Assessment of Galectin-1, Galectin-3, and PGE2 Levels in Patients with COVID-19

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Running Title: Galectin-1, Galectin-3, and PGE2 levels in COVID-19.
SUMMARY

It is important to determine the inflammatory biomarkers in the severity of Coronavirus Disease 2019 (COVID-19) with the emergence of the pandemic. Galectins and prostaglandins play important roles in the regulation of immune and inflammatory responses. Therefore, this study aimed to investigate Galectin-1 (Gal-1), Galectin-3 (Gal-3), and prostaglandin E2 (PGE2) levels in patients with COVID-19. Gal-1, Gal-3, and PGE2 serum concentrations were measured using enzyme-linked immunosorbent analysis (ELISA) on 84 COVID-19 patients (severe=29 and nonsevere=55) and 56 healthy controls. In this study, the increased levels of Gal-1 (median, 9.86, 6.35, 3.67 ng/ml), Gal-3 (median, 415.31, 326.33, 243.13 pg/ml) and PGE2 (median, 193.17, 192.58, 124.62 pg/ml) levels were found in patients with COVID-19 than healthy controls (p<0.001 for all). In the severe group, Gal-3 levels were higher while there were no differences in Gal-1 and PGE2 levels (p=0.011, p=0.263, p=0.921, respectively). There was a positive correlation between serum Gal-1 and Gal-3 levels (ρ=0.871, p<0.001). Gal-3, C-reactive protein, lymphocyte count, and age were found as independent predictors of the disease severity (p=0.002, p=0.001, p=0.007, and p=0.003, respectively). With the emergence of effective drug needs in the COVID-19 pandemic, differentiation of severe disease is important. Gal-3 could be a potential prognostic biomarker of COVID-19.

INTRODUCTION

The emergence of Coronavirus Disease 2019 (COVID-19) pandemic threat to human health around the world. Although most of the patients have a milder clinical course, some of them have severe disease. Evaluation and management of COVID-19 are linked to the severity of the disease. Therefore, it has great importance to determine the predictive indicators of severe
Numerous studies have shown increased blood concentrations of inflammatory markers in severe COVID-19. The role of different inflammation parameters in the disease of COVID-19 has been investigated especially lymphocyte count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP), D-dimer (2-4). Studies suggest CRP levels are a strong indicator to reflect the presence and severity of COVID-19. Biomarkers currently play important roles in research to understand COVID-19, including predicting the severity and monitoring the prognosis of COVID-19 (5). Some biomarkers such as serum amyloid A (SAA), presepsin have been determined in COVID-19 as severity indicators (6,7). Some studies mentioned about galectin inhibitors may an option in COVID-19 treatment (8,9). Then, the authors have been curious about the usefulness of galectin levels in the prognosis of the disease. Galectins, a family of β-galactoside-binding lectins, play multiple important roles in virus infections. Therefore, the endogenous effects of galectins in host-virus regulation remain valuable to investigate. Galectins can be sub-divided into three groups defined by structural differences. Galectin-1 (Gal-1; a prototype galectin is the first identified in the galectins family) and Galectin-3 (Gal-3; is uniquely a chimera-type galectin) play a key regulator of immune responses. Gal-1 and Gal-3 are up-regulated in response to infections (10-12). Revilla et al. presented evidence on the potential role of Galectin-3 in COVID-19 in the regulation of the inflammatory response and infection progression (13). At similar times, some studies have been published about the relation between prostaglandins (PGs) especially prostaglandin E2 (PGE2) and COVID-19 (14,15). Evidence a role for PGE2, generated by cyclooxygenase-2 (COX-2), in the regulation of viral replication and modulation of inflammatory responses to viruses (16). Robb et al. put forward elevated levels of PGE2 may enhance severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cell entry (15). Hong et al. proposed that PGE2 may be a key in the pathology of COVID-19 and COX-2 is the critical target for the treatment of COVID-19 (17).
In this research, the authors explore the usefulness of galectins and PGE2 as biomarkers in COVID-19. The levels of Gal-1, Gal-3, and PGE2 were measured and compared to other inflammation indicators in patients with COVID-19. Besides, the results were evaluated with the severity of the disease and compared to healthy controls.

In the absence of currently proven, effective anti-viral therapy, biomarkers have been important to estimate the disease severity. To the best of our knowledge, this was the first study to investigate Gal-1 and Gal-3 levels in patients with COVID-19.

MATERIALS AND METHODS

Study design and participants

The present study retrospectively enrolled 84 confirmed COVID-19 patients who were hospitalized in a tertiary hospital from 30 March to 30 May 2020. The diagnosis was confirmed by detecting SARS-CoV-2 RNA in oro-nasopharyngeal swab samples. Also, 58 healthy controls without any chronic disease and respiratory symptoms were recruited for the control group.

Demographic data and laboratory values were extracted from electronic medical records and patients’ files. The following variables were recorded for each COVID-19 patient: age, sex, severity assessment on admission, laboratory findings. All laboratory results of the patients within 24 hours of admission were recorded. A complete blood count (CBC) was performed using the ADVIA 2120 Hematology System (Siemens Healthcare Diagnostics, Erlangen, Germany). Biochemical parameters were measured using Atellica Solution Immunoassay & Clinical Chemistry Analyzers (Siemens Healthcare Diagnostics, Erlangen, Germany). Prothrombin time (PT), activated partial thromboplastin time, international normalized ratio (INR), and D-dimer was analyzed using the Sysmex CS-5100 System (Siemens Healthcare
Diagnostics, Erlangen, Germany). All laboratory parameters except IL-6 were routinely obtained for all patients. IL-6 was measured in 38 patients.

On admission, patients with COVID-19 were categorized into two groups (nonsevere and severe illness) according to the National Institutes of Health (NIH) classification based on disease severity (18).

The severe illness was defined as:

– Respiratory frequency >30 breaths per minute, SpO2 <94% on room air at sea level, a ratio of the arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300, or lung infiltrates >50%.

The primary outcome of the present study was evaluated to the Gal-1, Gal-3, PGE2 levels, and other laboratory parameters for distinguishing the predictors of severe prognosis. Patients diagnosed with COVID-19 in the general ward were included throughout the study period. Follow-up of 84 patients with COVID-19, the intensive care unit (ICU) admission was needed in 19 patients (22.6%), death occurred in 7 patients in ICU patients (36.8%). The hospital-fatality rate was 8.3% for 84 patients. All other patients were clinically improved and discharged.

Approval from the ethics committee of The Ankara City Hospital was obtained for this study (confirmation date and number: 21.05.2020/ E1-20-619). This study was conducted by the principles of the Declaration of Helsinki.

**Blood Collection and Quantification**

Blood samples were collected into yellow top blood tubes without anticoagulant (BD Vacutainer®, Becton, Dickinson and Company, NJ) within 24 hours of admission. All serum
samples were centrifuged in the cold at 1500 rpm for 10 min, the supernatant was taken into Eppendorf tubes and kept frozen at -80°C.

On the day of analysis, the serum samples were thawed by letting them stand at room temperature for at least 1 h. After inverting samples 10 times, analyses were performed within 4 h from the start of thawing. By the manufacturer's protocol, Galectin 1, Galectin 3, and PGE2 serum concentrations were measured using enzyme-linked immunosorbent analysis (ELISA) kits (Bioassay Technology Laboratory, Shanghai, China; Bioassay Technology Laboratory, Shanghai, China; Cloud-Clone Corp, Shanghai, China, respectively).

Statistical Analysis

All statistical calculations were made using the “SPSS for Windows version 26” software program (IBM Corporation, IL). Comparisons for categorical variables were executed using the Pearson chi-square test or Fisher’s exact test. Kolmogorov-Smirnov test was performed to check the normality of the continuous variables. Differences between the two groups were compared using the Mann-Whitney U test. Kruskal Wallis test was used for comparisons of more than two groups and the significant (p<0.05) results from the Mann-Whitney test (with Post hoc Bonferroni correction) were performed. Correlation analyses were performed using the Spearman correlation coefficient. Multivariate logistic regression analysis (The Forward LR) was performed which found significant during univariate logistic regression analysis were selected as independent variables to define the independent predictors of the disease severity. The odds ratio (OR) was calculated for significantly associated variables. Statistical significance was defined as p<0.05.

RESULTS

In total, 84 patients with COVID-19 and 56 healthy controls participated in the
study. Gal-1, Gal-3, and PGE2 levels of healthy controls and patients with COVID-19 are shown in Table 1.

Gal-1 levels were significantly higher than healthy controls in both nonsevere and severe groups (p<0.001, p<0.001; respectively); however there was no statistically significant difference between severe and nonsevere groups (p=0.263) (Table 1 and Fig. 1a).

Gal-3 levels were significantly higher than healthy controls in nonsevere groups and severe groups (p<0.001, p<0.001; respectively). The severe group had higher Gal-3 levels than nonsevere groups ( p=0.011) (Table 1 and Fig. 1b).

PGE2 levels were significantly higher than healthy controls in both nonsevere and severe groups (p<0.001, p<0.001; respectively); however there was no statistically significant difference between severe and nonsevere groups (p=0.921) (Table 1 and Fig. 1c).

Univariate logistic regression analysis was performed for the severity of COVID-19. Gal-1 ([OR]:1.04; 95% CI: 1.01-1.07, p=0.024) and Gal-3 ([OR]:1.1, 95% CI: 1.01-1.2, p=0.006) were significantly associated with the disease severity. But there was no relation between PGE2 ([OR]: 1.00; 95% CI: 0.98-1.03, p=0.911) and the disease severity.

Table 2 summarizes the correlations between galectins and PGE2 levels, and other laboratory parameters. Across all patients with COVID-19, there was a statistically significant positive correlation between serum Gal-1 and Gal-3 levels (ρ = 0.871, p < 0.001). When the groups were considered individually, serum Gal-1 and Gal-3 levels showed significant correlations both in the severe and nonsevere groups (ρ = 0.874, p <0.001 and ρ = 0.829, p <0.001, respectively). In the correlation analysis, there was no significant correlation between PGE2 and galectins. PGE2 levels were found to be negatively correlated with lymphocytes and monocytes (ρ= -0.217 p= 0.047; ρ= -0.267 p= 0.014; respectively). There were no statistically
significant correlations between serum CRP and serum Gal-1 or Gal-3 or PGE2 levels (Gal-1: ρ= 0.076; Gal-3: ρ= 0.032; PGE2: ρ= - 0.099, p >0.05 for all).

Compared with the nonsevere group, the severe group was significantly older (45.73±14.77 vs 60.76±14.46 years ; p < 0.001). The optimal cut-off value of age was found to be >54 when the ROC curve analysis was used (p < 0.0001, AUC: 0.762, sensitivity: 65.52, specificity: 76.36). The presence of chronic obstructive pulmonary disease (COPD) and lung infiltrates >50% were more common in the severe group significantly (p=0.042, p<0.001; respectively). Higher NLR, PLR, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine kinase (CK), interleukin-6 (IL-6), CRP, ferritin, D-dimer, procalcitonin, and lower lymphocytes, monocytes, red blood cell (RBC), hemoglobin were found in the severe group than nonsevere group (Table 3 and 4).

Logistic regression analysis showed that Gal-3, CRP, age, and lymphocyte were identified as independent predictors of the disease severity (Table 5).

DISCUSSION

This study investigated the Gal-1, Gal-3, and PGE2 (one of the most studied PGs) levels in the blood of patients with COVID-19. Our results show an increase in Gal-1, Gal-3, and PGE2 levels in patients with COVID-19 than healthy controls. Also, in severe patients, Gal-3 levels were found to be higher than the nonsevere group. The Gal-3 level was found an independent predictor for the severity of the disease.

The pathogenesis and severity of COVID-19 remain unknown, but many different methods, including routine laboratory parameters, biomarkers, genetic analyses in patients have been investigated. Compared to nonsevere patients, severe COVID-19 patients had remarkably elevated inflammatory biomarkers, such as CRP, D-dimer, NLR, PLR, lymphocytes-to-CRP ratio, and cytokines, for example, IL-1β, IL-6, and TNF-α (4,19-21). In the study, older age,
higher AST, LDH, CK, PT, INR, D-dimer, CRP, IL-6, ferritin, procalcitonin, NLR, PLR, and lower lymphocytes and monocytes levels were found in the severe group. Older age, CRP, and lymphocyte count were independent predictors for COVID-19 severity according to this study. A significant reduction in lymphocytes may play a role in the pathogenesis and contribute to the progression of severe COVID-19 (22). SAA can promote inflammatory response and Li et al. reported SAA, CRP, and lymphocytes were valuable indicators in evaluating the severity and prognosis of COVID-19 (6). Liu et al. revealed that age (>58), lymphocyte count, and IL-6 level were independent risk factors in patients with COVID-19 (2).

It was previously mentioned that a deficiency in the arachidonic acid (AA) pathway can make humans susceptible to COVID-19 and PGs levels should be measured in patients with COVID-19 (14). Hong et al. showed that the PGE2 levels in urine samples of COVID-19 patients were significantly higher than healthy individuals (170±40 ng/ml vs 18.8±3.8 ng/ml, p<0.01). They also used a COX-2 specific inhibitor (celecoxib) in the treatment of COVID-19 patients (n=25) and showed improved outcomes after discontinuation (17). It was mentioned that COX-2 inhibition might be a valuable treatment strategy considering the lack of definitive treatment and the importance of immunomodulation in COVID-19 (23).

Glucocorticoids, which inhibit PGE2 synthesis with other effects, are currently used in severe disease (24). In the study, PGE2 levels were found to be significantly higher than healthy controls in COVID-19 patients. Also, PGE2 levels were found to be negatively correlated with lymphocytes and monocytes. PGE2 is one of the lipid mediators produced in inflammatory reactions and can inhibit T and B lymphocyte proliferation (16). Lymphocyte subsets alteration such as the decrease of T, B lymphocytes, and lower monocytes were seen in severe COVID-19 patients. Pence reported that monocytes, which are macrophage precursors, would be principal players of cytokine storm in COVID-19 (21,25,26).
It is a query that Galectin inhibitors may an option in the treatment of COVID-19. Reville et al. provided that measuring plasma galectin-3 as a prognosis biomarker for COVID-19 patients and inhibition of galectin-3 represent a new therapeutical approach (13). Furthermore, a gal3 inhibitor (TD139, Galecto Biotech) has already been used with idiopathic pulmonary fibrosis in phase IIb clinical trial (NCT03832946) (27). Higher Gal-3 concentrations were found to be associated with lower lung volumes (28). The burden of fibrotic lung disease following SARS-CoV-2 infection is likely to be high; therefore, the antifibrotic treatment strategy may be considered as part of the treatment in severe COVID-19 (29).

Inflammatory processes and cytokine storms have been identified as a potential contributing factor to the severity of COVID-19 (30). The binding of extracellular Gal-1 or Gal-3 to the surface of airway epithelial cells can be severely dysregulated and potentially leading to hypercytokinemia (31). In immunomodulation, galectin-1 exhibits antiinflammatory activity by inhibiting leukocyte infiltration and migration, whereas galectin-3 displays pro-inflammatory activity by increasing macrophage survival (12,32). In the study, there was no significant difference in Gal-1 levels between severe and nonsevere groups, while Gal-3 levels were found to be higher in the severe group than the nonsevere group. When evaluated with the severity of the disease; Gal-1 and Gal-3 levels were significantly associated with the severity. Also, a correlation was found between these galectins. However, the Gal-3 level was an independent predictor for the severity according to our results. It might be explained by the excessive inflammatory response which promotes severe disease. An increase in Gal-3 levels leads to a dysregulated expression and release of pro-inflammatory cytokines (33). It was reported that SARS-CoV-2 spike protein shares some similarities with Gal-3. Gal-3 may boost the cytokine storm associated with severe COVID-19 (34).
In the present study, Gal-3, C-reactive protein, lymphocyte count, and age were found as independent predictors of the disease severity. However, no correlation was found between Gal-3 levels and CRP or lymphocyte count. It may be explained by many differences and factors in innate and adaptive immunity or small sample size. Gal-3 is thought to be associated in COVID-19 with macrophage-related hyper inflammation, virus entry to host cell, and lung fibrosis (13, 33). We still need to clarify the pathophysiology and immunity of the COVID-19.

This study presents some limitations. The first is that the sample size was relatively small, and our findings may not be generalizable. Second, it is a cohort study with a retrospective design. Third, this study evaluated predictors of the severity but did not examine the predictors of mortality. Last, parameters were performed only at the time of admission. If parameters are measured at multiple time points, the strength of results to predict the disease severity may increase.

In conclusion, the role of galectins and PGs in COVID-19 is an important research topic.

In this study, the increased levels of Gal-1, Gal-3, and PGE2 levels were found in patients with COVID-19. Gal-3 levels were higher in severe patients while there were no differences between Gal-1 and PGE2 levels. Gal-3 is an independent predictor of the disease severity.

As it is known, there is not a precise treatment agent for COVID-19 up to date. In light of the current pandemic and the emergence of effective drug needs, differentiation of severe disease than the milder clinical course is important. Gal-3, in particular, could be a potential prognostic biomarker of COVID-19.

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**Conflict of interest**

The authors declare no conflicts of interest.

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FIGURE LEGENDS

Fig. 1. a) Galectin-1 levels in patients with COVID-19 and healthy controls. b) Galectin-3 levels in patients with COVID-19 and healthy controls. c) PGE2 levels in patients with COVID-19 and healthy controls.
|                  | Median (Min.-Max.) |       | X²  | P      |
|------------------|--------------------|-------|-----|--------|
|                  | Nonsevere (n=55)   | Severe (n=29) | Healthy (n=56) |       |        |
| Galectin-1 (ng/mL) | 6.35 (0.33-54.75)  | 9.86 (0.47-95.33) | 3.67 (0.23-20.45) | 17.401 | <0.001 |
| Galectin-3 (pg/mL) | 326.33 (100.09-1271.04) | 415.31 (122.81-1622.23) | 243.13 (166.57-380.41) | 47.591 | <0.001 |
| PGE2 (pg/mL)      | 192.58 (107.09-222.16) | 193.17 (151.76-213.29) | 124.62 (43.6-224.59) | 24.676 | <0.001 |

Kruskal Wallis $H$ analysis.

Table 2. Correlation coefficients between Galectin-1, Galectin-3, and PGE2 levels, and other laboratory parameters in patients with COVID-19.

|                      | Galectin-1 (ng/mL) | Galectin-3 (pg/mL) | PGE2 (pg/mL) |
|----------------------|--------------------|--------------------|--------------|
|                      | ρ      | p                  | ρ      | p                  | ρ      | p                  |
| Galectin-1 (ng/mL)   | 1.000  |                    |        |                    |        |                    |
| Galectin-3 (pg/mL)   | 0.871  | <0.001             | 1.000  |                    |        |                    |
| PGE2 (pg/mL)         | 0.075  | 0.447              | 0.144  | 0.141              | 1.000  |                    |
| Leucocytes (/μl)     | -0.128 | 0.247              | -0.048 | 0.666              | -0.212 | 0.053              |
| Neutrophils (/μl)    | -0.086 | 0.438              | -0.007 | 0.946              | -0.140 | 0.204              |
| Lymphocytes (/μl)    | -0.144 | 0.191              | -0.098 | 0.375              | -0.217 | 0.047              |
| Monocytes (/μl)      | -0.085 | 0.440              | -0.108 | 0.330              | -0.267 | 0.014              |
| Platelets (10^9/μl)  | -0.121 | 0.273              | -0.179 | 0.104              | -0.199 | 0.070              |
| NLR                  | -0.017 | 0.875              | 0.046  | 0.675              | 0.096  | 0.387              |
| PLR                  | -0.001 | 0.995              | -0.024 | 0.830              | 0.081  | 0.464              |
| RBC (10^{12}/l)      | -0.072 | 0.513              | -0.060 | 0.588              | 0.110  | 0.320              |
| Hemoglobin (g/dl)    | -0.026 | 0.816              | 0.019  | 0.865              | 0.063  | 0.569              |
| CRP (g/l)            | 0.076  | 0.492              | 0.032  | 0.773              | -0.099 | 0.369              |
| D-dimer (mg/l)       | 0.045  | 0.684              | -0.043 | 0.701              | -0.019 | 0.866              |
| APTT (sec)           | -0.058 | 0.620              | -0.059 | 0.612              | 0.004  | 0.973              |
| ALT (U/l)            | 0.076  | 0.490              | 0.079  | 0.475              | -0.003 | 0.976              |
| AST (U/l)            | 0.073  | 0.510              | 0.107  | 0.333              | 0.062  | 0.573              |
| LDH (U/l)            | -0.004 | 0.975              | 0.015  | 0.894              | 0.037  | 0.739              |
| CK (U/l)             | 0.111  | 0.319              | 0.154  | 0.163              | 0.135  | 0.224              |
|                         | Spearman’s correlation analysis. PGE2; Prostaglandin E2. NLR; Neutrophil-to-lymphocyte ratio. PLR; Platelet-to-lymphocyte ratio. RBC; Red blood cells. CRP; C-reactive protein. APTT; Activated partial thromboplastin time. ALT; Alanine aminotransferase. AST; Aspartate aminotransferase. LDH; Lactate dehydrogenase. CK; Creatinine kinase. IL-6; Interleukine-6. |
Table 3. Demographic and clinical characteristics of patients with COVID-19.

|                                | Nonsevere (n=55) | Severe (n=29) | Total (n=84) | Z/X^2 | P       |
|--------------------------------|------------------|---------------|--------------|-------|---------|
| Age (years)                    | 46 (17-81)       | 63 (25-83)    | 51 (17-83)   | 3.939 | <0.001  |
| Length of hospitalization (days)| 7 (1-15)         | 15 (6-70)     | 8.5 (1-70)   | -5.079| <0.001  |
| Male gender                    | 31 (56.4)        | 17 (58.6)     | 48 (57.1)    | 0.039 | 0.842   |
| Diabetes                       | 7 (12.7)         | 7 (24.1)      | 14 (16.7)    | 1.78  | 0.223   |
| Hypertension                   | 12 (21.8)        | 11 (37.9)     | 23 (27.4)    | 2.479 | 0.115   |
| Coronary artery disease        | 2 (3.6)          | 4 (13.8)      | 6 (7.1)      | 2.953 | 0.175   |
| Chronic obstructive pulmonary disease | 4 (7.3)   | 7 (24.1)      | 11 (13.1)    | 4.746 | 0.042   |
| Lung infiltrates >50%          | 27 (50)          | 26 (89.7)     | 53 (63.9)    | 12.855| <0.001  |

Pearson’s X^2, Fisher-Exact test. Mann-Whitney U analysis. Data are n (%) or median (min-max).
Table 4. Laboratory parameters of patients with COVID-19.

|                    | Normal Range | Median (Min.-Max.) |    |    |    |    |    |    |    |
|--------------------|--------------|-------------------|----|----|----|----|----|----|----|
|                    |              | Nonsevere (n=55)  | Severe (n=29) | Total (n=84) |    |    | Z   |    |
| Leucocytes (/μl)   | 4200-10800   | 5680 (2150-14600) | 6220 (2810-16500) | 5890 (2150-16500) | -0.174 | 0.862 |
| Neutrophils (/μl)  | 1700-7900    | 3520 (230-11000)  | 4660 (1550-13770) | 3765 (230-13770) | -1.868 | 0.062 |
| Lymphocytes (/μl)  | 1500-4500    | 1400 (700-2660)   | 960 (370-2170)   | 1275 (370-2660)  | -4.229 | <0.001 |
| Monocytes (/μl)    | 100-900      | 410 (160-970)     | 300 (120-1020)   | 390 (120-1020)   | -2.085 | 0.037 |
| Platelets (10⁹/μl)| 160-385      | 227 (127-574)     | 180 (117-445)    | 221.5 (127-574)  | -1.628 | 0.104 |
| NLR                |              | 2.31 (0.2-9.14)   | 4.38 (1.36-14.78) | 2.94 (0.2-14.78) | -4.013 | <0.001 |
| PLR                |              | 0.15 (0-0.46)     | 0.22 (0.06-0.55) | 0.18 (0-0.55)    | -2.982 | 0.003 |
| RBC (10¹²/l)       | 4-5.65       | 4.91 (3.14-378)   | 4.68 (3.32-5.79) | 4.85 (3.14-378)  | -2.343 | 0.019 |
| Hemoglobin (g/dl)  | 13-16.6      | 14.3 (4.19-17.1)  | 13.2 (7.4-17.4)  | 13.65 (4.19-17.4) | -1.662 | 0.097 |
| CRP (g/l)          | 0-0.005      | 0.004 (0.001-0.164)| 0.066 (0.003-0.188) | 0.013 (0.001-0.188) | -5.018 | <0.001 |
|                          | Nonsevere Group | Severe Group       | Mann-Whitney U analysis | p values indicate differences between nonsevere and severe groups. |
|--------------------------|-----------------|--------------------|-------------------------|------------------------------------------------------------------|
| **D-dimer (mg/l)**       | < 0.55          | 0.44 (0.05-2.35)   | 0.62 (0.19-3.56)        | 0.51 (0.05-3.56)                                                 | -2.718 | **0.007** |
| **APTT (sec)**           | 21-32           | 25.1 (19.1-32.4)   | 24.9 (20.3-41.8)        | 25.05 (19.1-41.8)                                                | -0.016 | 0.987    |
| **ALT (U/l)**            | 35              | 27 (3-199)         | 33 (15-226)             | 28 (3-226)                                                       | -1.389 | 0.165    |
| **AST (U/l)**            | 50              | 21 (6-107)         | 35 (14-220)             | 25 (6-220)                                                       | -3.761 | **<0.001** |
| **LDH (U/l)**            | 120-246         | 204 (135-417)      | 304 (160-666)           | 231.5 (135-666)                                                 | -3.674 | **<0.001** |
| **CK (U/l)**             | 32-294          | 79 (14-424)        | 144.5 (30-1286)         | 94 (14-1286)                                                    | -3.795 | **<0.001** |
| **IL-6 (pg/dl)**         | 0-4.4           | 12.45 (2-62)       | 71 (16.5-1000)          | 32.1 (2-1000)                                                   | -3.889 | **<0.001** |
| **Ferritin (μg/l)**      | 10-291          | 98 (3-903)         | 527 (131-24176)         | 149.5 (3-24176)                                                 | -5.395 | **<0.001** |
| **Procalcitonin (μg/l)** | < 0.16          | 0.03 (0.01-0.15)   | 0.1 (0.03-15.85)        | 0.03 (0.01-15.85)                                               | -5.029 | **<0.001** |

NLR; Neutrophil-to-lymphocyte ratio. PLR; Platelet-to-lymphocyte ratio. RBC; Red blood cells. CRP; C-reactive protein. APTT; Activated partial thromboplastin time. ALT; Alanine aminotransferase. AST; Aspartate aminotransferase. LDH; Lactate dehydrogenase. CK; Creatinine kinase. IL-6; Interleukine-6.
| Parameters           | B      | p     | Exp(B) | 95% CI for Exp(B) |
|----------------------|--------|-------|--------|-------------------|
| Galectin-3           | 0.005  | **0.002** | 1.005  | 1.002 - 1.008     |
| Age                  | 0.096  | **0.003** | 1.100  | 1.034 - 1.171     |
| Lymphocytes          | -0.002 | **0.007** | 0.998  | 0.996 - 0.999     |
| C-Reactive Protein   | 0.030  | **0.001** | 1.031  | 1.012 - 1.050     |
| Constant             | -6.102 | **0.007** | 0.002  |                   |
