

Research Article

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Relationship of thrombospondin-1 and thrombospondin-2 with hematological, biochemical and inflammatory markers in COVID-19 patients

<https://doi.org/10.1515/tjb-2022-0265>

Received December 2, 2022; accepted May 9, 2023;

published online June 23, 2023

Abstract

Objectives: Roles of thrombospondin-1 (TSP-1) and thrombospondin-2 (TSP-2) in tissue repair and inflammation are well-documented, but the association of their serum expressions with the pathogenesis of COVID-19 remains unclear. We investigate the roles of TSP-1 and TSP-2 in COVID-19.

Methods: 106 SARS-CoV-2 infected patients and 23 healthy people were enrolled in our study. COVID-19 patients were divided into two groups as non-severe and severe. TSP-1 and TSP-2 concentrations were measured with an enzyme-linked Immunosorbent Assay, and blood markers were analyzed with routine laboratory techniques.

Results: COVID-19 patients had significantly higher TSP-1 and TSP-2 levels than healthy controls. TSP-1 and TSP-2 positively correlated with inflammatory markers, including ESR, CRP, PCT, ferritin, and biochemical parameters such as ALT, AST, BUN, CK, and LDH. In addition, TSP-1 and TSP-2 were negatively correlated with hematological markers such as LYM, EOS, and HGB. Receiver operating characteristic analyses revealed that COVID-19 may be predicted with TSP-1 levels over 189.94 ng/mL and TSP-2 levels higher than 0.70 ng/mL.

Conclusions: Our analysis suggests that TSP-1 and TSP-2 expressions at the systemic level may have clinical importance for COVID-19.

Keywords: biomarker; COVID-19; inflammation; thrombospondin-1; thrombospondin-2

Introduction

Coronavirus disease 2019 (COVID-19) is a fatal infectious illness affecting millions worldwide. This unprecedented outbreak has remained a tremendous social, healthcare, and economic burden since it was first reported in Wuhan, China [1]. More common symptoms of COVID-19 are cough, fatigue, fever, expectoration, and dyspnea [1–3]; other symptoms, such as loss of taste and smell and diarrhea, have also been reported [3, 4]. The progression of COVID-19 consists of three stages. In the first stage, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) generates an immune response by infecting lung tissue, then following proliferation and mild symptoms start. Then, the second (pulmonary) phase is characterized by respiratory dysfunction, activated inflammatory pathways, increased cytokine levels, and tissue damage. In the third final phase, systemic inflammation, caused by excessive inflammatory protein synthesis, leads to multi-organ failure, which may result in fatal complications such as coagulopathy and septic shock [1]. We see significant

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effort and progress in vaccine development and implementation and limited therapeutic approaches (with some clinical settings use) [5, 6]. However, there is still no effective and specific therapeutic option for COVID-19. To date, some studies have reported that routinely analyzed hematological and biochemical parameters might have diagnostic and prognostic value in the clinical management of COVID-19 [7–9]. Especially some parameters from the hematological panel could have a relationship with the disease severity of COVID-19. Increased Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) may reflect the presence and severity of COVID-19 infection [7, 9].

Moreover, it was reported in another study that the C-reactive protein-to-lymphocyte ratio might reflect the poor prognosis for COVID-19 [8]. Nevertheless, the above-mentioned parameters are insufficient for predicting the course and outcomes of the disease [8]. Hence, we need novel markers with high specificity and sensitivity that can be useful in developing tools for diagnosing and prognosis of COVID-19. Furthermore, despite many efforts to elucidate the molecular mechanism of COVID-19, its pathogenesis is not fully understood [1]. A better understanding and grasp of the inflammatory molecular mechanism of this deadly disease would be useful in preventing and treating COVID-19 [1, 10].

Thrombospondins (TSPs), a matricellular protein family which includes five members, are secreted by various cell types during regular physiological activity [11, 12]. Thrombospondin-1 (TSP-1) is crucial in several physiological events, including inflammation, apoptosis, coagulation, wound healing, and interaction with extracellular components, receptors, and cytokines [12, 13]. Furthermore, TSP-1, expressed in immune cells such as neutrophils and macrophages, is elevated in various inflammatory disorders [14]. TSP-1 regulates inflammatory cytokine synthesis in THP-1 cells by controlling Nuclear Factor kappa B signalling, suggesting that TSP-1 is closely related to this inflammatory pathway involved in many diseases [15]. Moreover, as a multifunctional protein, TSP-1 is synthesized in neutrophils in response to inflammation and promotes chemotaxis and phagocytosis by activating leukocytes and macrophages [14]. Thrombospondin-2 (TSP-2), similar to TSP-1 at the molecular structural level, is central in numerous pathological processes, e.g., tissue remodeling, angiogenesis, heart diseases, and inflammation [16–18]. Moreover, TSP-2 has anti-inflammatory features, and its upregulation alleviates lung damage and inflammation [18]. Action mechanisms of TSP-1 and TSP-2 in many illnesses are well-documented [14, 16]; however, their roles in the pathogenesis of COVID-19 remain unclear. Therefore, we aim to investigate the relationship between TSP-1 and TSP-2, biochemical alterations, and the severity of the disease. We also evaluate their diagnostic and prognostic potentials in COVID-19 patients.

Materials and methods

Study participants

Our study was approved by the clinical research Ethics Committee of Hatay Mustafa Kemal University (approval #: 2021/86). We conducted this research according to the Declaration of Helsinki between June 15, 2021, and October 15, 2021, and obtained an informed consent form from all individuals who participated in our study. We enrolled 106 SARS-CoV-2 infected patients at the University Hospital and 23 healthy people for our research. A physician diagnosed the patients according to WHO's interim COVID-19 guidelines, and each diagnosis was verified with the real-time quantitative polymerase chain reaction method (RT-qPCR) at a laboratory. Our analysis included demographic data such as age and gender and clinical data such as comorbidities and clinical symptoms.

COVID-19 severity classifications

We clinically classified patients according to the practices in the literature [17, 19] and divided them into four groups. The first (mild) group has symptoms of COVID-19, but they show no evidence of viral pneumonia or hypoxia. The second (moderate) group has no evidence of severe pneumonia, and their oxygen saturation (SpO_2) is higher than 90 % in room air. The third (severe) group has symptoms of severe pneumonia (e.g., cough and dyspnea) and SpO_2 not higher than 90 % in room air and/or a respiratory frequency higher than 30 breaths/minute. The last and the fourth (critically ill) group have septic shock and/or multiple organ dysfunction and ARDS, and also they are most likely to be treated in an intensive care unit with a ventilator.

Blood sample collection

We collected the venous blood samples from all participants (infected patients and healthy controls) and then transferred blood samples into test tubes containing gel for serum separation. Next, the blood samples were centrifuged (at $1,500 \times g$ for 10 min at 4 °C) and then stored at -80 °C until serum TSP-1 and TSP-2 analyses.

Data collection

We conducted the following analysis of our COVID-19 patients' blood samples. We analyzed hematological parameters such as white blood cells (WBC), red blood cells (RBC), hemoglobin (HMG), hematocrit (HCT), mean corpuscular volume (MCV), lymphocyte (LYM), monocytes (MON) and eosinophil (EOS). Our analysis used Mindray BC 6000 Hematology System (MINDRAY Medical International Co., Shenzhen, China). We measured coagulation markers, including D-dimer levels using VIDAS D-dimer Exclusion™ II (BioMérieux, France). For Fibrinogen (FIB) concentration detections, we used STA Compact Max (Stago, USA). To measure procalcitonin (PCT) levels and C-reactive protein (CRP), we used chemiluminescent microparticle immunoassay (ABBOTT I2000, USA) and nephelometric method (Siemens Dade Behring BN II System Marburg, Germany) respectively. We utilized an immunometric assay (Siemens Atellica IM 1600, USA) for ferritin levels. For all other biochemical markers measurements (such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA)), blood urea nitrogen (BUN), creatine kinase (CK), and

lactate dehydrogenase (LDH)) we used an autoanalyzer with the spectrophotometric method (Siemens Atellica CH 930 Analyser, USA).

Concentrations of TSP-1 and TSP-2

We measured concentrations of TSP-1 and TSP-2 in the serum samples of SARS-CoV-2 infected patients and healthy controls with the help of Enzyme-linked Immunosorbent Assay (ELISA) commercial kits (Elabscience, USA). We observed detection ranges of 7.81–500 ng/mL (TSP-1) and 0.313–20 ng/mL (TSP-2). Both intra- and interassay coefficients of variance (CV %) for analyzed proteins were <10 %.

Statistical analysis

We used the Kolmogorov-Smirnov test to verify the normal distribution assumption for the continuous data. Subsequently, we performed two and multiple groups and compared them with Mann-Whitney U and Kruskal Wallis H tests, respectively. We presented continuous data as medians (interquartile range) or mean $\pm$ standard deviation. Using the chi-square test, we analyzed the categorical data and demonstrated the results as percentages and numbers. For

correlation analysis of TSP1 and TSP-2 with other variables, we used Spearman's rho test. We tested potential diagnostic/prognostic values of TSP-1, TSP-2, and some blood markers in SARS-CoV-2 infected patients utilizing receiver operating characteristic (ROC) analysis. We utilized GraphPad Prism 8.0 software (GraphPad, USA) for statistical analyses and used 0.05 and lower for the significance of p values.

Results

Clinical characteristics and demographics of participants

Demographics and clinical characteristics of 106 confirmed SARS-CoV-2 infected patients and 23 healthy people are presented in Table 1. Considering the severity of the disease on clinical examination, we classified all infected patients into two main groups: non-severe (22 mild + 56 moderate) and Severe (15 severe + 13 critically ill). The average age in the healthy, non-severe, and severe

Table 1: Clinic characteristics and demographics of COVID-19 patients and healthy controls.

Variables	Control group (n=23)	All COVID-19 patients			p-Value
		Non-severe group (n=78)	Severe group (n=28)	All patients (n=106)	
Age, years	44 (41–50)	50 (39.8–58)	51 (46.25–55)	50 (41–56)	0.068
Gender, n (%)					
Male	12 (52.2)	45 (57.7)	20 (71.4)	65 (61.32)	0.321
Female	11 (47.8)	33 (42.3)	8 (28.6)	41 (38.68)	
Hospitalization, day		8.56 $\pm$ 3.45	15.25 $\pm$ 8.96	10.33 $\pm$ 6.17	<0.001
Comorbidities, n (%)					
Hypertension		10 (12.8)	6 (21.4)	16 (15.1)	0.068
Diabetes mellitus		6 (7.7)	10 (35.7)	16 (15.1)	<0.001
Chronic kidney disease		2 (2.6)	3 (10.7)	5 (4.7)	0.091
Malignancy		0 (0)	2 (7.1)	2 (1.88)	0.026
Cerebrovascular disease		2 (2.6)	2 (7.1)	4 (3.77)	0.311
Chronic liver disease		1 (1.3)	0 (0)	1 (0.94)	0.719
Pulmonary disease		2 (2.6)	2 (7.1)	4 (3.77)	0.311
Thyroid disease		2 (2.6)	0 (0)	2 (1.88)	0.515
Clinical symptoms, n (%)					
Fever		27 (34.6)	3 (10.7)	30 (28.3)	0.001
Cough		38 (48.7)	13 (46.4)	51 (48.11)	<0.001
Loss of appetite		1 (1.3)	1 (3.6)	2 (1.8)	0.563
Dyspnea		7 (9)	15 (53.6)	22 (20.75)	<0.001
Chest tightness		0 (0)	1 (3.6)	1 (0.94)	0.162
Fatigue		24 (30.8)	6 (21.4)	30 (28.3)	0.009
Myalgia		4 (5.1)	0 (0)	4 (3.77)	0.259
Diarrhea		1 (1.3)	2 (7.1)	3 (2.83)	0.151
Sore throat		9 (11.5)	0 (0)	9 (8.49)	0.042
Nausea or vomiting		1 (1.3)	2 (7.1)	3 (2.83)	0.151
Other symptoms		8 (10.3)	7 (25)	15 (14.15)	0.018
Deaths, n (%)		0 (0)	4 (14.3)	4 (3.77)	0.001

We presented continuous results as mean $\pm$ standard deviation or medians (interquartile range) and categorical data were as numbers (percentages). p-Values less than 0.05 were considered statistically significant and are shown in bold.

groups were 44.78 ± 4.12 , 49.24 ± 12.04 , and 49 ± 8.42 years, respectively.

Regarding gender, 41 (38.68 %) patients were male, and 65 (61.32 %) were female. As demonstrated in Table 1, the study groups were similar, and no considerable differences were found in age or gender. While fever, cough, and fatigue were the most frequent symptoms in the non-severe group, dyspnea and cough were the most common in the severe group. Most patients in the severe group had at least one or more comorbidity (89.1 %). The severe group had a higher mortality rate. Four patients died in severe cases ($p=0.001$).

Laboratory results of participants

Laboratory markers of healthy controls were observed within the normal range, while abnormal patterns of hematological, biochemical, and inflammatory parameters were detected in COVID-19 patients. We presented details of

laboratory results in Table 2. For instance, in hematological parameters, we found a significant increase in WBC values for the severe group compared to the healthy ($p<0.0001$) and non-severe groups. On the other hand, we did not find any significant differences in WBC counts between healthy and non-severe groups ($p=0.069$). Furthermore, compared to the healthy controls, we detected a considerable decrease in LYM, EOS, HGB, and HCT levels in the non-severe and severe groups ($p<0.001$). In addition, we detected a significant decrease in RBC levels in the severe group compared to the healthy and non-severe groups ($p<0.01$). No differences were found between the groups in MON and MCV levels ($p>0.05$). Moreover, our data showed that COVID-19 patients had significantly high coagulation markers, e.g., D-dimer and fibrinogen. Among the inflammation-related indicators, we also observed significant differences in CRP, ESR, ferritin levels, and PCT, and our results showed that these parameters were markedly increased in all patient groups compared to the healthy controls ($p<0.005$). Moreover, levels of biochemical parameters were altered in COVID-19

Table 2: Laboratory results of participants.

Parameters	Healthy controls (n=23)	COVID-19 patients			p-Value
		Non-severe group (n=78)	Severe group (n=28)	All patients (n=106)	
WBC, $10^3/\mu\text{L}$	6.50 (6.02–7.38)	5.70 (4.35–7.25)	11.37(8.80–15.01)	6.3 (4.62–10.35)	<0.0001^{a,c,d}=0.069^b
LYM, $10^3/\mu\text{L}$	2.20 (1.64–2.35)	1.45 (0.97–1.85)	0.66 (0.52–1.27)	1.26 (0.82–1.8)	<0.0001^{a, b, c, d}
MON, $10^3/\mu\text{L}$	0.42 (0.40–0.48)	0.42 (0.31–0.55)	0.50 (0.30–0.73)	0.43 (0.31–0.56)	$=0.227^a, =0.319^b, =0.583^c, =0.112^d$
EOS, $10^3/\mu\text{L}$	0.16 (0.14–0.29)	0.02 (0.00–0.05)	0.01 (0.00–0.04)	0.01 (0.0–0.04)	<0.0001^{a, b, c, d}=0.106^d
RBC, $10^6/\mu\text{L}$	4.51 (4.36–0.4.9)	4.77 (4.46–5.16)	4.32 (3.82–4.52)	4.61 (4.32–4.97)	<0.0001^{a, d}=0.115^b=0.002^c
HGB, g/dL	14.4 (13.2–15)	12.85 (12.4–14.1)	11 (9.3–12.5)	12.8 (11.5–13.62)	<0.0001^{a, b, c, d}
HCT, %	43.4 (39.7–46)	39.05 (37.37–41.9)	34.7 (28.75–38.95)	38.4 (35.67–38.4)	<0.0001^{a, c, d}=0.001^b
MCV, fL	89.4 (82.2–91.6)	84.55 (80.9–87.9)	84.35 (79.6–88.7)	84.55 (80.8–88.2)	$=0.036^a=0.013^b, 0.035^c=0.895^d$
ESR, mm/h	8 (7–10)	18.5 (10.5–30.25)	36.5 (19.25–49.5)	21 (12–38)	<0.0001^{a, b, c, d}
FIB, mg/dL	215 (202–238)	359 (301–474)	544.5 (435.75–674.25)	407 (309–500.75)	<0.0001^{a, b, c, d}
D-dimer, ng/mL	208 (152–256)	413.91 (247–664.20)	1,090 (662.50–3,150)	560.41 (290.98–1,045)	<0.0001^{a, b, c, d}
CRP, mg/L	1.4 (1.2–1.96)	15.2 (5.99–53.8)	35 (15.8–104.5)	22.7 (7.36–66.02)	<0.0001^{a, b, c, d}=0.031^d
PCT, ng/mL=0.004 ^b	0.014 (0.012–0.018)	0.02 (0.01–0.05)	0.1 (0.05–0.22)	0.04 (0.018–0.09)	<0.0001^{a, c, d}=0.004^b
Ferritin, ng/mL=0.004	16 (10–39)	96.1 (52.5–296.6)	370.9 (162.45–887.5)	150.8 (56.9–425.97)	<0.0001^{a, c, d}=0.004^b
LDH, U/L	126 (122–134)	240 (200.75–311)	323 (245–436.25)	258.5 (201–357)	<0.0001^{a, b, c, d}
ALT, U/L	16 (15–18)	33 (25–44)	29 (22–44)	32 (23.75–44)	<0.0001^{a, b, c, d}
AST, U/L	16 (13–19)	29 (21–42.5)	28 (19.25–53.75)	29.5 (21–44.25)	<0.0001^{a, b, c, d}
CREA, mg/dL	0.58 (0.53–0.65)	0.75 (0.59–0.98)	0.80 (0.54–0.92)	0.58 (0.53–0.65)	$=0.002^a<0.0001^b=0.013^c, =0.926^d$
BUN, mg/dL	8 (8–9)	13 (10–16)	21.5 (15–26.5)	14 (11–19)	<0.0001^{a, b, c, d}
CK, U/L	52 (45–65)	93 (57–156)	208 (121.25–263.25)	103 (76–204)	<0.0001^{a, b, c, d}

We presented continuous results as mean $\pm$ standard deviation or medians (interquartile range) and categorical data were as numbers (percentages). p-Values less than 0.05 were considered statistically significant and are shown in bold. ^aComparison among all groups. ^bComparison between non-severe group and healthy controls. ^cComparison between severe group and healthy controls. ^dComparison between severe and non-severe groups.

patients with a considerably elevated level of ALT, LDH, AST, BUN, CK and CREA.

TSP-1 and TSP-2 protein levels in COVID-19 patients

What are TSP-1 and TSP-2's likely roles as blood biomarkers? We measured serum TSP-1 and TSP-2 concentrations in COVID-19 patients and healthy controls to answer this question. We found that in healthy controls, TSP-1 and TSP-2 concentrations were 97.28 ± 96.33 and 0.69 ± 0.69 , respectively. Our data showed these patients had significantly higher levels of serum TSP-1 (314.6 ± 137.4) and TSP-2 (2.046 ± 1.64) than healthy controls ($p < 0.0001$, Figure 1A and B). Further, we observed that TSP-1 levels in non-severe and severe groups were 307.8 ± 139.2 and 333.2 ± 132.7 , respectively. TSP-2 concentrations in non-severe and severe groups were 1.88 ± 1.40 and 2.46 ± 2.1 , respectively. On the other hand, we did not find any significant differences between non-severe and severe cases (Figure 1A and B) in TSP-1 and TSP-2 concentrations. We also compared the serum levels in all subgroups to see whether TSP-1 and TSP-2 had any relationship with the severity of COVID-19. Our results showed no considerable differences in these protein levels between mild, moderate, severe, and critically ill groups (Figure 1C and D).

Table 3: Correlation between laboratory parameters and TSP-1 and TSP-2.

Variables	TSP-1		TSP-2	
	r	p-Value	r	p-Value
WBC, $10^3/\mu\text{L}$	0.101	0.255	0.102	0.270
LYM, $10^3/\mu\text{L}$	-0.257	0.003	-0.281	0.002
MON, $10^3/\mu\text{L}$	0.30	0.735	0.060	0.520
EOS, $10^3/\mu\text{L}$	-0.289	0.001	-0.381	<0.001
RBC, $10^6/\mu\text{L}$	-0.003	0.969	0.0	1
HGB, g/dL	-0.242	0.006	-0.399	<0.0001
HCT, %	-0.136	0.125	-0.167	0.070
MCV, fL	-0.041	0.643	-0.217	0.018
ESR, mm/h	0.316	<0.001	0.250	0.006
FIB, mg/dL	0.362	<0.001	0.349	<0.001
D-dimer, ng/mL	0.289	0.001	0.290	0.001
CRP, mg/L	0.344	<0.001	0.448	<0.0001
PCT, ng/mL	0.222	0.012	0.386	<0.0001
Ferritin, ng/mL	0.416	<0.001	0.315	<0.0001
ALT, U/L	0.216	0.014	0.278	0.002
AST, U/L	0.311	<0.001	0.281	0.002
CREA, mg/dL	0.177	0.046	0.104	0.259
BUN, mg/dL	0.344	<0.001	0.266	0.003
CK, U/L	0.371	<0.001	0.290	0.001
LDH, U/L	0.316	<0.001	0.483	<0.0001

The relationship of TSP-1 and TSP-2 with blood markers was evaluated with Spearman's rho test. We consider bolded p-values less than 0.05 statistically significant.

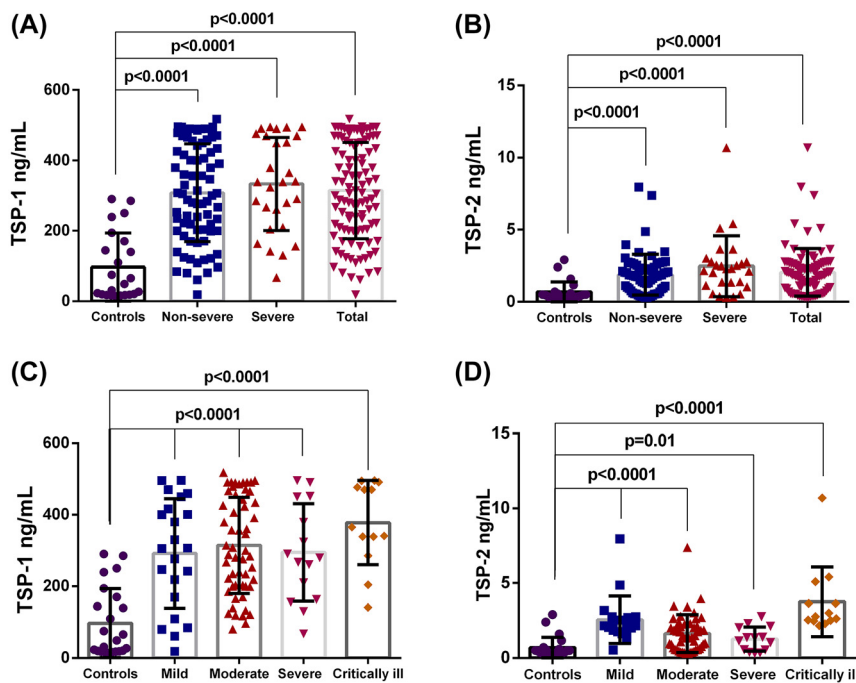


Figure 1: The TSP-1 and TSP-2 protein expression levels in patients with COVID-19 and healthy controls. Comparison of TSP-1 (A) and TSP-2 (B) protein expression in COVID-19 patients according to the classification of non-severe (mild, moderate) and severe (severe and critically-ill). Comparison of TSP-1 (C) and TSP-2 (D) protein expression in COVID-19 patients according to the classification of mild, moderate, severe and critically ill disease.

Table 4: ROC analysis and optimal thresholds of TSP-1, TSP-2, CRP, D-dimer, Fibrinogen and Ferritin in COVID-19 patients.

Variables	AUC (95 %CI)	Sensitivity %	Specificity %	Cut-off	p-Value
TSP-1	0.8936 (0.8260–0.9612)	79.0	78.3	189.94 ng/mL	<0.0001
TSP-2	0.7375 (0.7375–0.9319)	78.1	78.3	0.70 ng/mL	<0.0001
CRP	0.9996 (0.9982–1.001)	97.1	95.7	3.03 mg/mL	<0.0001
D-dimer	0.8798 (0.8219–0.9377)	80.8	82.6	260.50 ng/mL	<0.0001
Fibrinogen	0.9416 (0.9031–0.9800)	89.6	91.3	256.5 mg/dL	<0.0001
Ferritin	0.9286 (0.8823–0.9750)	87.7	87	43.3 ng/mL	<0.0001

AUC, area under the receiver operating characteristic curve; TSP-1, thrombospondin-1; TSP-2, thrombospondin-2; CRP, C-reactive protein.

TSP-1 and TSP-2 connections with biochemical parameters

To elucidate the possible association of TSP-1 and TSP-2 with blood parameters abnormalities in patients with COVID-19 regarding hematological, coagulation, inflammatory, and biochemical, we performed a Spearman rank correlation test and presented our results in Table 3. Our correlation analyses revealed that TSP-1 and TSP-2 had negatively correlated with hematological markers such as LYM, EOS, and HGB. Furthermore, we showed that TSP-1 and TSP-2 concentrations were positively correlated with inflammatory markers, including CRP, ESR, ferritin, and PCT. In addition, serum TSP-1 and TSP-2 are significantly correlated with some biochemical parameters such as AST, ALT, CK, LDH and BUN.

Predictive power and thresholds of TSP-1 and TSP-2 for COVID-19

To evaluate the predictive power and thresholds of thrombospondins (TSP-1 and TSP-2) and other blood markers in COVID-19 patients, we constructed ROC curves, detected optimal cut-off levels for those markers, and presented our results in Table 4 and Figure 2. Our analyses revealed that COVID-19 could be predicted with TSP-1 concentrations over 189.94 ng/mL ($p < 0.0001$), and the sensitivity and specificity for TSP-1 were 79 and 78.3, respectively. Furthermore, TSP-2 values higher than 0.70 ng/mL could distinguish the presence of COVID-19, and the sensitivity and specificity for TSP-2 were 78.1 and 78.3, respectively ($p < 0.0001$). In other blood markers, the D-dimer, CRP, ferritin, and fibrinogen thresholds were

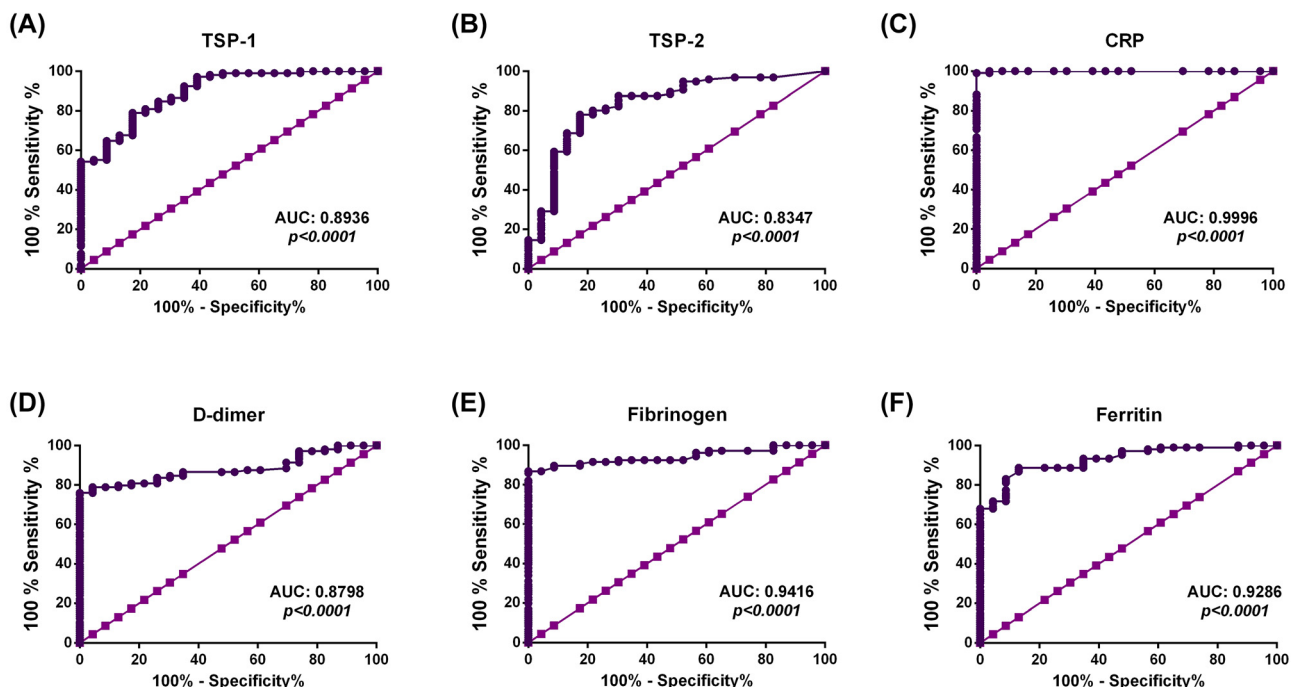


Figure 2: The evaluation of potential predictive values of TSP-1 (A), TSP-2 (B), CRP (C), D-dimer (D), fibrinogen (E) and ferritin (F) in COVID-19 patients with ROC curve analyses.

260.50 ng/mL, 3.03 mg/mL, 43.3 ng/mL, and 256.5 mg/dL, respectively. Our results show that TSP-1 and TSP-2 may provide diagnostic and prognostic value for COVID-19 outcomes.

Discussion

Despite the currently available potential therapeutic and preventive approaches, no effective options exist to treat and prevent COVID-19 [1]. The elucidation of the molecular mechanism of SARS-CoV-2 infection will contribute to development of novel preventive strategies for COVID-19. In this paper, we examined whether the thrombospondin proteins are related to the pathogenesis of COVID-19. To the best of our knowledge, ours is the first study exploring and showing how TSP-1 and TSP-2 have relationships with inflammation, coagulation, and biochemical alterations in COVID-19 patients. Our results revealed that the patients had significantly higher TSP-1 and TSP-2 levels than healthy controls. Furthermore, our ROC analyses showed that TSP-1 and TSP-2 may have clinical importance in improving the management and outcomes of COVID-19.

Recent studies on COVID-19 have shown that SARS-CoV-2 infection causes an inflammatory response and lung damage [1]. Furthermore, injured lung tissue uncontrollably stimulates the extravagant secretion of pro-inflammatory mediators, also known as a cytokine storm, which leads to life-threatening complications such as septic shock and multi-organ dysfunction. TSP-1, synthesized mainly by immune cells, including neutrophils and macrophages, is a multifunctional protein that has a role in many physiological processes, such as inflammatory response, phagocytosis, apoptosis, and fibrosis [14]. Furthermore, TSP-1 has a crucial role in inflammatory processes by regulating cytokine secretion and the functional capacity of immune cells (e.g., macrophages and dendritic cells) [14, 20]. Previous studies showed that elevated TSP-1 levels in several clinical conditions provide a defense mechanism against disease by enhancing cytokine release and promoting the phagocytosis of injured cells [14]. Our results in this paper suggest that increased serum TSP-1 concentrations may result from lung tissue injury caused by viral infection. Moreover, our research shows that TSP-1 expressions are positively associated with inflammatory markers such as D-dimer and CRP, suggesting that the increased TSP-1 synthesis could arise from its essential role in inflammatory mechanisms. Considering the relevance of TSP-1 in lung homeostasis and inflammation [14, 21], we may speculate that elevated TSP-1 might have protective effects against excessive multi-organ damage caused by Coronavirus infection.

TSP-2, a multifunctional glycoprotein, is crucial in numerous physiological activities, e.g., apoptosis,

inflammation, proliferation, and angiogenesis [16–18]. Previous studies have emphasized that TSP-2 involves various sicknesses, e.g., heart failure, renal disorder, and rheumatoid arthritis [16, 17, 22]. Furthermore, it has been reported in some studies that circulating TSP-2 as a biomarker might reflect the severity and prognosis of some diseases [23, 24]. However, no available data is investigating the role of TSP-2 in COVID-19. A recent study investigating the role of TSP-2 on inflammation and apoptosis in lung tissues has shown that TSP-2 could regulate inflammation and might have therapeutic potential against ARDS [18]. In the same study, their histopathological analyses indicated that upregulation of TSP-2 alleviated endothelial damage and suppressed neutrophil function [18]. In addition, the results showed that upregulation of TSP-2 inhibits pro-inflammatory cytokines synthesis (IL-6 and TNF- α), while it stimulates the synthesis of anti-inflammatory cytokine (IL-10) [18]. Another study reported that TSP-2 expressions were elevated in inflammatory conditions accompanying tissue damage [25]. Similarly, Tian et al. showed that TSP-2 knockout mice cause elevated inflammation [26]. These observations highlight that TSP-2 might protect against tissue damage by suppressing the inflammatory processes.

Our data revealed that COVID-19 patients had abnormal hematological marker levels during disease progression, consistent with previous studies [19, 27]. Another exciting result of our study is that serum concentrations of TSP-1 and TSP-2 and hematological markers such as LYM, EOS, and HGB had negatively correlated. On the other hand, recent studies reported that COVID-19 patients had dramatically increased inflammatory markers, e.g., ESR, CRP, PCT, and Ferritin, caused by the cytokine storm [19, 28]. Our correlation analyses showed that these inflammatory markers and TSP-1 and TSP-2 had a relationship, suggesting that TSP-1 and TSP-2 are closely linked to cytokine storms. Coagulation abnormalities frequently were seen in COVID-19 patients [29, 30]. Consistent with these results, D-dimer and Fibrinogen coagulation markers were elevated in our COVID-19 patients. We also observed that Fibrinogen and D-dimer were positively correlated with TSP-1 and TSP-2 levels.

Like any research, our study also has a few limitations, and the results presented here might not represent all COVID patients. Our study's sample size was relatively small since we conducted this research in a single-center study. Each patient participating in the study was receiving treatment, and the effect of current therapy on TSP-1 and TSP-2 is still unclear. Last but not least, we could not investigate serum TSP-1 and TSP-2 concentrations at different points in time for each patient.

Our results, for the first time, suggest that elevated TSP-1 and TSP-2 expressions may be diagnostic and prognostic markers for COVID-19. TSP-1 and TSP-2 can regulate immune cell function and cytokine secretion in COVID-19.

Furthermore, modulation of TSP-1 and TSP-2 expressions at a systemic level may have therapeutic importance for alleviating the clinical severity of COVID-19. We hope this and the following studies will enable researchers to determine any diagnostic/prognostic and therapeutic capabilities of TSP-1 and TSP-2 expressions for COVID-19 and related diseases. Further functional research should focus on the association between thrombospondins and cytokine storms to better understand the pathogenesis of COVID-19.

Research funding: None declared.

Author contributions: SD and HMO contributed substantially to the concept and design of the study. SD, TB, and MC contributed to the collection of data. SD and HMO contributed substantially to laboratory and statistical analysis. SD, HMO, TB, MC and MAB wrote, edited and revised the manuscript and interpreted the results. SD and HMO created tables, graphs and figures. As authors of this manuscript, we approved the final version submitted for publication and take responsibility for statements made in the published article. All authors contributed to the agreement to be accountable for all aspects of the work to ensure that the data accuracy or integrity of any part of the work is appropriately investigated.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Our study was approved by the clinical research Ethics Committee of Hatay Mustafa Kemal University (approval #: 2021/86).

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