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ORIGINAL ARTICLE

Galectin-3 as a Novel Biomarker for Predicting Clinical Outcomes in Hospitalized COVID-19 Patients

Derya Baykiz [1], Samim Emet [1], Elif Ayduk-Govdeli [1], Murat Kaytaz [2], Mustafa L. Yavuz [1], Pelin Karaca-Ozer [1], Ekrem B. Karaayvaz [1], Alpay Medetalibeyoglu [3], Ali Elitok [1], Sema Genc [2], Zehra Bugra [1], Berrin Umman [1]

[1] *Department of Cardiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey*
[2] *Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey*
[3] *Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey*

SUMMARY

Background: **Galectin-3 has been shown to play a key pathophysiological role in pulmonary associated inflammatory response and lung fibrosis in COVID-19 and is a mediator for viral adhesion. However, there is limited data about its potential role in severity and prognosis of COVID-19. This study aimed to investigate the predictive role of serum galectin-3 concentrations in the severe clinical outcomes of hospitalized COVID-19 patients: the severity of pneumonia, in-hospital mortality, and the need for intensive care unit (ICU) admission.**

Methods: **This single-center study included 68 patients with laboratory- and radiologically-confirmed COVID-19 admitted to our emergency department. The study population was divided into patients with primary clinical outcomes (n = 32) and those without (n = 36). The need for ICU admission and/or in-hospital mortality were the primary clinical endpoints. The study group was also classified based on pneumonia severity: severe or mild/moderate. Blood samples were collected within 48 hours of admission to estimate serum galectin-3 concentrations.**

Results: **Multivariate regression analysis showed that lower concentrations of galectin-3 and arterial oxygen saturation (SpO_2) were independently associated with the primary clinical outcomes (OR = 0.951, p = 0.035; OR = 0.862, p = 0.017, respectively); increased concentrations of galectin-3 were an independent predictor of severe pneumonia (OR = 1.087, p = 0.016). In the receiver operating characteristics curve analysis, serum galectin-3 concentrations at hospital admission predicted pneumonia severity with 52.1% sensitivity and 90% specificity with a cutoff of 38.76 ng/mL.**

Conclusions: **Circulating galectin-3 at hospital admission could be a useful biomarker for identifying COVID-19 patients at high risk for severe pneumonia.**

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Correspondence:
Derya Baykiz, Lecturer MD
Department of Cardiology
Istanbul Faculty of Medicine
Istanbul University
Topkapi
Turgut Özal Millet Cad
34093, Fatih
Istanbul
Turkey
Phone: + 90 212 414 20 00
Fax: + 90 212 635 85 22
Email: derya.baykiz@hotmail.com
ORCID ID: 0000-0003-0666-6631

KEYWORDS

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INTRODUCTION

In December 2019, in Wuhan Province in China, a novel coronavirus (severe acute respiratory syndrome coronavirus-2 [SARS-CoV-2]) arose with cases of treatment-resistant pneumonia. Its prevalence rapidly expanded, causing the first pandemic of the 21st century [1,2]. It was officially determined in our country on March 11, 2020.

With the increasing domain of presentations in patients with coronavirus disease 2019 (COVID-19), the full range of manifestations has not yet been clarified. Although the classical clinical symptoms for COVID-19 patients are dry cough, dyspnea, high fever, and severe pneumonia, many patients have been found to have additional signs because of multiorgan systems failure. At the same time, COVID-19 is responsible for a large number of direct or indirect cardiovascular (CV) complications, such as myocarditis, myocardial injury, arrhythmia, and thromboembolism [3,4], and in some case reports, COVID-19 has been reported to be presented as the onset of heart failure, acute myocardial infarction (MI), myocarditis, or even sudden cardiac arrest [1,3,4]. However, the main cause of death in COVID-19 is severe acute respiratory failure [1,3,4]. Due to variations in the clinical presentation and the complexity of management of the disease, numerous factors have been evaluated to identify fragile patients. In this respect, inflammatory parameters play an important role. Various inflammatory markers have been identified as being associated with disease severity and mortality in hospitalized patients. Increased levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), serum ferritin, D-dimer, and interleukin-6 (IL-6) have been reported to play a predictive role for poor outcomes in cases of severe COVID-19 [5].

Recently, galectin-3, which is a β-galactoside-binding lectin, has garnered some interest as an inflammation marker of pulmonary damage and a potential target in COVID-19 pneumonia [6]. Its expression can be induced under conditions of tissue injury or stress. Several studies have revealed that galectin-3 participates in different physiological processes, such as inflammation, immune responses, oxidative stress, cell proliferation, and migration, regulation of cell growth, and apoptosis [7-9]. Galectin-3 is involved in the pathophysiological processes of inflammation and fibrosis with several diseases. Galectin-3 has also been associated with cardiac fibrosis, heart failure, atherothrombosis, or chronic renal failure [7,10,11]. Moreover, galectin-3 can play a role in several important pathophysiological mechanisms in COVID-19. After lung injury, galectin-3 has been shown to play key roles in pulmonary associated inflammatory response and lung fibrosis and is also a mediator in viral adhesion [6]. However, the relationship between serum galectin-3 concentration and the prognosis and pathogenesis in COVID-19 has not yet been sufficiently clarified.

In this study, we aimed to investigate the predictive role of serum galectin-3 concentrations in the severity of pneumonia, in-hospital mortality, and the need for invasive mechanical ventilation or intensive care unit (ICU) admission in hospitalized COVID-19 patients. We also assessed the possible associations between serum galectin-3 concentrations and biochemical parameters regarding myocardial injury and heart failure.

MATERIALS AND METHODS

Study population

A total of 68 patients admitted to the Emergency Department of Istanbul Faculty of Medicine were included in this cross-sectional study between January 2, 2021, to March 20, 2021. The diagnosis of COVID-19 was confirmed by real-time reverse-transcription-polymerase chain reaction (RT-PCR) on the samples taken from nasal and oropharyngeal swabs and sputum (Bio-Speedy® qPCR Detection Kit). The patients were hospitalized based on one of following conditions; severe pneumonia (bilateral infiltration > 50% and/or multiple mottling and sub-pleural ground-glass opacity) or respiratory rate ≥ 30/minute with hypoxemia; hemodynamic instability, hypotension (< 90/60 mm Hg) or tachypnea or hypoxemia (arterial oxygen saturation (SpO_2) < 93% on room air); mild/moderate pneumonia (unilateral infiltration < 50%), but not clinically stable.

Primary endpoint definition

The patients who were admitted to ICU and/or died during hospitalization (in-hospital mortality) were defined as having primary clinical endpoints. Severity of COVID-19 pneumonia identified secondary outcomes. Patients who had severe pneumonia or mild/moderate pneumonia were defined as having secondary clinical outcomes.

Study design

The study population was classified into two groups: 32 patients who were primary endpoint (admitted to ICU or died) and 36 patients discharged. The study group was also classified depending on the severity of pneumonia; severe pneumonia (n = 48) and mild/moderate pneumonia (n = 20).

The clinical and laboratory parameters which are indicative for the patients to be taken to ICU were dyspnea with SpO_2 < 90% and PaO_2 < 65 mmHg (with > 5 L/minute nasal oxygen), severe respiratory distress requiring endotracheal intubation, hypotension (systolic blood pressure (SBP) < 90 mmHg or mean arterial pressure (MAP) < 65 mmHg), tachycardia (> 110 beats/minute), and peripheral hypoperfusion and multiorgan failure with increased CRP, ferritin, LDH, D-dimer (sepsis or septic shock).

The patients with severe chronic kidney disease (CKD) (estimated GFR < 30 mL/minute/1.73 m^2), a history of MI, acute coronary syndrome within 3 months, heart

failure with reduced ejection fraction (EF) (< 50%), severe valvular heart disease, chronic inflammatory and autoimmune diseases, malignancy, abnormal thyroid function, chronic liver disease, pancreatitis, uncontrolled diabetes mellitus with organ damage were excluded from the study.

Blood sampling and serum galectin-3 measurement

Venous blood samples were collected into K2-EDTA tubes for complete blood counting (CBC), serum separator tubes for serum glucose, albumin, high sensitivity troponin T (hsTnT), prohormone B-type natriuretic peptide (proBNP), and ferritin and into Na-citrated tubes (109 M, 3.2%) for D-dimer and fibrinogen measurements. Tubes were centrifuged for 15 minutes at 2,000 x g, and supernatants were separated. CBC, fibrinogen and D-dimer levels were measured in the same day within three hours. Ten milliliters of peripheral venous blood were taken within 48 hours of admission for the measurement of galectin-3, and the serum samples were stored at -80°C until the study.

The CBC analyses were performed using the LH780 Hematology Analyzer (Beckman Coulter, Inc., CA, USA), serum glucose and albumin levels were measured by Cobas 6000 and serum hsTnT, proBNP, and ferritin levels were assessed by Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany). Fibrinogen and D-dimer concentrations were measured using STA R Max coagulation analyzer (Stago, Asnieres sur Seine, France). Serum galectin-3 concentration was measured by ELISA method (Elabscience Biotechnology Inc., Houston, TX, USA).

Baseline demographic and clinical and laboratory findings both at hospital admission and during hospitalization were collected from the hospital medical records. The presence or severity of pneumonia was determined with a positive RT-PCR and the reports of the thorax computed tomography (CT) scan. All data were carefully reviewed and recorded in the computer database by two independent investigators. The local ethics committee approved this single center study (Decision no. 2020/11/1512). Written informed consent was provided by all patients before enrolling in the study.

Statistical analysis

All statistical tests were performed using the Statistical Package for the Social Sciences 26.0 for Windows (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to analyze the normality of the data. Continuous data are indicated as mean ± standard deviation (SD) and categorical data are represented as percentages. A chi-squared or Fisher's exact test were used to assess the differences in the categorical variables between the groups. Student's *t*-test or the Mann-Whitney U test was used to compare the unpaired samples. The relationships among the parameters were assessed using Pearson's or Spearman's correlation analyses according to the normality of the data. Univariate and multivariate logistic regression analyses were used to identify the independent predictors for primary endpoint and severe pneumonia. After performing univariate analysis, the stepwise method was used to select the significant variables for use in the multivariate logistic regression analysis. The results of the univariate and multivariate regression analyses are presented as odds ratios (OR) with 95% confidence intervals (CIs). The receiver operating characteristic (ROC) curve, sensitivity, specificity, and area under the curve (AUC) were calculated to evaluate the ability of the galectin-3 concentration to predict severe pneumonia. Significance was taken at a two-sided $p < 0.05$. The sample size was determined using G power (version 3.1.9.4) based on the results found in the literature [12], assuming 80% power and an alpha level of 0.05 for comparing between independent samples. A total of 68 subjects were required.

RESULTS

Clinical characteristics of the patients based on the severity of pneumonia

The baseline and clinical characteristics and laboratory findings of the study participants are shown in Table 1. The study consisted of a total of 68 participants, 66.2% were male and 33.8% were female (Figure 1). The average age of the study population was 61.68 ± 14.4 years. Among the 68 patients, 48 (70.5%) had severe pneumonia and 20 (29.5%) had mild/moderate pneumonia based on CT scan. Hypertension was seen more frequently in the severe pneumonia group compared to mild/moderate pneumonia ($p = 0.032$), SpO_2 of the patients with severe pneumonia was significantly lower compared with mild/moderate pneumonia (86.1 ± 10.9% vs. 95.4 ± 2.8%, $p < 0.001$) (Table 1). No statistically significant differences were obtained in heart rate, body temperature, and SBP between the patients with severe and mild/moderate pneumonia (Table 1).

The laboratory findings at admission and the levels during hospitalization are presented in Table 1 and supplementary Table 1. Serum blood urea nitrogen (BUN), fasting plasma glucose, LDH, CRP, D-dimer, ferritin, hs-TnT, pro-BNP, procalcitonin, fibrinogen, and CBC parameters, including white blood cell (WBC), neutrophil, and lymphocyte counts were also significantly different in the patients with severe pneumonia than those of mild/moderate pneumonia (Table 1, supplementary Table 1). The serum galectin-3 concentrations were also significantly higher in severe pneumonia compared with mild/moderate pneumonia (31.46 ± 18 vs. 24.02 ± 12.3 ng/mL, respectively; $p = 0.044$) (Table 1, Figure 2). The adverse effects such as acute renal failure and myocardial injury, during hospitalization, were also higher in patients with severe pneumonia compared with mild/moderate pneumonia ($p = 0.009$, $p < 0.001$, respectively; supplementary Table 1).

Table 1. Baseline clinical characteristics, and laboratory findings of the study participants based on pneumonia severity.

		Total patients (n = 68)	Severe Pneumonia (n = 48)	Mild/Moderate Pneumonia (n = 20)	p-value
Clinical characteristics					
Age (years)		61.68 ± 14.4	63.08 ± 15.2	58.3 ± 11.9	0.091
Gender	male (%)	45 (66.2%)	29 (60.4%)	16 (80%)	0.120
	female (%)	23 (33.8%)	19 (39.6%)	4 (20%)	
HT (%)		27 (39.7%)	23 (47.9%)	4 (20%)	0.032 *
DM (%)		20 (29.4%)	15 (31.3%)	5 (25%)	0.606
HR (bpm)		87.86 ± 19.3	86.59 ± 19.1	90.8 ± 19.8	0.298
Temperature (°C)		36.76 ± 0.6	36.68 ± 0.5	36.95 ± 0.8	0.091
SBP (mmHg)		120.15 ± 18.7	119.41 ± 18.9	121.85 ± 18.6	0.823
SpO_2 (%)		89 ± 10.2	86.13 ± 10.9	95.45 ± 2.8	< 0.001 *
Laboratory findings at admission					
Fasting plasma glucose (mg/dL)		137.35 (76 - 710)	145.85 (81.8 - 710.4)	128 (76 - 321.9)	0.273
BUN (mg/dL)		17.9 (5.42 - 155.28)	20.35 (5.42 - 155.28)	16.17 (6.45 - 27.43)	0.021 *
Creatinine (mg/dL)		1.1 ± 0.9	1.18 ± 1.1	0.92 ± 0.2	0.706
CKD-EPI (mL/min/1.73 m^2)		78.77 ± 31.5	71.68 ± 36.5	86.15 ± 17.28	0.030 *
Urea (mg/dL)		37.4 (11.6 - 332.3)	42.1 (11.6 - 332.3)	32.4 (13.8 - 54.3)	0.047 *
Uric acid (mg/dL)		5.37 ± 2.2	5.62 ± 2.4	5.04 ± 1.2	0.510
Sodium (mmol/L)		136.6 ± 5.9	136.52 ± 6.2	137 ± 3.78	0.337
Potassium (mmol/L)		4.48 ± 0.6	4.46 ± 0.7	4.39 ± 0.4	0.649
AST (IU/L)		32.8 (8 - 181)	37 (8 - 181)	27.85 (13 - 155.4)	0.106
ALT (IU/L)		25 (5 - 194)	27.3 (5 - 194)	24.55 (12 - 139.5)	0.540
LDH (IU/L)		338 (138 - 925)	372 (142 - 925)	266 (138 - 489)	0.001 *
CRP (mg/L)		90.04 (2.63 - 404.64)	104.63 (19.73 - 404.65)	36.75 (0.75 - 206.98)	0.001 *
D-dimer (µg/L)		990 (330 - 5,460)	1,326.55 (392.9 - 7,810)	642.6 (330 - 2,500)	0.002 *
Ferritin (ng/mL)		678.5 (20.99 - 6,162)	700.9 (20.99 - 3,116)	383 (23.23 - 6,162)	0.062
Hs-TnT (pg/mL)		5.75 (3 - 366.2)	9.04 (3 - 394.2)	4.07 (3 - 23.18)	0.014 *
Pro-BNP (pg/mL)		287.5 (16.93 - 35,000)	507.65 (36.41 -35,000)	128.9 (16.93 - 2,418)	0.001 *
Procalcitonin (ng/mL)		0.16 (0.02 - 2.38)	0.19 (0.03 - 2.38)	0.10 (0.02 - 1.73)	0.026 *
Fibrinogen (mg/dL)		631 (285 - 1,164)	640.5 (385 - 1,164)	503.7 (285 - 750.7)	0.003 *
Albumin (g/dL)		3.56 ± 0.5	3.49 ± 0.5	3.79 ± 0.6	0.031 *
Hgb (gr/dL)		11.56 ± 2.3	11.63 ± 2	11.87 ± 3	0.408
Hematocrit (%)		34.81 ± 6.4	35.09 ± 5.9	35.39 ± 7.8	0.567
WBC (10^3/µL)		9.17 ± 6.2	10.03 ± 6.1	7.18 ± 5.9	0.027 *
RBC (10/µL)		4.08 ± 0.7	4.12 ± 0.7	4.13 ± 0.9	0.956
Neutrophil (10^3/µL)		5.9 (0.5 - 26.7)	6.75 (0.5 - 22)	3.5 (0.5 - 26.7)	0.002 *
Lymphocyte (10^3/µL)		0.7 (0.1 - 2.2)	0.6 (0.2 - 2)	0.9 (0.1 - 2.9)	0.002 *
Lymphocyte (%)		11.95 ± 8.4	9.61 ± 7.6	19.68 ± 10.1	< 0.001 *
Neutrophil (%)		79.32 ± 13.5	82.62 ± 12.9	69.34 ± 12.8	< 0.001 *
Platelet (10^3/µL)		260 (31 - 590)	266.5 (91 - 590)	225 (31 - 582)	0.086
Galectin-3 (ng/mL)		29.67 ± 16.7	31.46 ± 18	24.02 ± 12.3	0.044 *

Abbreviations: HT - hypertension, DM - diabetes mellitus, HR - heart rate, SBP - systolic blood pressure, SpO_2 - arterial oxygen saturation, BUN - blood urea nitrogen, CKD-EPI - estimated glomerular filtration rate, AST - aspartate transaminase, ALT - alanine transaminase, LDH - lactate dehydrogenase, CRP - C reactive protein, Hs-TnT - high-sensitivity troponin T, Pro-BNP - prohormone B-type natriuretic peptide, Hgb - hemoglobin, WBC - white blood cell, RBC - red blood cell.

Table 2. Baseline clinical characteristics and laboratory findings of the patients with and without primary composite endpoint.

		Total patients (n = 68)	Patients with primary composite endpoint (n = 32)	Patients without primary composite endpoint (n = 36)	p-value
Clinical characteristics					
Age (years)		61.68 ± 14.4	64.75 ± 15.1	58.94 ± 13.4	0.046 *
Gender	male n (%)	45 (66.2%)	23 (71.9%)	22 (61.1%)	0.349
	female n (%)	23 (33.8%)	9 (28.1%)	14 (38.9%)	
HT (%)		27 (39.7%)	15 (46.9%)	12 (33.3%)	0.255
DM (%)		20 (29.4%)	10 (31.3%)	10 (27.8%)	0.754
HR (bpm)		87.86 ± 19.3	91.83 ± 20.6	84.56 ± 17.8	0.128
Temperature (°C)		36.76 ± 0.6	36.71 ± 0.6	36.8 ± 0.6	0.554
SBP (mmHg)		120.15 ± 18.7	116.73 ± 18.8	123 ± 18.4	0.292
SpO_2 (%)		89 ± 10.2	83.59 ± 12.2	93.36 ± 5.2	< 0.001
Pneumonia severity	mild/moderate (%)	20 (29.4%)	1 (3.1%)	19 (52.8%)	< 0.001 *
	severe (%)	48 (70.6%)	31 (96.9%)	17 (47.2%)	
Laboratory findings at admission					
Fasting plasma glucose (mg/dL)		137.35 (76 - 710)	143.6 (81.8 - 388)	132.65 (76 - 710)	0.990
BUN (mg/dL)		17.9 (5.42 - 155.28)	20.05 (5.42 - 155.28)	17.69 (6.45 - 55.28)	0.457
Creatinine (mg/dL)		1.1 ± 0.9	1.2 ± 1.2	1.0 ± 0.5	0.493
CKD-EPI (mL/min/1.73 m^2)		78.77 ± 31.5	76.03 ± 36.3	75.86 ± 29.6	0.902
Urea (mg/dL)		37.4 (11.6 - 332.3)	39.2 (11.6 - 332.3)	37.4 (13.8 - 118.3)	0.957
Uric acid (mg/dL)		5.37 ± 2.2	5.3 ± 2.3	5.59 ± 2	0.683
Sodium (mmol/L)		136.6 ± 5.9	136.97 ± 6.7	136.39 ± 4.3	0.648
Potassium (mmol/L)		4.48 ± 0.6	4.39 ± 0.6	4.48 ± 0.6	0.567
AST (IU/L)		32.8 (8 - 181)	37.45 (8 - 181)	31.65 (13 - 155.4)	0.242
ALT (IU/L)		25 (5 - 194)	28.6 (5 - 150.3)	25.65 (8 - 194)	0.332
LDH (IU/L)		338 (138 - 925)	340 (142 - 925)	308 (138 - 714)	0.075
CRP (mg/L)		90.04 (2.63 - 404.64)	100.07 (18.07 - 312.16)	73.16 (0.75 - 404.64)	0.134
D-dimer (µg/L)		990 (330 - 5,460)	1,435 (450 - 7,810)	724.45 (330 - 4,897.3)	0.006 *
Ferritin (ng/mL)		678.5 (20.99 - 6,162)	758.85 (20.99 - 3,116)	610.45 (26.93 - 6,162)	0.196
Hs-TnT (pg/mL)		5.75 (3 - 366.2)	18.52 (3 - 366.2)	4.39 (3 - 23.18)	< 0.001
Pro-BNP (pg/mL)		287.5 (16.93 - 35,000)	627.6 (37.06 - 35,000)	222.8 (16.93 - 2,418)	0.011 *
Procalcitonin (ng/mL)		0.16 (0.02 - 2.38)	0.18 (0.04 - 1.83)	0.13 (0.02 - 2.38)	0.111
Fibrinogen (mg/dL)		631 (285 - 1,164)	631 (422 - 1,164)	648 (285 - 955)	0.526
Albumin (g/dL)		3.56 ± 0.5	3.47 ± 0.5	3.64 ± 0.5	0.104
Hgb (gr/dL)		11.56 ± 2.3	11.82 ± 2.1	11.59 ± 2.5	0.694
Hematocrit (%)		34.81 ± 6.4	35.69 ± 6.1	34.72 ± 6.8	0.544
WBC (10^3/µL)		9.17 ± 6.2	10.03 ± 5.6	8.44 ± 6.6	0.089
RBC (10/µL)		4.08 ± 0.7	4.17 ± 0.7	4.08 ± 0.7	0.597
Neutrophil (10^3/µL)		5.9 (0.5 - 26.7)	6.7 (0.6 - 22)	5.1 (0.5 - 26.7)	0.040
Lymphocyte (10^3/µL)		0.7 (0.1 - 2.2)	0.6 (0.2 - 2.1)	0.8 (0.1 - 2.9)	0.129
Lymphocyte (%)		11.95 ± 8.4	9.14 ± 6.3	15.63 ± 10.9	0.009 *
Neutrophil (%)		79.32 ± 13.5	82.99 ± 12.3	74.92 ± 14.7	0.010 *
Platelet (10^3/µL)		260 (31 - 590)	249.5 (91 - 572)	283 (31 - 590)	0.749
Galectin-3 (ng/mL)		29.27 ± 16.8	24.65 ± 17.7	33.38 ± 15	0.034 *
Hospital stay duration (days)		16 (2 - 55)	25 (7 - 51)	13 (2 - 55)	< 0.001

Abbreviations: HT - hypertension, DM - diabetes mellitus, HR - heart rate, SBP - systolic blood pressure, SpO_2 - arterial oxygen saturation, BUN - blood urea nitrogen, CKD-EPI - estimated glomerular filtration rate, AST - aspartate transaminase, ALT - alanine transaminase, LDH - lactate dehydrogenase, CRP - C reactive protein, Hs-TnT - high-sensitivity troponin T, Pro-BNP - prohormone B-type natriuretic peptide, Hgb - hemoglobin, WBC - white blood cell, RBC - red blood cell.

Table 3. Clinical Outcomes of the Patients.

	Total patients (n = 68)	Severe Pneumonia (n = 48)	Mild/Moderate Pneumonia (n = 20)	p-value
Clinical Outcomes of the Patients				
Hospital stay duration (days)	16 (2 - 55)	19 (5 - 55)	12 (2 - 25)	< 0.001 *
Mortality (%)	12 (17.6%)	12 (25%)	0 (0%)	0.013 *
Intensive care unit admission (%)	28 (41.2%)	27 (56.3%)	1 (5%)	< 0.001 *

Table 4A. Correlation of galectin-3 with inflammatory markers and cardiac injury biomarkers in patients without primary composite endpoint.

	Variable	r	p
Galectin-3 (without primary composite endpoint)	LDH (at admission)	0.349	0.040 *
	LDH (peak levels)	0.279	0.105
	CRP (at admission)	0.194	0.256
	CRP (peak levels)	0.137	0.427
	Procalcitonin (at admission)	0.045	0.799
	Procalcitonin (peak levels)	0.064	0.720
	Pro-BNP (at admission)	0.273	0.113
	Pro-BNP (peak levels)	0.321	0.056
	hs-TnT (at admission)	-0.082	0.639
	hs-TnT (peak levels)	0.105	0.555
	D-dimer (at admission)	0.138	0.438
	D-dimer (peak levels)	0.201	0.247

Abbreviations: LDH - lactate dehydrogenase, CRP - C reactive protein, Pro-BNP - prohormone B-type natriuretic peptide, Hs-TnT - high-sensitivity troponin T.

Table 4B. Correlation of galectin-3 with inflammatory markers and cardiac injury biomarkers in patients with primary composite endpoint.

	Variable	r	p
Galectin-3 (with primary composite endpoint)	LDH (at admission)	0.055	0.770
	LDH (peak levels)	-0.058	0.757
	CRP (at admission)	-0.230	0.205
	CRP (peak levels)	-0.307	0.093
	Procalcitonin (at admission)	-0.354	0.050*
	Procalcitonin (peak levels)	-0.154	0.410
	Pro-BNP (at admission)	-0.379	0.033*
	Pro-BNP (peak levels)	-0.323	0.076
	Hs-TnT (at admission)	-0.080	0.665
	Hs-TnT (peak levels)	-0.097	0.605
	D-dimer (at admission)	0.106	0.563
	D-dimer (peak levels)	-0.029	0.875

Abbreviations: LDH - lactate dehydrogenase, CRP - C reactive protein, Pro-BNP - prohormone B-type natriuretic peptide, Hs-TnT - high-sensitivity troponin T.

Table 5A. Multivariate regression analysis to predict independent variables for primary composite endpoint.

Variable	OR	95% Confidence interval	p-value
SpO_2	0.862	0.764 - 0.973	0.017 *
LDH (peak)	1.003	0.998 - 1.009	0.255
CRP (peak)	1.007	0.996 - 1.018	0.227
Hospital stay duration	1.069	0.989 - 1.155	0.093
Acute renal failure	1.127	0.164 - 7.748	0.903
Galectin-3	0.951	0.907 - 0.996	0.035 *

Abbreviations: SpO_2 - arterial oxygen saturation, LDH - lactate dehydrogenase, CRP - C reactive protein, OR - odds ratio.

Table 5B. Multivariate regression analysis to predict independent variables for pneumonia severity.

Variable	OR	95% Confidence interval	p-value
SpO_2	0.703	0.526 - 0.938	0.017 *
LDH (peak)	1.015	1.002 - 1.028	0.019 *
CRP (peak)	0.989	0.970 - 1.008	0.248
Acute renal failure	6.045	0.425 - 8.596	0.184
Fibrinogen (peak)	1.007	1.00 - 1.014	0.048 *
Galectin-3	1.087	1.015 - 1.163	0.016 *

Abbreviations: SpO_2 - arterial oxygen saturation, LDH - lactate dehydrogenase, CRP - C reactive protein, OR - odds ratio.

Table 6. Comparison of galectin-3 levels between the groups according to clinical outcomes.

Galectin-3			
		Mean ± SD	p-value
Pneumonia severity	mild/moderate pneumonia	24.02 ± 12.3	0.044 *
	severe pneumonia	31.46 ± 18	
Mortality	death (-)	32.89 ± 15.1	< 0.001 *
	death (+)	12.4 ± 14.1	
ICU admission	ICU (-)	30.83 ± 16.5	0.328
	ICU (+)	27.05 ± 17.3	
Oxygen treatment	via mask	29.98 ± 15	0.352
	NIMV/ HFNC	31.5 ± 18.3	
	orotracheal intubation	23.72 ± 18.7	
Acute renal failure	ARF (-)	28.28 ± 17.3	0.476
	ARF (+)	30.82 ± 16.7	
Acute liver failure	ALF (-)	27.76 ± 18.6	0.475
	ALF (+)	30.27 ± 16	
Myocardial injury	MI (-)	30.87 ± 13.9	0.632
	MI (+)	28 ± 19.6	
Cerebrovascular accident	CVA (-)	28.9 ± 16.5	0.154
	CVA (+)	1.39	

Abbreviations: ICU - intensive care unit, NIMV - non invasive mechanical ventilation, HFNC - high flow nasal cannula, ARF - acute renal failure, ALF - acute liver failure, MI - myocardial injury, CVA - cerebrovascular accident, SD - standard deviation.

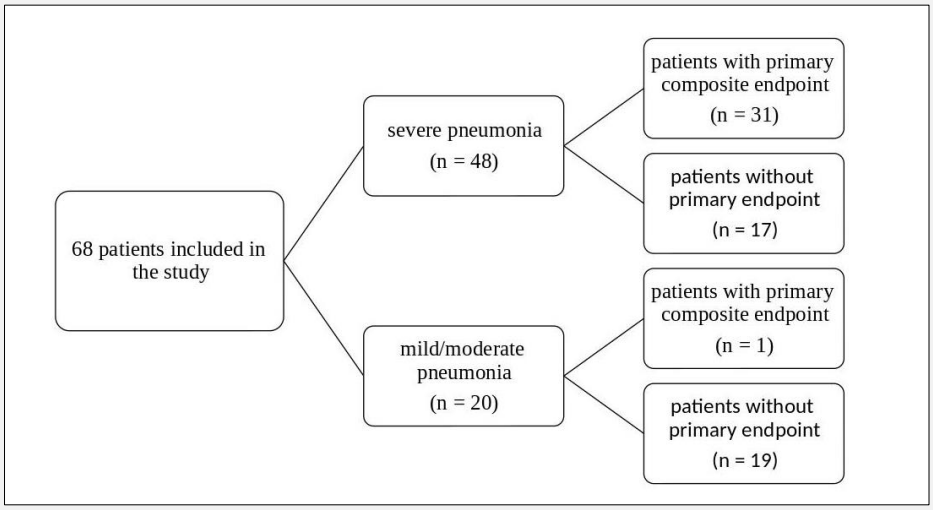

Figure 1. Flow chart of the study design.

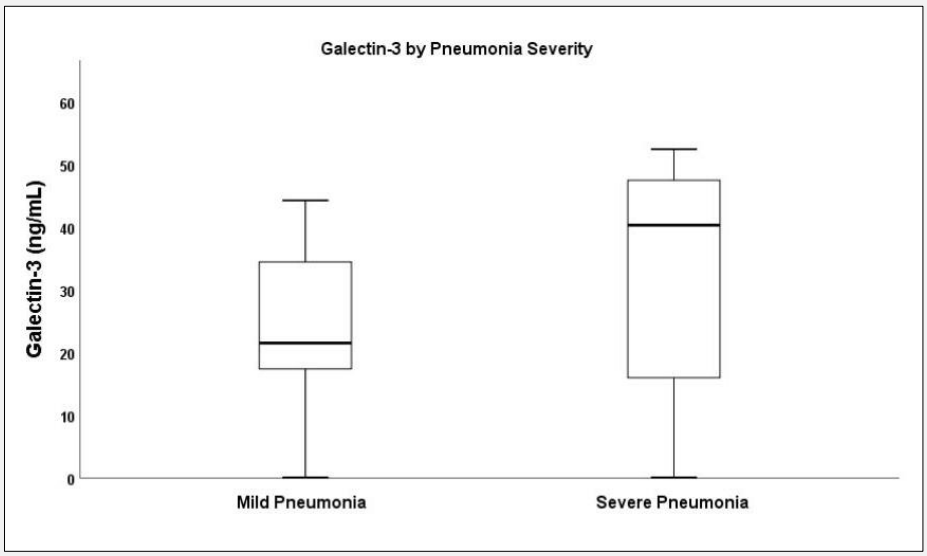

Figure 2. Serum galectin-3 levels according to pneumonia severity of the study group.

Clinical characteristics of the patients with and without the primary composite endpoints

The clinical and baseline characteristics of the patients with and without the primary composite endpoints are presented in Table 2. Thirty-two patients (47%) reached the primary composite endpoint (Table 2). The patients with primary composite endpoints were mostly males (71.9%), and the average age of this group was older than the group with no primary endpoint (64.75 ± 15.1 vs. 58.94 ± 13.4 years, p = 0.046). Of the patients with primary composite endpoints, 96.9% had severe pneumonia. Also, the SpO_2 of this group was significantly lower compared to the group with no primary endpoint ($p < 0.001$). However, no significant differences were

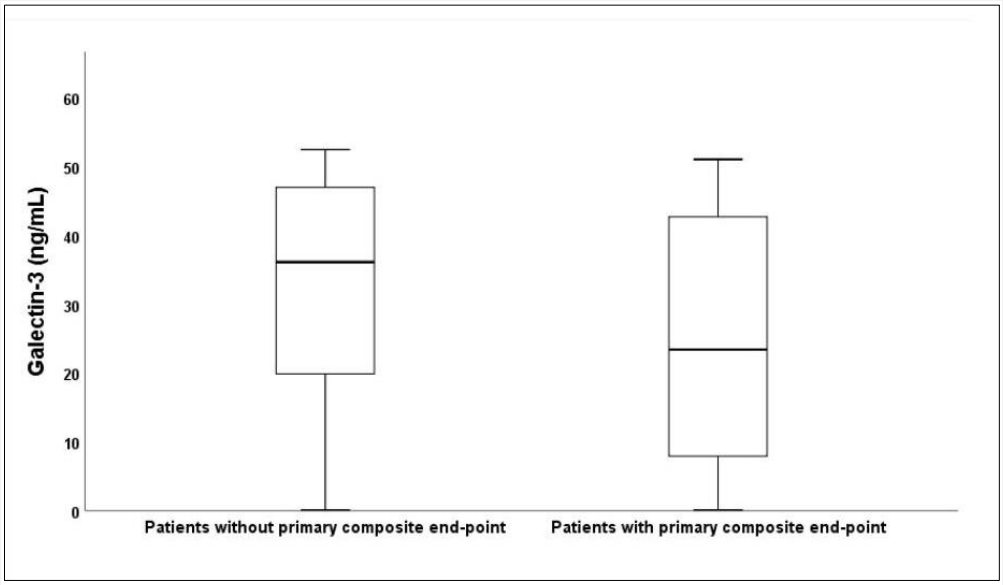

Figure 3. Serum galectin-3 levels according to primary composite endpoint of the study group.

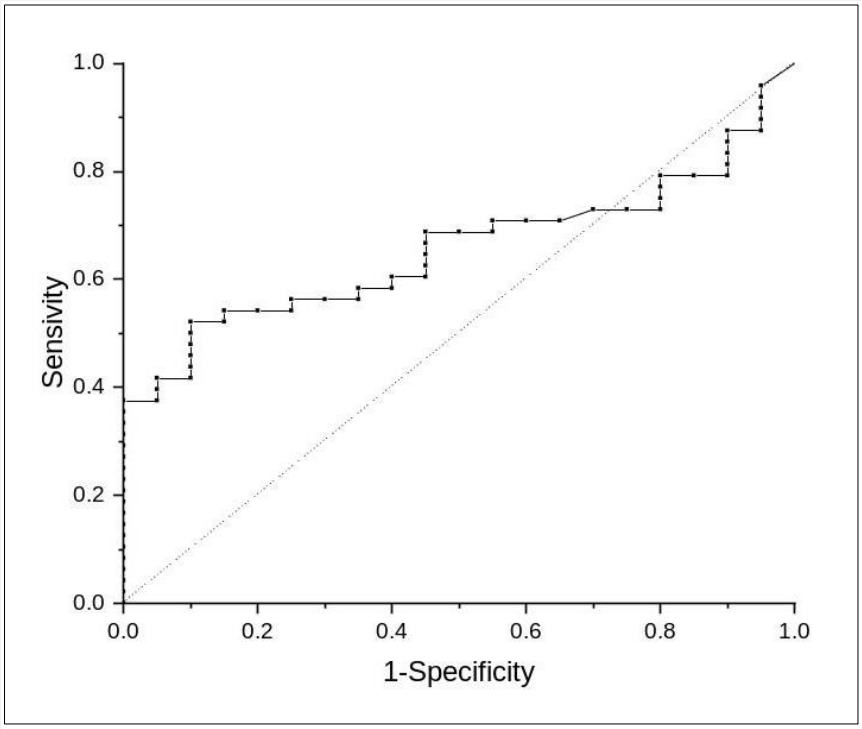

Figure 4. The receiver operating characteristics (ROC) curve analysis for predicting pneumonia severity by galectin-3 at hospital admission.

obtained in terms of diabetes mellitus, hypertension, heart rate, and systolic blood pressure between the groups.

Clinical outcomes of the study group

Hospital stay duration was significantly longer in the patients with primary composite endpoints in comparison to those without (25 [7 - 51] versus 13 [2 - 55] days, respectively, $p < 0.001$) (Table 2). In our study, 12 patients (17.6%) died and 28 patients (41.2%) were admitted to the ICU during hospitalization period (Table 3). Ultimately, 32 patients (47%) reached the primary composite endpoint.

Treatment regimens

The treatments received by the patients were significantly different between the groups (supplementary Table 1, supplementary Table 2). Of the total population, 33 (48.5%) patients required oxygen treatment via a face mask, 22 (32.4%) required high flow nasal cannula or non invasive mechanical ventilation, and 13 (19.1%) underwent orotracheal intubation.

The laboratory findings of the groups at admission and during hospitalization are shown in Table 2 and supplementary Table 2. The serum galectin-3 concentrations were significantly different between the patients with and without primary endpoints (24.65 ± 17.7 vs. 33.38 ± 15 ng/mL, $p = 0.034$, respectively) (Table 2, Figure 3).

The number of patients who developed acute renal failure and myocardial injury during hospitalization were significantly different between the groups ($p = 0.015$, $p < 0.001$, respectively) (supplementary Table 2).

In the correlation analysis, no significant positive correlations were obtained between the serum galectin-3 concentrations, pro-BNP, and hs-TnT in patients with and without primary composite endpoints (Table 4A, B). Multivariate regression analysis showed that lower concentrations of galectin-3 and SpO_2 were independently related to the primary composite endpoints (OR = 0.951, 95% CI 0.907 to 0.996, $p = 0.035$; OR = 0.862, 95% CI 0.764 to 0.973, $p = 0.017$, respectively; Table 5A). Additionally, in the regression analysis, increased levels of LDH, fibrinogen, and galectin-3 and lower levels of SpO_2 were found to be independent predictors of severe pneumonia ($p = 0.019$, $p = 0.048$, $p = 0.016$, $p = 0.017$, respectively) (Table 5B).

According to the ROC analysis, the cutoff value for predicting severe pneumonia at hospital admission was 38.76 ng/mL for serum galectin-3 concentration with sensitivity of 52.1% and specificity of 90% ($p = 0.044$, AUC 0.656, 95% CI 0.530 - 0.782) (Figure 4). The serum galectin-3 concentration was significantly lower in patients who died compared with survived ($p < 0.001$). Regarding ICU admission, intubation, and adverse events during hospitalization, no statistically significant differences were found in the serum galectin-3 concentrations between the groups (Table 6).

DISCUSSION

Currently, the lack of an effective anti-viral therapy has made biomarkers attractive for assessing the disease severity and predicting the prognosis of COVID-19 disease. Galectin-3, which plays key roles in various inflammatory disease processes, has been used as an important inflammatory and profibrotic biomarker. However, there is limited data about the role of galectin-3 in the pathogenesis of COVID-19. In this study, the role of the serum galectin-3 concentration in predicting the prognosis and severity of COVID-19 was investigated. According to our findings, higher serum galectin-3 concentrations have been found in patients with severe pneumonia in comparison to those with mild/moderate pneumonia and is an independent predictor for the severity of the disease. The cutoff value of serum galectin-3 for predicting severe pneumonia at hospital admission was 38.76 ng/mL with a sensitivity of 52.1% and a specificity of 90%. This cutoff value distinguishes severe pneumonia from mild-moderate ones. Alvarez et al. also reported higher galectin-3 levels at admission related with severe outcomes similar to our findings, and their cutoff value for galectin-3 was 30.99 ng/mL [13]. They also emphasized that serum albumin and CRP together with galectin-3 increased the ability to predict severe outcomes with mortality [13]. Our findings were also similar to that of Kazancioglu et al., who reported that galectin-3 concentrations increased in severe pneumonia patients [14]. In another recent study, galectin-3 was reported to be a potential prognostic biomarker of COVID-19 [5]. Garcia-Revilla reported that elevated concentrations of galectin-3 can play important roles in the inflammatory response associated with disease severity and subsequent lung fibrosis in COVID-19 patients [6]. Galectin-3 has been shown to exhibit its proinflammatory action by stimulating macrophages [14]. In another study, Cabala et al. also found higher serum galectin-3 concentrations in patients with COVID-19 pneumonia; however, no significant differences were reported between the patients with COVID-19 and the healthy controls [15].

We also found higher CRP, LDH, ferritin, procalcitonin, proBNP, and fibrinogen levels together with high galectin-3 levels in patients with severe pneumonia compared with mild/moderate pneumonia. However, only high levels of LDH, fibrinogen, and galectin-3 were associated with severe pneumonia using multivariate regression analysis. Kazancioglu et al. also found no significant correlation between serum galectin-3 concentrations and inflammatory biomarkers similar to our results [14]. However, in the study of Cabala et al., significant correlations between galectin-3 concentrations and inflammatory biomarkers were reported [15]. The differences between the active period of the disease, the times of blood collection, and patients' characteristics may explain the different results between studies.

Also, during hospitalization, the development of adverse effects including cardiac failure, and acute renal

failure were found higher in patients with severe pneumonia leading to poor out-come in our study.

Galectin-3 is expressed both in alveolar epithelial and endothelial cells and is secreted by inflammatory cells, mainly macrophages, endothelial cells, and epithelial cells. It has been suggested that higher galectin-3 concentration is specifically indicative of parenchymal and vascular damage of lung during COVID-19 disease [6, 15]. Galectin-3 is also potentially a profibrotic marker and regulates the activity of fibroblasts and macrophages in inflamed tissues [16]. Even in different cancer types, an association between high galectin-3 levels and cancer stage, progression, and metastases were demonstrated [17].

Galectin-3 induces cytokine release in human cells, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [18]. It has been reported to regulate its pro-inflammatory effect by activating transcription factor NF-κB, inducing the TNF-α and IL-6 and regulating immune cell function, cell growth, and apoptosis [15,19]. An increase in galectin-3 concentrations has been suggested to lead to release of pro-inflammatory cytokines and may trigger the cytokine storm associated with severity of COVID-19 [20]. As shown in various studies, in the pathogenesis of the SARS-CoV-2 infection, pro-inflammatory cytokines and chemokines play a key role causing a cytokine storm presented with high IL-6, TNF-α, IL-1 beta, and IFN-gamma concentrations [13, 21,22] so that COVID-19 may lead to many comorbidities, including endothelial damage, coagulopathies, thromboembolic events, multiple organ failure, acute respiratory distress syndrome (ARDS), and also cardiovascular complications [23,24]. The diffuse alveolar damage of the lung in ARDS happens due to the acute severe inflammation of the lung leading to severe outcomes [13]. Nevertheless, the other most serious complication of SARS-CoV-2 is myocardial injury caused by hyperinflammatory response syndrome, resulting in myocardial dysfunction and acute heart failure [21,23, 24]. Heart failure is a significant cause of death in patients with COVID-19 and it may occur as a result of direct myocardial injury by viral infiltration, direct or indirect inflammatory damage, hypoxemia, and an increase in thromboembolic events due to endothelial damage, hypercoagulability, or plaque instability [21, 23,24]. Myocardial injury is associated with a worse prognosis and a higher rate of mortality in COVID-19, and it causes an increase in serum concentrations of troponin and pro-BNP [23,25]. In both conditions, galectin-3 has emerged to mediate the inflammatory response by activating immune cells and inducing the inflammatory cytokines [13]. Revilla et al. also demonstrated that increased galectin-3 in proliferative T cells is associated with severe conditions of COVID-19 patients [6]. Additionally, the mediating role of galectin-3 in viral adhesion has been revealed [6]. It was suggested that the structural similarities of galectin-3 with the SARS-CoV-2 spike protein and its receptor ACE-2, which are required for virus-host interaction, may be effective in the role of galectin-3 in the SARS-CoV-2-host interaction [6].

There are also studies to show the relationship of increased expression of galectin-3 with cardiac fibrosis [10,26,27], as an active contributor to cardiac remodeling and the development of heart failure [28]. An increased concentration of galectin-3 has been shown to stimulate cardiac fibroblast proliferation, collagen deposition, and ventricular dysfunction [19]. Also, some studies have explained the association of high galectin-3 levels with COVID-19 disease progression and lung fibrosis [6]. In our study, we also evaluated patients who developed adverse effects during hospitalization period including myocardial injury, acute renal or liver failure and compared serum galectin-3 concentrations. However, no significant alterations in serum galectin-3 concentrations were obtained between patients. Based on our findings, serum galectin-3 concentrations did not differ between the patients with and without myocardial injury, and no significant correlations were obtained between the galectin-3 concentrations and the myocardial injury biomarkers, including pro-BNP and troponin. Although galectin-3 expression is rapidly induced during myocardial injury, several studies pointed out the conflicting results of the peak time of galectin-3. In an experimental study, galectin-3 mRNA levels have been reported to reach its peak value at 14 days [29], while in another study the peak levels of galectin-3 were determined at 1-week post-MI [30]. However, our blood samples were collected within the first 48 hours of hospital admission for measurement of serum galectin-3 concentrations. Also, the small sample size of our study may have caused these results.

We also examined the predictors of the severe clinical outcomes consisting of the need of ICU treatment and in-hospital mortality. As an interesting finding, we obtained lower galectin-3 concentrations in patients who died from COVID-19 than those who survived, and the lower galectin-3 and SpO_2 levels were detected to be the independent predictors of the primary clinical outcomes. Contrary to our results, Portacci et al. found that high serum galectin-3 concentrations were associated with a greater risk of death [5], and they reported that Galectin-3 was a better predictive marker for mortality. However, they found a similar cutoff value of 35.3 ng/mL as our results to determine the high risk of ICU admission, disease severity, and mortality. The inconsistency between our results and those of Portacci et al. might be explained with the small sample size of our cohort of patients who died or the potentially confounding factors [5]. First, we suggested that, in the initial stages after lung injury, expression of galectin-3 could be protective, but over time, galectin-3 overexpression may result in the excessive development of fibrosis resulting in adverse fibrotic conditions, ventricular dysfunction and, consequently, heart failure. Therefore, we assume that it may be essential to give a “window of opportunity” for the potential effects of galectin-3 in clinical settings.

As a result, we presented the results of galectin-3 in different clinical situations of COVID-19 in hospitalized patients, herein. Although sample size of our study is limited, it may be suggested that our study made an important contribution to clinical practice about COVID-19 by emphasizing the importance of galectin-3 in the development of complications and the severity of disease.

Strengths of study

Our study has some strengths. First, we only included hospitalized patients with COVID-19. Second, we compared serum galectin-3 concentrations between the groups according to the severity of pneumonia and also the primary clinical outcomes including the need for ICU admission and the mortality. We also examined the predictors of pneumonia severity and prognosis. As distinct from other studies, the developing adverse effects during hospitalization period including myocardial injury, acute renal or liver failure were evaluated, and serum galectin-3 concentrations were compared between patients. Also, the correlations between galectin-3 and pro-BNP and troponin were investigated.

Study limitations

Our study has some limitations. First, as a single-center study, a multicenter analysis will be needed to increase the discriminative role of this marker. In addition, the small sample size of the study and the deficiency to measure multiple galectin-3 concentrations during the hospitalization period of patients are the main limitations of the study. Finally, we could not compare our results with healthy controls, instead, we compared severe cases with mild/moderate cases.

CONCLUSION

Circulating galectin-3 could be a useful biomarker for identifying severe pneumonia and disease outcomes in COVID-19 patients. However, possible confounding factors, including the sample collection time and multiple samplings, should be taken into consideration to increase the predictive power of galectin-3 for prognosis and severe outcomes of COVID-19. Further studies with larger sample sizes are needed to confirm our findings.

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The authors report no conflict of interest.

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