respiratory pathogens were performed. All the patients were treated according to the Guidelines of the COVID-19 Diagnosis and Treatment (GCDT), issued by the National Health Committee of China.

2.3 | Criteria of clinical assessment

According to GCDT, we classified COVID-19 into three clinical subtypes: mild, moderate, and severe. Patients that just had slight clinical symptoms without radiological changes are classified as mild. Patients that had fever, respiratory distress, and a signs of pneumonia after CT image are classified as moderate. Patients that had any of the following are classified as severe: (1) respiratory rate > 30 times/min; (2) $Sp_{O2} \leq 93\%$; (3) $Pa_{O2}/ Fi_{O2} \leq 300$ mmHg; or (4) CT scan showing pulmonary lesions developed quickly within 1–2 days. Patients that required mechanical ventilation because of respiratory failure, with signs of septic shock or even multiple organ failure are critically severe cases, which are also include in the severe group.

CT imaging findings, as indicators of disease severity, were classified into the following four types: (1) healthy type, which did not exhibit alterations on pulmonary imaging; (2) mild type, which manifested ground-glass opacities and consolidation as well as thin and small subpleural patches in either single or bilateral lobes; (3) progressive type, which showed large lesions and multiple lung lobes that were involved in the bilateral lungs, accompanied by bronchial retraction, bronchiectasis, and interlobular pleural thickening; and (4) severe type, in which the bilateral lungs exhibited diffuse lesions with uneven distribution of density and large areas of ground-glass opacities. Large lung lesions resulted in a "white lung," with or without thickened interlobular pleura, bilateral pleura, and pleural effusion. Specifically, CT imaging was critical dependence for clinical severity assessment (Figure S1). In this study, healthy, mild, progressive, and severe types of CT imaging were scored as 0, 1, 2, and 3, respectively.

Based on the clinical progression, the outcomes of COVID-19 were classified as 4 types: fully recovered, improved, exacerbation, and death.

2.4 | Statistical analysis

We used SPSS (IBM SPSS software) and GraphPad Prism 5 (GraphPad Software) for statistical analysis. The normality test for continuous-type variables was performed using Kolmogorov–Smirnov test. We used the two-tailed unpaired Student's *t* test to evaluate the differences between two groups, and the chi-square test for non-parameter test among multiple groups.

We used the receiver operating characteristic (ROC) curve to assess the sensitivity and specificity of disease-associated cytokine factors and serum markers, in which a more substantial area under the ROC curve (AUC) indicated a higher accuracy. Since these markers can reflect disease severity, we used the ROC curve to evaluate the independent or joint sensitivity of the markers for disease progression. The judgment of disease progression was based on clinical assessment combined with CT imaging evaluation, regarding the mild/moderate type as negative (score: 0), whereas the severe/critically severe type was positive (score: 1). Hospitalization duration was more than 2 weeks, and the ROC curve calculations were repeated at three time points each week. In addition, we also adopted a linear correlation model to analyze the correlations between these serum markers. $p < 0.05$ was considered statistically significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, no significant difference.

3 | RESULTS

3.1 | Demographic characteristics

From January 2020 to March 2020, a total of 54 patients were hospitalized in the First Affiliated Hospital of Bengbu Medical College. In this patient cohort, there were 23 mild cases (42.6%), 22 moderate cases (40.7%), and 9 severe/critically severe cases (16.7%) according to the initial evaluation of clinical severity. In addition, age and sex distributions were as follows: 31 younger patients (57.3%, y < 60) and 23 older patients (42.7%, y ≥ 60); 22 female patients (40.7%) and 32 male patients (59.3%). Their demographic characteristics are shown in Table 1.

3.2 | Clinical classifications and their associated laboratory test and CT imaging results

In the total patient cohort, we tested the positivity of SARS-CoV-2 RNA at the first, second, and third weeks after hospitalization and stratified the positivity ratio into the mild, moderate, and severe groups. In this stratification, the mild group had fewer positivity days than the moderate and severe groups, but the comparison did not reach significance (Table 2). In addition, sputum had three times the positivity ratio than throat swabs, which were sampled and detected at the same time 68 times. This result suggests that the sputum test was more accurate and reliable (Figure 1A,B). Curiously, the Ct value of the COVID-19 ORF 1ab gene (second and third weeks) and the N gene (second week) in the sputum between the two groups indicated that the moderate and severe groups had lower virus replication than the mild group (Table 2).

In addition, the laboratory findings of blood cells and serum markers indicated that disease severity was negatively correlated with the counts of lymphocytes and monocytes and albumin levels but positively correlated with the levels of D-dimer, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen, creatine kinase (CK), LDH, total bilirubin, IL-6, glucose and CRP ($p < 0.05$; Table 3). Notably, the reduction of lymphocyte or monocyte counts indicated the potential immune cell exhaustion that represented disease severity.

3.3 | Dynamic profiling of blood cells and serum markers

COVID-19 progression is a dynamic process wherein lymphocytes and the levels of serum cytokines and markers change dynamically. Therefore, we evaluated the typical indexes involved in this

TABLE 1 Baseline Characteristics of Patients Infected With COVID-19.

	Severe ($n = 9$)	Moderate ($n = 22$)	Mild ($n = 23$)	p Value	Total ($n = 54$)
Characteristics					
Age					
x ± s, y	65.44 ± 14.16	57.32 ± 14.82	55.17 ± 12.10	$0.048_{(Sev:Mil)}$	57.76 ± 13.83
distribution					
<60 y	4(7.4%)	13(24.0%)	14(25.9%)	0.69	31(57.3%)
≥60 y	5(9.3%)	9(16.7%)	9(16.7%)		23(42.7%)
total	9(16.7%)	22(40.7%)	23(42.6%)		54(100%)
Sex					
Female-n(%)	1(1.9%)	9(16.7%)	12(22.2%)	0.10	22(40.7%)
Male-n(%)	8(14.8%)	13(24.0%)	11(20.4%)		32(59.3%)
total	9(16.7%)	22(40.7%)	23(42.6%)		54(100%)

Abbreviations: Mil, Mild; Sev, Severe.

TABLE 2 Results of Real-Time Polymerase Chain Reaction Testing for the COVID-19.

	Sample types	Severe & Moderate ($n = 13$)	Mild ($n = 23$)	p Value
0–7 d.a.h				
Positive rate (n/N,%)	Throat	6/8(75%)	8/19(42.1%)	0.12
	Sputum	6/9(66.7%)	28/34(82.4%)	0.58
Ct values(mean, range)				
ORF 1ab gene	Throat	36.83(35–38)	34.56(28.5–39)	0.08
	Sputum	31.40(25–38)	34.87(24–41)	0.19
N gene	Throat	33.00(31–36)	33.75(28–41)	0.94
	Sputum	29.20(23–39)	32.52(25–41)	0.17
8–14 d.a.h				
Positive rate (n/N,%)	Throat	2/14(14.3%)	3/19(15.8%)	0.91
	Sputum	11/23(47.8%)	21/40(52.5%)	0.72
Ct values(mean, range)				
ORF 1ab gene	Throat	38.00(35–41)	39.83(39–41)	0.50
	Sputum	37.91(35–41)	33.91(21–41)	0.04
N gene	Throat	34.30(31–37.6)	37.33(35–39)	0.37
	Sputum	34.77(31–38)	31.24(21–36)	0.008
≥15 d.a.h				
Positive rate (n/N,%)	Throat	0/2(0%)	0/17(0%)	-
	Sputum	9/23(39.1%)	22/50(44%)	0.70
Ct values(mean, range)				
ORF 1ab gene	Sputum	39.25(33–41)	34.02(22–41)	0.015
N gene	Sputum	33.50(29–36)	32.21(22–36)	0.33
Positive duration(Days)		13.69	11.46	0.27
Median num of specimens for each patient		6.23(3–13)	7.78(2–20)	0.26

Abbreviations: Ct, cycle of threshold; d.a.h, Days after hospital admission.

progression as follows: lymphocyte count, neutrophil count, white blood cell count, CRP, LDH, D-D, and NLR. In those indexes, we found that lymphocyte count reduction and the severe group had significantly lower lymphocyte counts than the moderate and mild groups, and the lymphocyte quantity gradually recovered in the following 2 weeks and reached a healthy level in the third week (Figure 2A). Serum levels of CRP, LDH, D-D, and NLR were increased but gradually decreased to the normal level with disease recovery

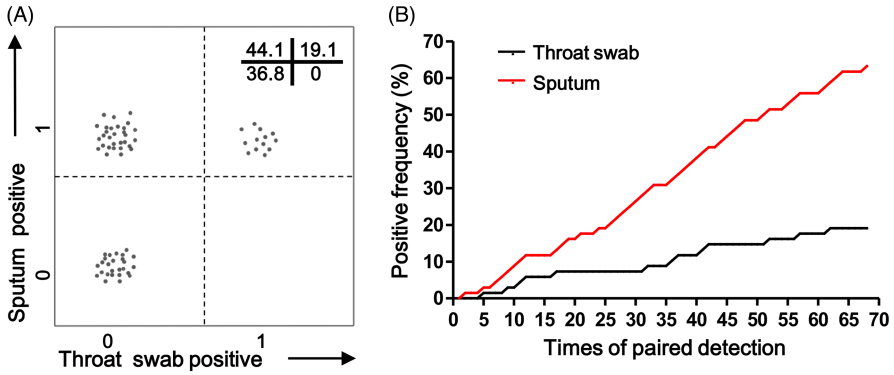

FIGURE 1 Detection of SARS-CoV-2 RNA was performed in the 68 pairs of throat swabs and sputum of 25 COVID-19 patients. (A) The image shows the incidence of sputum positivity and throat swab positivity in the 68 paired tests, wherein only the sputum was positive but the throat swab was negative for 30 times, double negative for 25 times, and double positive for 13 times. Score 0: negative, score 1: positive. (B) The image shows their increasing trends. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; COVID-19: coronavirus disease-2019.

TABLE 3 Laboratory Findings of COVID-19 patients on Admission to Hospital.

		Median (IQR)			
	Normal Range	Severe ($n = 9$)	Moderate ($n = 22$) (p Value$_{(Sev:Mod)}$)	Mild ($n = 23$) (p Value$_{(Sev:Mil)}$)	p Value (Mod:Mil)
White blood cell count, $\times 10^9$/L	3.5–9.5	9.92	5.49 (0.002)	7.04(0.12)	0.14
Red blood Cell count, $\times 10^{12}$/L	4.3–5.8	4.03	4.31(0.17)	4.17(0.54)	0.35
Neutrophil count, $\times 10^9$/L	1.8–6.3	9.02	4.14(0.0005)	5.10(0.04)	0.36
Lymphocyte count, $\times 10^9$/L	1.1–3.2	0.57	0.94(0.08)	1.33(0.002)	0.03
Monocyte count, $\times 10^9$/L	0.1–0.6	0.28	0.34(0.38)	0.50(0.014)	0.011
Hemoglobin, g/L	130–175	123.44	131.32(0.21)	132.48(0.18)	0.82
Platelet count, $\times 10^9$/L	125–350	157.89	222.14(0.11)	233.70(0.02)	0.67
Prothrombin time, s	9.8–12.1	14.39	11.91(0.006)	11.90(0.005)	0.98
Activated partial thromboplastin time, s	25–31.3	31.31	26.32(0.02)	26.36(0.03)	0.97
D-dimer, mg/L	0–0.55	26.91	1.94(0.003)	1.20(0.002)	0.41
Albumin, g/L	40–55	33.61	35.61(0.20)	39.58(0.001)	0.004
Alanine aminotransferase, U/L	9–60	265.33	40.77(0.08)	27.83(0.06)	0.21
Aspartate aminotransferase, U/L	15–45	562.78	47.27(0.04)	27.22(0.03)	0.051
Blood urea nitrogen, mmol/L	3.6–9.5	21.90	4.24(0.001)	4.05(0.0009)	0.67
Creatinine, μmol/L	57–111	276.67	60.82(0.03)	63.44(0.03)	0.47
Creatine kinase, U/L	50–310	321.89	160.68(0.27)	126.95(0.11)	0.69
Creatine kinase-MB, U/L	0–25	28.22	12.14(0.04)	13.19(0.06)	0.62
Lactate dehydrogenase, U/L	120–250	1136.25	483.14(0.001)	357.71(0.0001)	0.19
Hypersensitive Troponin I, pg/ml	<0.03	3.38	0.31(0.052)	0.79(0.04)	0.10
Total bilirubin, mmol/L	2–22	21.32	12.78(0.02)	9.15(0.0005)	0.08
IL-6, pg/ml	<7	90.09	20.17(0.006)	15.67(0.007)	0.65
Procalcitoninn, ng/ml ≥0.05, No.(%)	<0.5	8.50	0.19(0.04)	0.23(0.03)	0.51
Glucose, mmol/L	3.9–6.1	10.68	8.50(0.17)	6.86(0.04)	0.15
CRP, mg/L	0–10	150.44	66.05(0.001)	42.36(0.0001)	0.18

(Figure 2B–E). The CRP level in the severe group was high in the initial 5 days, sharply reduced on days 6–9, then interstitially rebounded to a high level on days 10–13, and finally reached the normal level on days 15–21 (Figure 2B). The LDH level showed a steady declining trend in the severe and moderate groups, while it was consistently low in the mild group (Figure 2C). Furthermore, the D-D level and

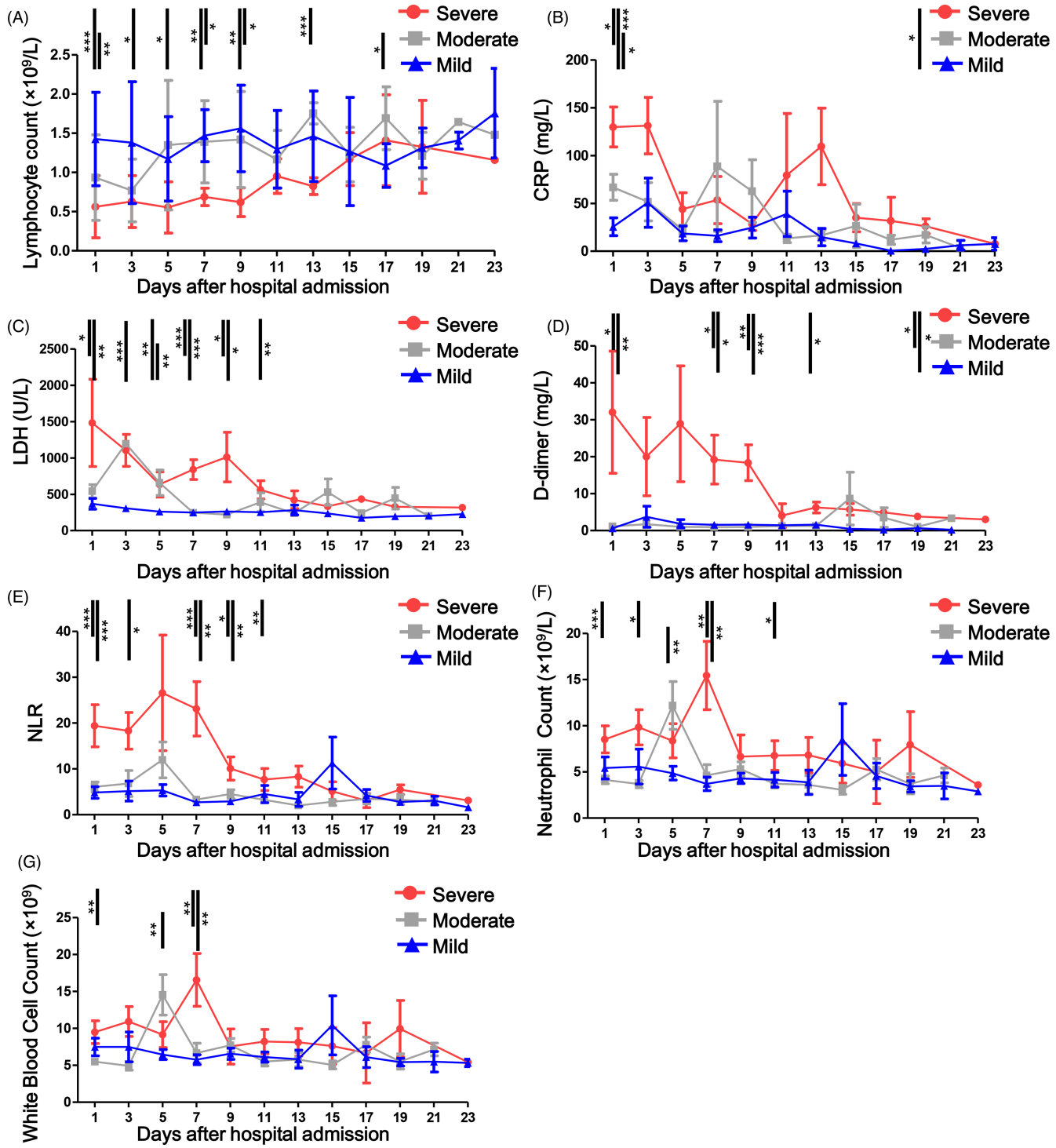

FIGURE 2 Dynamic profiles of laboratory parameters in 54 patients with SARS-CoV-2 infection. Timeline charts illustrate the differences in lymphocyte counts (A), CRP level (B), LDH level (C), D-D level (D), NLR (E), neutrophil count (F), and white blood cell count (G) in the severe (red line), moderate (gray line), and mild (blue line) groups every other day. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CRP: C-reactive protein; LDH: lactate dehydrogenase; D-D: D-dimer; NLR: neutrophil-to-lymphocyte ratio.

NLR were high in the severe group; they gradually declined on days 1–10 and reached a steady level in the following 11–23 days; these indexes remained steady and relatively low in the mild and moderate groups (Figure 2D,E). The neutrophil count and white blood cell count were not steady and did not represent significant changing trends among the three subgroups (Figure 2F,G).

3.4 | Linear correlations between serum markers and blood cells

The linear correlation model showed that the serum markers and lymphocyte count had intrinsic correlations with disease progression. In correlation analysis, CRP and lymphocyte count (L), LDH and

L, and NLR and L were negatively correlated (Figure 3A–C), while the remaining markers (LDH and CRP, D-D and CRP, CRP and IL-6, NLR and CRP, neutrophil count and CRP, NLR and IL-6, procalcitonin and LDH, NLR and LDH, neutrophil count and NLR, neutrophil count and D-D, and NLR and procalcitonin) were positively correlated (Figure 3D–N). These significant correlations indicated that the combined indexes might be better indicators of disease severity.

3.5 | Independent and joint sensitivities of COVID-19-associated markers

In this study, we found that either higher levels of CRP, LDH, D-D, and NLR or lower lymphocyte counts suggested more severe disease. Therefore, we used the ROC curve to calculate their sensitivity in detecting COVID-19 progression, regarding the mild/moderate type as negative and the severe/critical severe type as positive. The ROC curve showed that CRP had the highest independent sensitivity in predicting the disease severity. The area under the curve (AUC) values from high to low were as follows: CRP (0.705), LDH (0.695), D-D (0.650), PCT (0.648), IL-6 (0.624), and NLR (0.590) (Figure 4A). LDH combined with IL-6 had the highest joint sensitivity as a disease severity indicator. The joint sensitivities of these markers were as follows: LDH + IL-6 (0.729), CRP + PCT (0.719), D-D + IL-6 (0.719), CRP + LDH + IL-6 (0.710), LDH + D-D (0.705), and CRP + D-D (0.700) (Figure 4B). Furthermore, when SARS-CoV-2 RNA detection became negative in sputum, as an indicator of virus positivity in patients, the ROC curve also demonstrated that LDH had the highest sensitivity in distinguishing critically ill patients from mild ones independently. The independent sensitivity from high to low was as follows: LDH (0.792), NLR (0.701), CRP (0.649), and D-D (0.617) (Figure 4C). In addition, four indicators (NLR, CRP, LDH, and D-D) had the highest sensitivity in this study. The joint sensitivities were as follows: NLR + CRP + LDH + D-D (0.838), NLR + CRP + LDH (0.818), CRP + LDH (0.818), LDH + D-D (0.792), NLR + LDH (0.786), NLR + CRP (0.734), NLR + D-D (0.727), and CRP + D-D (0.656) (Figure 4D).

4 | DISCUSSION

SARS-CoV-2 can infect multiple organs and result in MODS, and this virus causes COVID-19.[21,22] Although most patients had no syndromes or mild syndromes, fatal MODS can develop rapidly within a few days in severe cases.[23] Therefore, COVID-19 treatments essentially require practical evaluation of the disease condition and expected judgment of disease progression, and both the evaluation and judgment urgently require laboratory evidence for clinical guidance.[24]

According to our paired detection results of sputum and throat swab samples of the same patients assessed 68 times, the accuracy of sputum detection is significantly higher than that of throat swabs. Upper respiratory tract samples are now widely used to detect viral RNA for the diagnosis of COVID-19, we must also remind that throat swabs are more suitable for broad-spectrum screening. For suspected cases, sputum and throat swab samples must be combined to improve the detection rate.

COVID-19 disease progression (incubation, syndromic, and recovery periods) involves virus-host interactions through which the host immune system recognizes and presents the virus-specific antigen to effective T and B cells and thereby clears the virus. In this process, pyroptosis of infected epithelial cells can release many DAMPs and PAMPs, thereby attracting lymphocyte infiltration.[6] Furthermore, extensive and severe infection sites could rapidly attract excessive lymphocyte infiltration within a short time, thereby reducing the quantity of blood lymphocytes.[25] Specifically, this reduction was mainly attributed to the lymphocyte decrease and NLR increase in blood cell counts, and recovered patients usually had lymphocyte restoration. Moreover, the cell count of neutrophils increased 5–9 days after viral infection and then gradually decreased. This rise and fall of neutrophil counts may be associated with bacterial infection that stimulates the bone marrow to produce neutrophils instantly, and bacterial infections usually occur 1 week after the onset of viral infections. Therefore, a continuous reduction in lymphocyte counts and increased NLR indicate a worsening trend of disease progression, and an increased number of neutrophils suggests potential bacterial infection in COVID-19.

In this study, we also found that inflammatory cytokines and serum markers were correlated with COVID-19 tissue damage and lymphocyte counts. LDH is a cytoplasmic glycolytic enzyme expressed in almost every tissue and could represent the extent of tissue damage in COVID-19,[26,27] in which severe pneumonia has a high level of LDH[28] and associated DAMPs and PAMPs. Alveolar macrophages can recognize the DAMPs and PAMPs released by the pyroptosis of endothelial cells, thereby initiating cytokine secretion (e.g., IL-1β, IL-18, and TNF-α). The IL-1β, TNF-α, and Toll-like receptor signaling pathways can activate innate immune cells and effective T cells to produce IL-6,[29] which circulates to the liver and induces an extensive range of acute-phase proteins, such as CRP, serum amyloid A (SAA), haptoglobin, fibrinogen, and α1-antichymotrypsin.[30] In addition, IL-6 can promote the final maturation of B cells into antigen-specific antibody-producing plasma cells.[31] Therefore, excessive inflammation,[32] which occurs as a high level of LDH, may result in macrophage pyroptosis and lymphocyte exhaustion,[33] and a large amount of IL-6 is produced in this process. Increased IL-6 expression[34] was correlated with high levels of CRP, SAA, and D-D (fibrinogen degradation product) and lymphocyte reduction (decreased lymphocyte count and increased NLR). It is difficult to define which one is the earliest and decisive factor, but our data demonstrate that CRP, LDH, and D-dimer levels are significantly higher in severe patients than in mild patients in the first 2 weeks. These indicators may lead to the formation of the cytokine storm in severe patients and COVID-19 exacerbation.

The inflammatory cytokines and serum markers analyzed in this study had individual specificity and may be used to evaluate specific

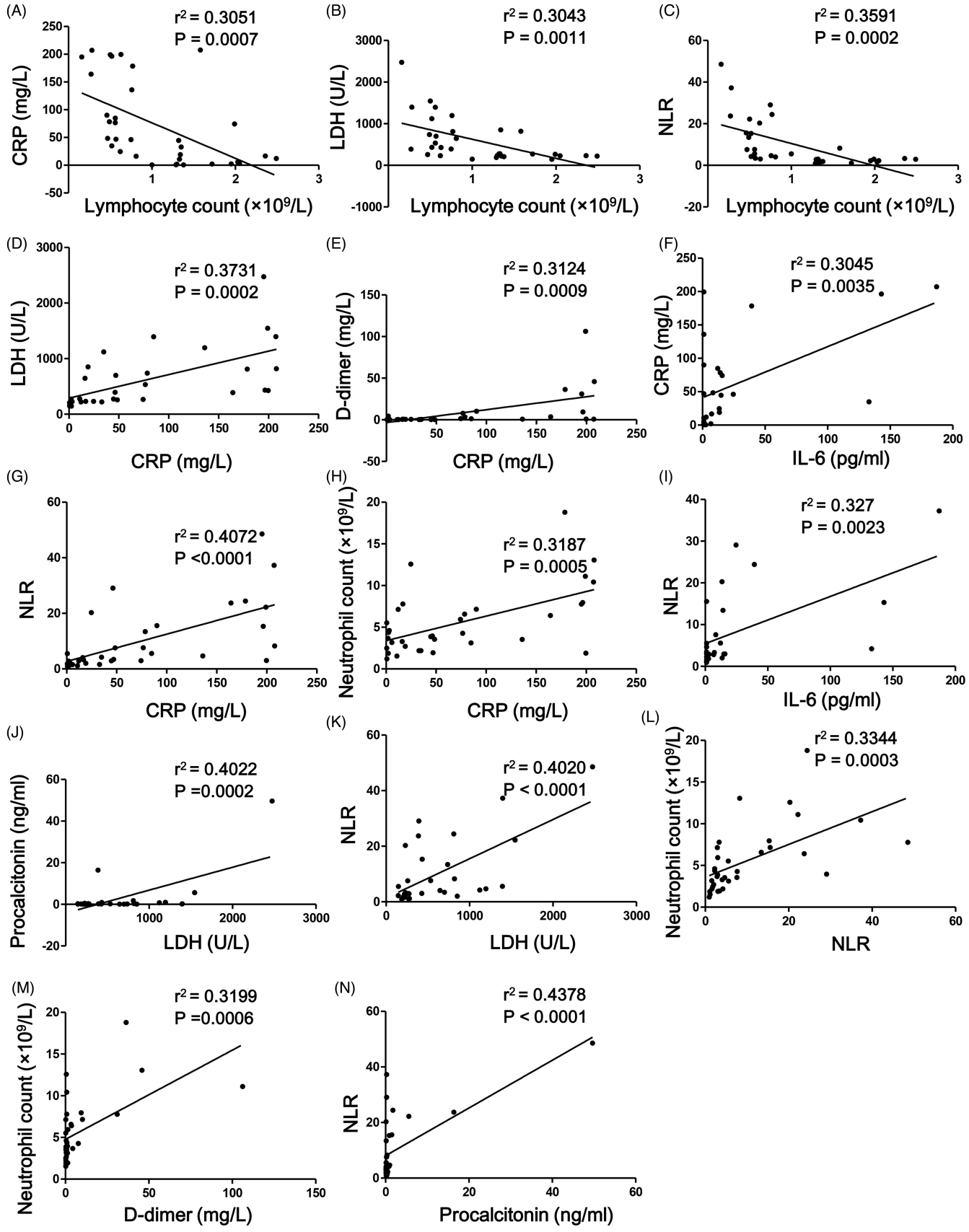

FIGURE 3 Linear correlations between the serum markers and blood cells. Linear correlations between (A) CRP and lymphocyte count, (B) LDH and lymphocyte counts, and (C) NLR and lymphocyte count were negatively correlated, while (D) LDH and CRP, (E) D-D and CRP, (F) CRP and IL-6, (G) NLR and CRP, (H) neutrophil count and CRP, (I) NLR and IL-6, (J) procalcitonin and LDH, (K) NLR and LDH, (L) neutrophil count and NLR, (M) neutrophil count and D-D, and (N) NLR and procalcitonin were positively correlated. CRP: C-reactive protein; LDH: lactate dehydrogenase; D-D: D-dimer; NLR: neutrophil-to-lymphocyte ratio; IL-6: interleukin-6.

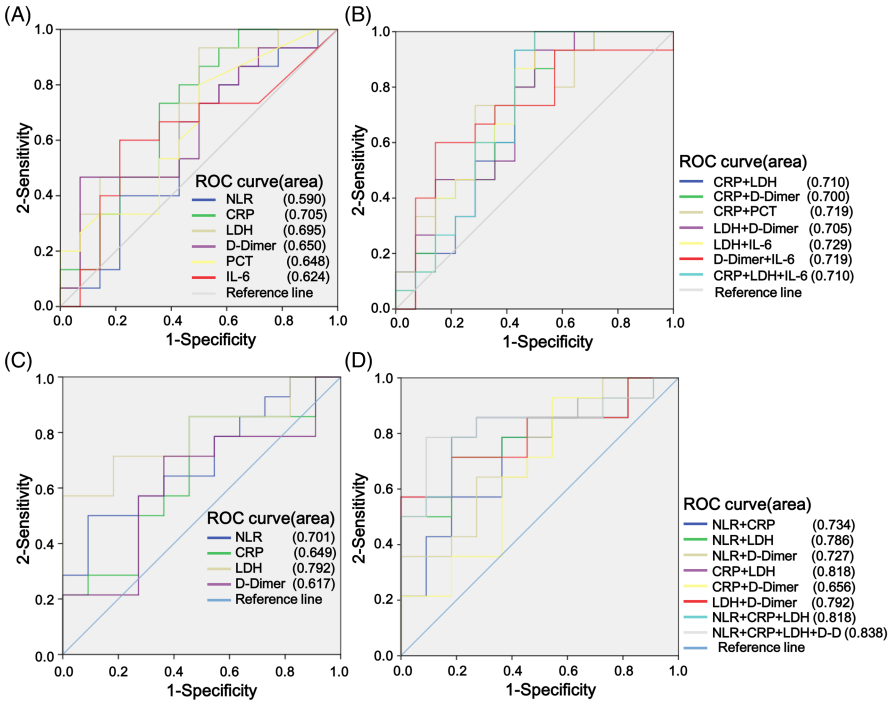

FIGURE 4 Independent and joint sensitivities of COVID-19-associated markers. (A) The ROC curve to evaluate the relationship between NLR, CRP, LDH, D-D, PCT, IL-6 and the progression of COVID-19 independently and (B) The joint sensitivity of these markers. (C) The ROC curve to evaluate the independent sensitivity of LDH, NLR, CRP and D-D with the timing of SARS-CoV-2 RNA detection became negative in sputum and (D) The joint sensitivities of these markers. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; COVID-19: coronavirus disease-2019; CRP: C-reactive protein; LDH: lactate dehydrogenase; D-D: D-dimer; NLR: neutrophil-to-lymphocyte ratio; PCT: procalcitonin; ROC: receiver operating characteristic.

disease progression time points, which may help better understand disease progression.[35] However, this study contained only a limited sample size, which is a limitation. Therefore, studies with more samples are warranted for further validation.

5 | CONCLUSIONS

Our study validated the changing trends of lymphocytes that correlated with inflammatory cytokines and serum markers, in which decreased lymphocytes were correlated with increased CRP, LDH, and NLR. Therefore, when SARS-CoV-2 RNA detection became negative, LDH independently and combined indexes of NLR, CRP, LDH, and D-D jointly could best represent disease severity with the highest sensitivity. Monitoring the dynamic changes in NLR, CRP, LDH, IL-6, and D-D, combined with CT imaging and viral RNA detection in sputum, could aid in severity evaluation and prognosis prediction, thereby facilitating the treatment of COVID-19.

AUTHOR CONTRIBUTIONS

J. Li and M. Tang take responsibility for the integrity of the data and accuracy of the data analysis. D. Liu and Z. Xie contributed to data analysis. Y. Yang designed the research, wrote the manuscript, and supervised the project. F. Wang designed the study and supervised the project. All authors have read and approved the manuscript.

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CONFLICT OF INTEREST

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.

DATA AVAILABILITY STATEMENT

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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