Research Article

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Prognostic value of total thiol and D-dimer in patients hospitalized with COVID-19

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Abstract

Objectives: The lack of specific treatment for COVID-19 and the fact that the clinical course differs between individuals makes it difficult to predict the prognosis. The aim was to investigate the prognostic value of total thiol, D-dimer, procalcitonin (PCT), ischemia-modified albumin (IMA), and complete blood count (CBC) in patients hospitalized with COVID-19.

Methods: 100 consecutive patients were hospitalized with COVID-19, confirmed by RT-PCR between December 2021-March 2022 and 30 healthy control participated in the study. According to the World Health Organization guideline, two groups were created as critical and non-critical. D-dimer, PCT, IMA, total thiol levels, and CBC were analyzed. Receiver-operating characteristic curves (ROC) were utilized to determine an optimum cut-off value for distinction.

Results: We defined a cut-off value of 1,030 µg/L for D-dimer (Area Under Curve, AUC): 0.691; p=0.001) and 148 µmol/L for total thiol (AUC: 0.749; p<0.001) via ROC analysis. The combination of D-dimer and total thiol reached 65% positive predictive value (PPV) and 80% negative predictive value (NPV).

Conclusions: D-dimer and total thiol may help predict critical patients with COVID-19.

Keywords: COVID-19; D-dimer; ischemia-modified albumin; prognosis; thiol.

Introduction

A new coronavirus, called SARS-CoV-2, continues to threaten human health worldwide [1]. While the disease was initially thought to be associated only with acute respiratory distress syndrome (ARDS), it has many unexplained atypical clinical characteristics, such as coagulopathy and cytokine storm [2].

The angiotensin-converting enzyme 2 (ACE2) receptor, which functions in the entry of SARS-CoV-2 into the cell, is expressed in endothelial and alveolar epithelial cells [3]. Infection of endothelial cells with SARS-CoV-2 and resulting inflammatory responses damage the integrity of the endothelium. As a result, the vascular barrier function is impaired, thrombogenesis is activated, and microcirculation disorders develop [4]. There is a specific sequence of Asp-Ala-His-Lys in the N-terminal region of albumin, and these amino acid residues bind transition metals such as cobalt (II), copper (II), and nickel (II) [5]. This sequence undergoes structural changes, and its capacity to tie transition metals decreases due to endothelial and extracellular hypoxia, acidosis, and free radical damage in the presence of ischemia. Therefore, this albumin is termed ischemia-modified albumin (IMA) [5, 6].

Respiratory viral infections such as COVID-19 induce stimulation of the innate immune response and secretion of inflammatory cytokines. All these processes are associated with the development of oxidative stress, which plays an important role in the pathogenesis of viral infections.

Antioxidants such as N-acetyl cysteine have been incorporated into clinical protocols to treat moderate and severe COVID-19. It is thought that antioxidant treatments can prevent organ and tissue damage caused by cytokine storms and oxidative stress [7]. It is stated that thiol levels, a good indicator of cellular redox, provide an important response to COVID-19 infection [8].

D-dimer is a fibrin degradation product released into the circulation due to fibrinolysis of the blood clot [9]. Researchers have suggested that high D-dimer levels may

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predict disease severity and pulmonary and thromboembolic complications [10].

The lack of specific treatment for COVID-19 and the fact that the clinical course differs between individuals makes it difficult to predict the prognosis. Prognostic markers can identify patients who will have a critical clinical course. Thus, the process can be successfully managed by following the patients closely and planning the necessary treatment early. However, there are few studies investigating thiol levels and IMA in COVID-19.

We planned a study investigating the importance of total thiol, D-dimer, IMA levels, procalcitonin (PCT), and complete blood count (CBC) in predicting patients hospitalized with COVID-19 infection whose clinical course will get worse.

Materials and methods

Study design

One hundred consecutive patients (48 female, 52 male) who applied to Erciyes University Hospital with positive COVID-19, confirmed by RT-PCR between December 2021-March 2022 and 30 healthy individuals were placed in the study. Considering clinical progression, patients hospitalized were divided into two groups, critical (n=28) and severe/non-severe (non-critical) COVID-19 (n=72), concerning the World Health Organization guideline [11]. Critical COVID-19 was defined by the criteria for acute respiratory distress syndrome (ARDS), sepsis, septic shock, or other conditions that would typically require the provision of life-sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor treatment. Severe or non-severe COVID-19 was defined as an absence of any criteria for critical COVID-19 [11].

Patients under 18 years of age with HIV positivity and immunodeficiency diseases were excluded from the study.

The local Clinical Research Ethics Committee approved this study and informed consent was obtained from all participants.

Blood samples were taken into tubes with and without anticoagulants within the first 24 h of hospitalization. CBC was analyzed, and after centrifugation at 2,000×*g* for 10 min, serum PCT and plasma D-dimer values were determined on the same day. Aliquoted serum samples were stored at −80 °C for total thiol and IMA analysis.

Laboratory analysis

D-dimer values were determined by an automatic blood coagulation analyzer (Sysmex CS-2500). PCT and CBC parameters were measured using a Roche Cobas immunoassay analyzer (Roche Diagnostic, Mannheim, Germany) and Sysmex XN 9000 (Sysmex, Kobe, Japan), respectively.

Albumin-adjusted IMA values were used to correct false high IMA results caused by low albumin. The individual's serum albumin level was divided by the population's mean serum albumin level, and the coefficient obtained was multiplied by the IMA value [12]. Total thiol concentrations were determined with a spectrophotometric assay defined by Hu et al. [13].

Statistical analysis

Statistical analyses were performed on SPSS 22.0 package program. Histograms and the Shapiro-Wilk test were used to assess whether the data were normally distributed. Study groups were compared with one-way analysis of variance (ANOVA) and Kruskal–Wallis analysis. Bonferroni was used for post hoc multiple comparisons. Independent samples t-test and Mann-Whitney U test were used to compare continuous variables according to distribution characteristics. The statistics of categorical variables were given as percent (%), and the exact chi-square test was used to compare these variables. Spearman correlation analysis was used to evaluate the relationship between variables. Optimum cut-off values were detected by Receiver-Operating Characteristic (ROC) analysis to distinguish critical and non-critical patients, and the area under the curve (AUC) values were calculated. Logistic regression analysis was applied to define the variables affecting the prognosis. P values below 0.05 were considered statistically significant.

Results

The age, gender distribution, comorbidities, and laboratory findings of the study groups were shown in Table 1. Of the 28 patients classified as critical, 14 (50%) died, of which 9 (64%) were male. All patients who died had comorbidities, and no difference was observed between the critical and non-critical groups regarding comorbidity.

When control, critical, and non-critical COVID-19 groups were compared, significant differences were found for D-dimer, lymphocyte, neutrophil, the ratio of neutrophil to lymphocyte (NLR), total thiol, albumin, and IMA values.

According to post hoc comparisons, total thiol, D-dimer, albumin, and IMA levels showed statistically significant differences between critical and non-critical COVID-19 patients. D-dimer and IMA levels were significantly higher ($p=0.011$ and $p=0.024$, respectively); total thiol and albumin levels were lower in patients with critical COVID-19 than in non-critical patients ($p<0.001$ and $p<0.001$, respectively). Only total thiol levels were similar in healthy controls and non-critical COVID-19 patients (Table 1).

When albumin correction was applied for IMA, a significant difference disappeared between the critical and non-critical COVID-19 patient groups ($p=0.201$). In addition, PCT levels were higher in the critical COVID-19 patient group than in the non-critical ($p=0.013$).

Total thiol concentrations were negatively correlated with age, adjusted IMA, and PCT. D-dimer levels showed a significantly positive correlation with PCT in COVID-19 patients (Table 2).

Decreased total thiol levels were associated with critical illness (Table 3). ROC analysis determined optimal

Table 1: Comparison of control, critical, and non-critical COVID-19 patients' age, gender, comorbidities, and laboratory findings.

Variables		Control (n=30)	Non-critical group (n=72)	Critical group (n=28)	p-Value
Age, years		56.76 ± 9.54	58.98 ± 15.59	62.81 ± 12.18	0.238
Gender, M,F	n, %	15 (50%), 15 (50%)	36 (50%), 36 (50%)	16 (57)%, 12 (43%)	0.521
Comorbidities			51 (71%)	21 (75%)	0.677
Hypertension			21 (29%)	10 (36%)	0.525
Cardiovascular disease			4 (5.5%)	3 (11%)	0.364
Diabetes			14 (19.4%)	6 (21.4%)	0.824
Chronic lung disease			11 (15%)	5 (17.8%)	0.752
Chronic kidney disease			6 (8.3%)	1 (3.5%)	0.402
Malignancy			4 (5.5%)	2 (7%)	0.764
Laboratory findings					
D-dimer, µg/L		216 (193–296)	670 (365–1,015)	1,130 (640–2090)	**<0.001**
Lymphocyte, 10^3/µL		2.14 (1.82–2.51)	1.13 (0.76–1.77)	0.88 (0.68–1.51)	**<0.001**
Neutrophil, 10^3/µL		3.46 (2.97–3.95)	4.91 (2.92–7.20)	5.76 (3.55–8.65)	**0.003**
NLR		1.64 (1.44–1.80)	3.70 (2.29–6.60)	6.08 (4.53–8.16)	**<0.001**
MPV, fL		10.53 (9.70–10.80)	10.52 (9.78–11.06)	10.55 (10.00–11.16)	0.443
Total thiol, µmol/L		203 (178–244)	186 (142–237)	123 (98–178)	**<0.001**
Albumin, g/dL		4.16 ± 0.27	3.66 ± 0.49	3.11 ± 0.48	**<0.001**
IMA (ABS U)		0.395 ± 0.055	0.519 ± 0.139	0.591 ± 0.112	**<0.001**
Adjusted IMA (ABS U)		–	0.484 ± 0.129	0.518 ± 0.096	0.201
PCT, ng/mL		–	0.108 (0.053–0.240)	0.165 (0.095–0.475)	**0.013**

MPV, mean platelet volume; IMA, ischemia modified albumin; PCT, procalcitonin; NLR, ratio of neutrophil to lymphocyte. Critical vs non-critical patient groups for D-dimer and IMA p=0.011 and p=0.024, respectively. Critical vs non-critical patient groups for total thiol and albumin p<0.001 and p<0.001, respectively. Critical and non-critical patient groups vs control group for neutrophil p=0.001, p=0.006, respectively. Critical and non-critical patient groups vs control group for IMA p<0.001 and p<0.001, respectively. Non-critical patients vs control group for total thiol p>0.05.

Table 2: Significance and correlation between variables in COVID-19 patients.

	PCT	D-dimer	Adjusted IMA	Total thiol
Age	0.077	0.126	0.157	−0.217[a]
PCT	–	0.389[c]	0.243[a]	−0.257[b]
D-dimer		–	0.190	−0.172
Adjusted IMA			–	−0.270[b]

PCT, procalcitonin. Correlation Coefficients (rho) were presented, [a]for p<0.05, [b]for p<0.01, [c]for p<0.001. IMA, ischemia-modified albumin.

Table 3: Binary lojistic regresion.

Variables	Odds ratio	95% CI	P
PCT	2.896	0.584–14.361	0.193
D-dimer	1.000	1.000–1.000	0.350
Total thiol	0.988	0.980–0.995	0.002

p=0.498 for the Hosmer-Lemeshow test.

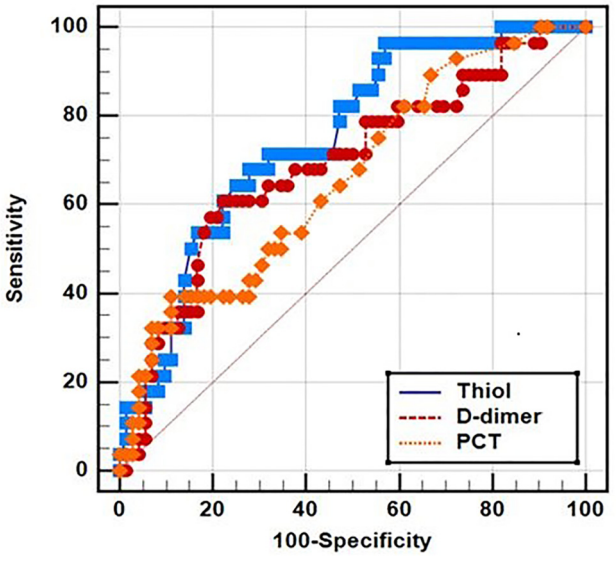

Figure 1: ROC curves for total thiol, D-dimer and PCT.

Table 4: Prognostic performance of total thiol, D-dimer and PCT in distinguishing critically from non-critical patients.

Variables	Optimal Cut-off values	Sensitivity	Specificity	PPV	NPV	AUC	95% CI	p-Value
Thiol, μmol/L	≤148	67.86	72.22	47.3	83.8	0.749	0.652–0.830	<0.001
D-dimer, μg/L	>1,030	60.71	77.78	51.5	83.5	0.691	0.590–0.779	0.001
PCT, ng/mL	>0.34	39.29	88.89	52.3	78.4	0.660	0.559–0.752	0.007

AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 5: Prognostic performance of combination of D-dimer and total thiol.

D-dimer and total thiol	Critical patients	Non-critical patients
>1,030 μg/L and ≤148 μmol/L	11	6
≤1,030 μg/L and >148 μmol/L	17	66
Total	28	72

PPV, positive predictive value; NPV, negative predictive value. Sensitivity=39%, Specificity=92%, PPV=65%, NPV=80%.

cut-off values and AUC for total thiol, D-dimer, and PCT (Figure 1).

D-dimer and PCT had a significant discrimination power (AUC: 0.691, p=0.001; AUC: 0.660, p=0.007, respectively). With a cut-off of 148 μmol/L, total thiol showed the best performance in distinguishing critically from non-critical patients infected with COVID-19 (Table 4).

A combination of D-dimer and total thiol had a higher positive predictive value (PPV) in identifying patients with critical clinical course infected with COVID-19 (Table 5).

Discussion

The current study indicated that total thiol level was lower, D-dimer and PCT levels were higher in patients with critical COVID-19 than in non-critical. Total thiol at a cut-off value of 148 μmol/L had the best performance in predicting the prognosis of COVID-19 patients compared with D-dimer or PCT. The combination of D-dimer and total thiol had a higher PPV.

Recent studies have indicated that D-dimer elevation and lymphopenia are correlated with the seriousness of COVID-19 [14–16] and are reliable indicators for identifying patients with poor prognoses. The sensitivity was 73%, and specificity was 91% in differentiating severe and non-serious patients with COVID-19 when a cut-off of 2000 μg/L was taken for D-dimer [17].

Additionally, it has been observed that the lymphocyte count was lower in COVID-19 patients treated in the intensive care unit compared to other clinical services [18]. However, the cut-off values for lymphocyte count and D-dimer differ between studies. Zhan et al. [19] reported that diagnostic sensitivity and specificity of D-dimer for mortality were 43–93% and 64–96%, respectively.

In another study, patients with a D-dimer >1,000 μg/L had a higher risk of death than those with a low D-dimer [20]. Xiaokang et al. [21] found that AUC for D-dimer, cut-off 765 μg/L, was 0.661 in predicting survival and mortality at the initial admission. In another study, high D-dimer levels were an independent indicator of mortality at first admission [22].

We obtained similar results with the literature regarding the importance of D-dimer as a prognostic factor. However, the relative expensiveness of the D-dimer kit and the observed increase in D-dimer test demands have led to increased interest in other parameters such as IMA or thiol.

A study detected that the binding affinity of ACE2 and SARS-CoV/CoV-2 spike proteins is significantly impaired when disulfide bonds are reduced to thiol groups [23]. In another study, serum total thiol levels were lower in patients with severe clinical course, and the AUC was 0.705 for thiol in adults. Therefore, their findings showed that thiol levels could be used as an indicator of COVID-19 severity [24]. Also, Erel et al. [25] reported that thiol levels decreased as the disease severity increased. Ducastel et al. [26] found that thiol was the best indicator to predict ICU admission, with an optimum cut-off at 154 μmol/L. We emphasize that the prognostic value of thiol status, observed in previous studies in the first year of the COVID-19 pandemic, may also be effective in later periods when different variants are dominant.

The above-mentioned study also showed high IMA levels in patients with COVID-19. IMA concentrations significantly increased in patients admitted to ICU [26]. Another study emphasized that IMA was an indicator of oxidative stress [27, 28]. So the researchers thought there was a relationship between oxidative stress and COVID-19. Higher IMA levels were determined in severe/critical patients with COVID-19 than in moderate patients [24]. We found that IMA levels were significantly higher in COVID-19 patients compared to

controls and in critically ill patients compared to non-critical patients. Although this finding supported that IMA may be related to the pathogenesis and prognosis of COVID-19 disease, the significant difference disappeared after albumin correction. This finding indicated that IMA would not be prognostically useful. Unlike other studies, albumin correction was applied to IMA levels in the present study since there was a significant difference between the albumin levels of the groups. Although the prognostic significance of total thiol and D-dimer were investigated separately, to our knowledge, the prognostic performance of the combination of total thiol and D-dimer in COVID-19 patients was demonstrated for the first time in this study. We obtained a higher PPV with the combination of total thiol and D-dimer in identifying patients with critical clinical course infected with COVID-19.

Limitations

Laboratory parameters have not been followed in the following days. Also, the sample sizes were relatively small.

In conclusion, total thiol levels may be a good indicator for predicting COVID-19 patients with a critical clinical course. Early identification of critical patients may provide an appropriate therapeutic approach and decrease mortality.

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