Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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SCUBE1 is associated with thrombotic complications, disease severity, and in-hospital mortality in COVID-19 patients

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ABSTRACT

Introduction: COVID-19 disease, which has recently become an important cause of mortality and morbidity all over the world, is remarkably associated with thrombotic complications. Although many factors are responsible for these increased thrombotic complications in COVID-19 disease, its relationship with a marker that increases the risk of thrombosis such as Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1) has not yet been clarified. This is the first study to examine the potential diagnostic and prognostic value of SCUBE1 levels in patients with COVID-19. In this study, we aimed to clarify the relationship between the increased risk of thrombosis and SCUBE1 in the course of COVID-19 disease.

Materials and methods: 553 patients with COVID-19 and 553 healthy controls were compared in terms of SCUBE1 levels. Additionally, patients with COVID-19 were divided into two groups according to their SCUBE1 levels and compared in terms of severity of disease, thrombotic complications and ın-hospital mortality.

Results: SCUBE1 levels were significantly higher in patients with COVID-19 compared to the control group ($p < 0.001$). Plasma SCUBE1 levels were significantly higher in patients with severe disease and thrombotic complications, those with mild to moderate disease, and those without thrombotic complications ($p < 0.001$, for both). In addition, SCUBE1 was found to be an independent predictor of in-hospital mortality ($p < 0.001$).

Conclusions: SCUBE1 may be one of the major determinants of thrombotic complications, which is an increased cause of mortality and morbidity in COVID-19 patients so inhibition of this peptide may be among the therapeutic targets in patients with COVID-19.

1. Introduction

COVID-19 disease is a systemic disease that has recently caused major mortality and morbidity all over the world [1]. The COVID-19 virus can affect almost all systems in the body [2]. Among these, thrombotic complications can be observed at an increased rate even during the course of the disease or even after the disease has survived [3]. Although many pathophysiological mechanisms have been blamed for this predisposition, the exact cause has not been fully elucidated [4]. Recent studies have shown that vasculopathy accompanied by platelet and endothelial dysfunctions plays a key role in susceptibility to thrombosis during the course of COVID-19 disease [5,6]. However, the relationship between this pro-thrombotic state in COVID-19 patients and SCUBE1 (signal peptide-CUB-epidermal growth factor [EGF] domain-containing protein 1) levels, which have been closely associated with pro-thrombotic states in previous studies, has not been studied so far [7,9].

SCUBE1 is a cell surface glycoprotein expressed and secreted from platelet and endothelial cells during early embryogenesis [10]. When SCUBE1 is overexpressed, it can be secreted into the conditioned medium as a soluble protein or bound to the cell surface as a peripherally associated membrane protein [11]. SCUBE1 binds to the plasma membrane by electrostatic and lectin-glycan interactions and is responsible for different functions depending on soluble or membrane protein distribution [10–13]. Besides endothelial cells, SCUBE1 is also produced and stored in thrombocyte, is released on the cell surface, undergoes

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proteolysis upon activation by adenosine diphosphate (ADP), thrombin or collagen stimulation, and is incorporated into the thrombus [8–12]. It is thought that plasma SCUBE1 actively participates in platelet aggregation by bridging activated platelets that come together and aggregate during thrombus formation [8]. It has been shown in experimental studies that soluble SCUBE1 is a biomarker for platelet activation and also an active participant in thrombosis, and plays a critical role in arterial thrombosis through its in vivo adhesive EGF-like repeats of platelet-derived SCUBE1 [7,8]. In a clinical study, it was shown that the plasma SCUBE1 level released from activated platelets is significantly increased in acute coronary syndrome and acute ischemic stroke, and therefore SCUBE1 may be a biomarker of platelet activation [9].

Both the increased prothrombogenic environment in COVID-19 patients and the active role of SCUBE1 in platelet activation made us think that there may be a relationship between COVID-19 and SCUBE1. Since the relationship between COVID-19 and SCUBE1 has not been examined before, we aimed to reveal the possible relationship between them in this study.

2. Materials and methods

2.1. Study design and subjects

In this observational, prospective study, 553 patients hospitalized for COVID-19 disease between May 2020 and December 2021, and 553 healthy volunteers matched for age and sex who did not have COVID-19 disease, who applied to the outpatient clinic for routine check-ups as the control group, were included. In-hospital mortality and thrombotic complications of patients were recorded.

During the course of the disease, 76 patients requiring intensive care unit (ICU) follow-up were evaluated in the severe disease category, and 477 patients requiring hospitalization and clinical service follow-up were evaluated in the mild to moderate disease category. Patients who use antiaggregant or anticoagulant for any reason, have any previous history of thrombotic complications, have had COVID-19 vaccine, history of active cancer or take chemotherapeutic drugs, genetically known coagulation disorders, hematologic and endocrine disorders, autoimmune diseases and chronic inflammatory diseases, advanced kidney or liver failure, and patients who could not obtain consent were excluded from the study. Baseline demographic and clinical characteristics were recorded for all patients and the control group. The study was approved by the local ethics committee and was performed in accordance with the Declaration of Helsinki (date: 05.04.2020, no: 20/05/02).

2.2. Biochemical measurements

For SCUBE1 measurement, venous blood samples were taken from the patients and control group at the time of admission, before any treatment was started. Blood samples were collected into tubes containing K_2EDTA or SST™ II as an anticoagulant and serum separator. Plasma and serum specimens were obtained after centrifugation at 2500 $\times g$ for 10 min. Routine biochemistry analyses, complete blood counts and coagulation tests were carried out on blood samples. Complete blood counts were assessed by an automated blood cell counter (Coulter LH 780 Hematology Analyzer, Beckman Coulter Corp, Hileh, Florida). Serum creatinine and blood urea nitrogen levels were measured using the Architect Plusci 4100 m (Abbott Laboratories, Abbott Park, Illinois, USA). D-dimer serum levels were measured using an STA Compact analyzer (Houston, USA). The reference range of D-dimer is 0–0.55 mg/L and results are expressed in mg/L. Serum samples for SCUBE1 analysis were frozen, and kept at −80 °C until testing. SCUBE1 levels were measured by using commercial SCUBE1 Enzyme-Linked Immunosorbent Assay (ELISA) kit (Molecular Devices, Sunnyvale, CA, USA) according to the manufacturer's instructions. The absorbance of samples was measured at 450 nm on a VERSA max tunable microplate reader. Results are presented in ng/mL for SCUBE1; intra-assay and inter-assay coefficients of variation were < 10 %.

2.3. Diagnostic and treatment modalities

The diagnosis was made in accordance with current guidelines according to clinical signs, reverse transcription-polymerase chain reaction (RT-PCR), chest computed tomography findings, IgM and IgG antibody tests [14]. Patients were categorized as mild, moderate and severe illness according to their present symptoms as follows; Mild illness: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging; Moderate illness: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation measured by pulse oximetry (SpO_2) ≥94 % on room air at sea level; Severe illness: Individuals who have SpO_2 < 94 % on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2 /FiO_2) <300 mmHg, a respiratory rate > 30 breaths/min, or lung infiltrates >50 %. Those with mild and moderate illness were followed up in the inpatient service, and those with severe illness were followed up in the intensive care unit [15].

We diagnosed disseminated intravascular coagulation (DIC) based on low platelet count, high D-dimer concentration, decreased fibrinogen concentration, and prolongation of clotting times such as prothrombin time (PT) [16]. ST elevation myocardial infarction (STEMI) is diagnosed if there is >1–2 mm of ST elevation in two contiguous leads on the electrocardiography (ECG) or new left bundle branch block (LBBB) with a clinical picture consistent with ischemic chest pain [17]. Non-ST elevation myocardial infarction (NSTEMI) is diagnosed through elevated levels of creatine kinase-myocardial band (CK-MB), troponin I, and troponin T and an ECG [18]. The diagnosis of pulmonary embolism was diagnosed by detecting a filling defect in the pulmonary arteries on computed tomography (CT) pulmonary angiography [19]. Duplex ultrasonography was used as the standard imaging test to diagnose deep vein thrombosis (DVT) [20]. CT venography (CT-V) protocol was used in the diagnosis of cerebral vein thrombosis [21]. The treatment of the patients was arranged according to the current guidelines [15]. Antithrombotic medication was started in all patients during hospitalization in accordance with current guidelines [15].

2.4. Prospective follow-up and primary end point

In-hospital mortality and thrombotic complications of patients were recorded. All-cause mortality was considered the primary end point.

2.5. Statistical analysis

Statistical Program for Social Sciences 26 (IBM SPSS, Chicago, IL, USA) was used for all statistical calculations. Kolmogorov-Smirnov test was used to determine whether the data fit the normal distribution. Continuous variables that fit the normal distribution were expressed as means ± standard deviation (SD), and those that did not fit the normal distribution were expressed as median with interquartile range (IQR). Comparisons between groups were analyzed using the Mann-Whitney *U* test, independent *t*-test and one-way analysis of variance (ANOVA), where appropriate. Chi-square test was applied to categorical variables. Pearson correlation coefficient was used to determine the relationship between SCUBE1 levels and other variables. Multivariate regression analyses were performed to determine the independent predictors of presence of thrombotic complications. Baseline variables with significant significance ($p < 0.05$) by univariate analysis were included in the multivariate logistic regression analysis.

To determine the predictive value of SCUBE1 in predicting thrombotic complications and in-hospital mortality, a pairwise comparison with D-dimer and fibronogen was performed by performing Receiver

Table 1
Demographic, clinical and biochemical characteristics of COVID-19 patients and control group.

Variables	Control Group ($n = 553$)	COVID -19 Group (n = 553)	p value
Demographics and medical history			
Age, years	61.7 ± 12.3	62.0 ± 12.7	0.662
Gender,male,n (%)	368 (66.5)	393 (71.0)	0.174
BMI, kg/m^2	24.34 ± 3.04	24.45 ± 3.91	0.290
Diabetes mellitus, n (%)	73 (13.2)	93 (16.8)	0.102
Hypertension, n (%)	317 (57.3)	344 (62.2)	0.098
Dyslipidemia, n (%)	401 (72.5)	442 (79.9)	0.004
Smoking, n (%)	261 (47.1)	315 (56.9)	0.001
Laboratory results			
FPG, (mg/dL)	122.6 (118.4–127.5)	132.3 (127.1–138.4)	<0.001
Creatinine, (mg/dL)	0.85 ± 0.30	0.88 ± 0.37	0.166
Uric acid, (mg/dL)	5.48 ± 2.68	5.22 ± 1.53	0.054
Albumin, (mg/dL)	4.49 ± 3.75	4.23 ± 1.67	0.143
LDH, (mg/dL)	250.0 (202.0–330.0)	262 (225.0–351.0)	0.003
Triglycerides, (mg/dL)	151.0 (108.0–216.0)	139.0 (86.5–180.0)	0.078
TC, (mg/dL)	183.0 (149.0–217.0)	175.0 (141.0–201.0)	0.609
HDL-C, (mg/dL)	35.0 (30.0–41.0)	35.0 (29.0–41.0)	0.609
LDL-C, (mg/dL)	111.5 (84.0–137.0)	107.0 (83.0–130.0)	0.176
CRP, (mg/dL)	0.57 (0.46–0.76)	1.55 (1.30–1.83)	<0.001
e-GFR, (ml/min)	91.12 ± 18.09	86.77 ± 19.88	<0.001
WBC, (×1000/mm^3)	10.1 (8.2–13.5)	10.2 (7.6–14.1)	0.028
Lymphocyte, (×1000/mm^3)	2.50 (2.20–2.87)	1.40 (1.13–1.70)	<0.001
Monocytes, (×1000/mm^3)	0.57(0.53–0.60)	0.57 (0.40–0.72)	0.323
Neutrophil, (×1000/mm^3)	5.90 (4.44–7.80)	6.75 (4.41–10.07)	<0.001
Phosphorus, (mg/dL)	3.20 (2.80–3.70)	3.10 (2.60–3.60)	0.001
Calcium, (mg/dL)	9.20 (8.90–9.60)	9.0 (8.61–9.38)	<0.001
Hemoglobin, (mg/dL)	14.2 ± 2.78	13.80 ± 1.90	0.001
Hematocrit, (%)	44.01 ± 7.99	43.09 ± 9.15	0.076
Platelet count, (×1000/mm^3)	261.0 (224.0–308.0)	288.0 (217.5–363.0)	<0.001
Fibrinogen, (mg/dL)	163.0 (154.0–228.8)	288.0 (241.0–350.0)	<0.001
D-dimer, mg/L	1.10 (0.21–1.55)	2.10 (1.50–2.80)	<0.001
SCUBE1, ng/mL	0.70 (0.18–1.47)	2.30 (1.56–2.84)	<0.001

Values are mean ± SD, n (%), or median (interquartile range) unless otherwise stated.
Abbreviations: BMI: body mass index; CRP: C- reactive protein; FPG: Fasting plasma glucose; eGFR: estimated glomerular filtration rate; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; WBC: White blood cell.

Table 2
Demographic, clinical and biochemical characteristics of patients with COVID-19 according to SCUBE1 levels.

Variables	Groups of SCUBE1		p-Value
	Lower SCUBE1 (≤2.30 ng/mL) (n:276)	Higher SCUBE1 (>2.30 ng/mL) (n:277)	
Demographics and medical history			
Age, years	61.50 ± 12.66	62.67 ± 12.78	0.196
Gender,male,n (%)	194 (70)	199 (72)	0.687
BMI, kg/m^2	24.76 ± 2.98	25.59 ± 4.11	0.158
Diabetes mellitus, n (%)	39 (14.1)	54 (19.4)	0.092
Hypertension, n (%)	159 (57.6)	185 (66.7)	0.026
Dyslipidemia, n (%)	226 (81.8)	216 (77.9)	0.252
Smoking, n (%)	139 (50.3)	176 (63.5)	
Laboratory results			
FPG, (mg/dL)	114.0 (94.0–141.0)	117.0 (100.0–143.0)	0.210
Creatinine, (mg/dL)	0.90 ± 0.46	0.86 ± 0.24	0.204
Uric acid, (mg/dL)	5.27 ± 1.58	5.17 ± 1.48	0.262
Albumin, (mg/dL)	4.33 ± 2.33	4.13 ± 0.43	0.085
LDH, (mg/dL)	261.0 (220.5–344.5)	262.0 (225.0–378.0)	0.249
Triglycerides, (mg/dL)	126.0 (90.25–183.0)	115.0 (84.0–176.0)	0.097
TC, (mg/dL)	174.0 (140.0–199.0)	176.0 (144.0–203.0)	0.549
HDL-C, (mg/dL)	35.0 (30.0–40.7)	35.0 (28.5–41.0)	0.909
LDL-C, (mg/dL)	105.4 (81.0–131.7)	109.0 (86.0–129.0)	0.653
CRP, (mg/dL)	0.49 (0.15–1.06)	0.54 (0.16–2.28)	0.013
e-GFR, (ml/min)	86.41 ± 20.56	87.13 ± 19.21	0.862
WBC, (×1000/mm^3)	10.0 (7.4–14.2)	10.7 (8.40–14.4)	0.016
Lymphocyte, (×1000/mm^3)	1.65 (1.25–1.80)	1.30 (0.98–1.57)	<0.001
Monocytes, (×1000/mm^3)	0.55 (0.40–0.72)	0.58 (0.40–0.73)	0.697
Neutrophil, (×1000/mm^3)	5.90 (4.09–8.97)	7.80 (5.15–11.0)	<0.001
Phosphorus, (mg/dL)	3.10 (2.70–3.70)	3.00 (2.50–3.50)	0.007
Calcium, (mg/dL)	9.10 (8.70–9.40)	9.00 (8.50–9.36)	0.166
Hemoglobin, (mg/dL)	13.69 ± 1.87	13.92 ± 1.92	0.246
Hematocrit, (%)	42.41 ± 5.42	43.478 ± 11.72	0.173
Platelet count, (×1000/mm^3)	308.0 (237.2–369.0)	260.0 (204.5–350.5)	<0.001
Fibrinogen, (mg/dL)	258.0 (211.7–308.0)	322.0 (258.0363.5)	<0.001
D-dimer, mg/L	1.59 (1.21–2.11)	2.59 (1.66–3.11)	<0.001
Severity of disease, severe cases, n (%)	24 (8.6)	52 (18.7)	0.001

Values are mean ± SD, n (%), or median (interquartile range) unless otherwise stated.
Abbreviations: BMI: body mass index; CRP: C- reactive protein; FPG: Fasting plasma glucose; eGFR: estimated glomerular filtration rate; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; WBC: White blood cell.

operating characteristic (ROC) analysis. The predictive validities were quantified as the area under the ROC curve (c statistics), and these comparisons were done using MedCalc 16 statistic software (MedCalc Software Ltd., Ostend, Belgium). The optimal cut-off value was calculated from the point of maximum sensitivity and specificity (Youden's index). Also, patients' baseline characteristics were classified as lower SCUBE1 and higher SCUBE1 based on their admission SCUBE1 level. The median value of 2.30 ng/mL of SCUBE1 levels of patients with COVID-19 was taken as the cut-off value to categorize patients according to SCUBE1 levels.

The cumulative events of these two groups were evaluated using Kaplan-Meier curves and compared using the log-rank test.Overall survival time was calculated to the in-hospital day of death or the date of last discharge. In addition, univariate and multivariate Cox proportional hazards model analysis was used to evaluate the relationship between admission SCUBE1 levels and all-cause in-hospital deaths. Five models were created to show the effects of possible confounders on the relationship between SCUBE1 level and in-hospital mortality. The model 1 was unadjusted. Model 2 was adjusted for sex and age. Based on model 2, model 3 was further adjusted for status of smoking, history of diabetes mellitus, hypertension, dyslipidemia. Based on model 3, model 4 was further adjusted for white blood cell, lymphocyte, neutrophil, phosphorus and platelet. Based on model 4, model 5 was further adjusted for D-dimer and fibrinogen. Results of Cox regression analysis was presented as hazard ratio (HR) and 95 % confidence interval (CI). Two-tailed p-values of <0.05 were considered to be statistically significant.

3. Results

553 patients who had COVID-19 and were hospitalized and 553 healthy subjects were included in the study as the control group (mean age 62.0 ± 12.7, 71.0 % male; 61.7 ± 12.3 years, 66.5 % male; respectively). These two groups were compared in terms of baseline demographic, clinical, biochemical characteristics and SCUBE1 levels (Table 1). Cardiovascular comorbidities, dyslipidemia, and smoking were more common in the COVID-19 group compared to the control group ($p = 0.004$, $p = 0.001$; respectively). In the group with COVID-19,

Table 3
Thrombotic complications seen in patients.

Disseminated intravascular coagulation, n	4
Non-ST elevation myocardial infarction, n	12
ST elevation myocardial infarction, n	2
Pulmonary embolism (without DVT), n	3
Deep vein thrombosis, n	8
Central vein thrombosis, n	1

fasting plasma glucose (FPG), lactate dehydrogenase (LDH), C-protein (CRP), white blood cell (WBC), neutrophil, platelet counts, fibrinogen, D-dimer and SCUBE1 levels were higher than in the control group, in addition, the estimated glomerular filtration rate (e-GFR), lymphocyte, phosphorus, calcium and hemoglobin levels were lower ($p < 0.05$, for all). In the patient group, hypertension was more common in the higher SCUBE1 group than in the lower SCUBE1 group ($p = 0.026$) and WBC, neutrophil counts and fibrinogen, D-dimer levels were higher, lymphocyte, platelet counts and phosphorus levels were lower ($p < 0.05$, for all).

In addition, the higher SCUBE1 group had more severe cases than the lower SCUBE1 group (52 [18.7 %] vs. 24 [8.6 %]; $p = 0.001$) (Table 2).

Thrombotic complications were seen in 30 patients during admission and hospitalization. Of these, 4 had disseminated intravascular coagulation (DIC), 12 had Non-ST-leveled myocardial infarction (NSTEMI), 2 had ST-elevation myocardial infarction (STEMI), 3 had pulmonary embolism (without evidence of DVT), 8 had deep vein thrombus (DVT), central vein thrombus was detected in 1 of them (Table 3). SCUBE1 levels were significantly higher in patients with COVID-19 than in patients without ($p < 0.001$), and SCUBE1 levels were significantly higher in patients with severe disease than those with mild to moderate disease ($p < 0.001$) (Fig. 1A). In addition, SCUBE1 levels were significantly higher in patients with thrombotic complications than in patients without ($p < 0.001$) (Fig. 1B).

A positive correlation was found between SCUBE1 and D-dimer (Fig. 2A) and fibrinogen (Fig. 2B) in the correlation analysis ($r = 604$, $p < 0.001$; $r = 458$, $p < 0.001$; respectively). D-dimer, Severity of Disease, SCUBE1 were determined as independent predictors for thrombotic complications in multivariate analysis (OR 2.499, 95 % CI 1.240–5.038, $p = 0.010$; OR 5.708, 95 % CI 1.746–18.660, $p = 0.004$; OR 4.343, 95 % CI 1.818–10.373, $p = 0.001$; respectively) (Table 4). When the predictive value of thrombotic complications of SCUBE1, D-dimer and fibrinogen were compared pairwise with ROC analysis, there was no statistically significant difference between SCUBE1 and D-dimer ($p = 0.3601$), while the difference between SCUBE1 and fibrinogen was significant ($p = 0.0044$) (Fig. 3A). At the same time, when the in-hospital mortality predictive values of SCUBE1, D-dimer and fibrinogen were compared pairwise with ROC analysis, there was no statistically significant difference between SCUBE1 and D-dimer ($p = 0.0867$), but the difference between SCUBE1 and fibrinogen was significant (0.0033) (Fig. 3B).

The Kaplan-Meier cumulative survival curve shows that in-hospital mortality was significantly higher in the higher SCUBE1 group than in

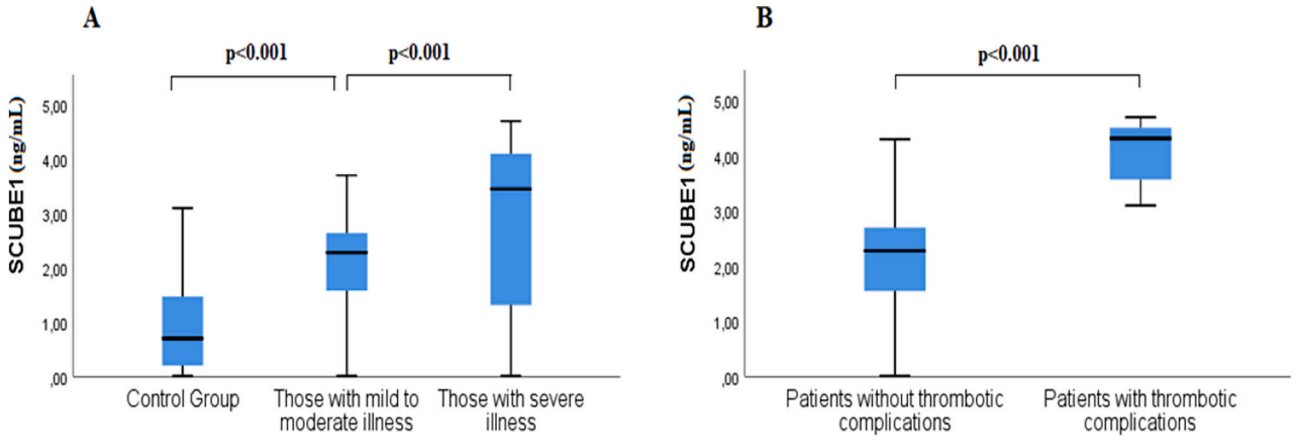


Fig. 1. In patients with COVID-19, SCUBE1 levels were significantly higher in patients with both mild to moderate and severe disease than in the control group, and SCUBE1 levels were significantly higher in patients with severe disease than in those with mild to moderate disease (A), Patients with COVID-19 also had significantly higher SCUBE1 levels in the group with thrombotic complications compared to the group with no thrombotic complications (B).

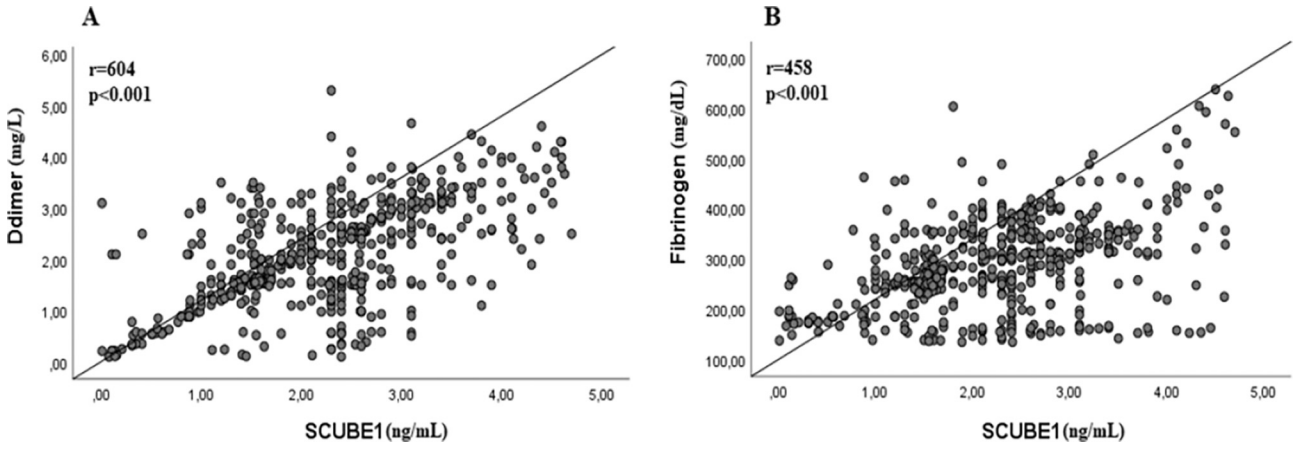


Fig. 2. Scatter/dot plots showing positive correlation of SCUBE1 with D-dimer (A) and Fibrinogen (B).

Table 4
Univariate and Multivariate Logistic Regression Analysis for Identifying Independent Predictors of Thrombotic Complications.

Variables	Univariate analyses		Multivariate analyses	
			Nagelkerke R^2 in final step = 0.607	
	OR (95 % CI)	p-Value	OR (95 % CI)	p-Value
Fibrinogen	1.011 (1.007–1.015)	<0.001	1.003 (0.998–1.008)	0.193
Platelet	0.989 (0.984–0.994)	<0.001	0.997 (0.991–1.002)	0.271
D-dimer	6.184 (3.531–10.830)	<0.001	2.499 (1.240–5.038)	0.010
Severity of disease	29.137 (11.936–71.127)	<0.001	5.708 (1.746–18.660)	0.004
SCUBE1	13.886 (6.828–28.243)	<0.001	4.343 (1.818–10.373)	0.001

Abbreviations: OR: Odds ratio; CI: Confidence Interval.

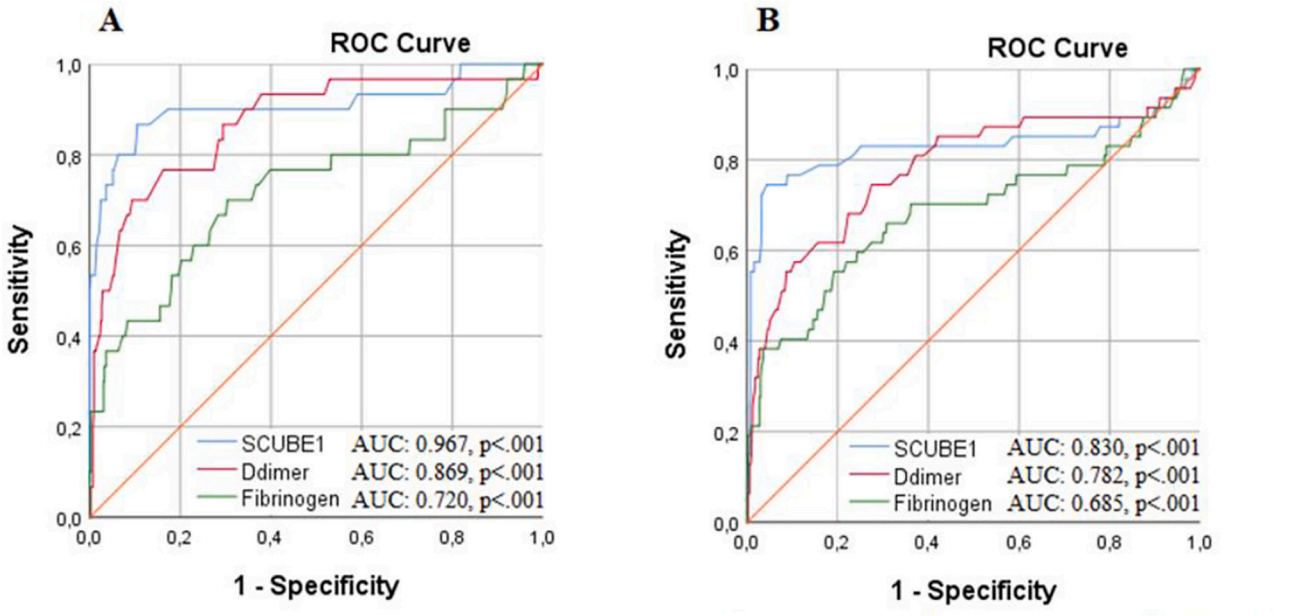
the lower SCUBE1 group (Log-rank test: $p < 0.001$) (Fig. 4). Severe cases, thrombotic complications, and in-hospital mortality rates were significantly higher in the higher SCUBE1 group than in the lower SCUBE1 group ($p = 0.001$, $p < 0.001$, $p < 0.001$; respectively) (Table 5). The Cox regression analysis for in-hospital mortality related with the plasma SCUBE1 levels are presented in Table 6. Even after adjustment for multiple confounding factors (model 5), SCUBE1 was found to be an independent predictor of in-hospital mortality (HR = 2.692, 95 % CI: 1.879–3.858; $p < 0.001$) (Table 6).

4. Discussion

First, the major finding of this study is that SCUBE1 levels were found to be higher in patients who had COVID-19 compared to those who did not. In addition, patients with severe disease (patients requiring intensive care follow-up) had higher levels of SCUBE1 in plasma than those with mild to moderate disease. Second, thrombotic complications and in-hospital mortality during the disease course were higher at higher SCUBE1 levels and elevated SCUBE1 levels were found to be an independent predictor for thrombotic complications and in-hospital mortality in patients with COVID-19. To the best of our knowledge, this is the first study to investigate the relationship between SCUBE1 and thrombotic complications and in-hospital mortality during the course of COVID-19.

The COVID-19 pandemic has heavily affected the world in recent years as a major cause of mortality and morbidity [1]. Thrombotic complications are prominent among these increased mortality and morbidities [16]. These complications may be the manifestation of a systemic involvement, or they may accompany an isolated pulmonary involvement alone [17]. Although many mechanisms have been suggested for this prethrombogenic environment seen during the course of COVID-19 disease, the main cause has not been understood and it has been mentioned that vasculopathy, which is the result of the interaction of many thrombogenic parameters, may play an important role in this process [18].

The primary targets of the COVID-19 are endothelial cells, pneumocytes, immune cells [19]. The main causes of acute lung failure in COVID-19 are virus-induced alveolar damage and thrombosis in the pulmonary microvascular bed [20]. Vasculopathy occurring during the course of the disease results from the activation of a pathological process directly caused by the COVID-19 infection and involving the immune system, including thrombo-inflammatory responses [20]. As a result of



Pairwise comparison of ROC curves according to thrombotic complications

Compared Variables	ΔAUC	95%CI	p
SCUBE1 & D-dimer	0.0380	-0.043-0.119	0.3601
SCUBE1 & Fibrinogen	0.1870	0.058-0.317	0.0044
D-dimer & Fibrinogen	0.1490	0.022-0.276	0.0209

Pairwise comparison of ROC curves according to in-hospital mortality

Compared Variables	ΔAUC	95%CI	p
SCUBE1 & D-dimer	0.0479	-0.006-0.103	0.0867
SCUBE1 & Fibrinogen	0.1450	0.048-0.242	0.0033
D-dimer & Fibrinogen	0.0972	-0.005-0.200	0.0643

Fig. 3. Pairwise comparison of ROC curves of SCUBE1, D-dimer and Fibrinogen to predict thrombotic complications (A), Pairwise comparison of ROC curves of SCUBE1, D-dimer and Fibrinogen to predict in-hospital mortality (B). Abbreviations: AUC: Area under the curve; ΔAUC: Difference between areas under the curve; ROC: Receiver operating characteristics; CI: Confidence Interval.

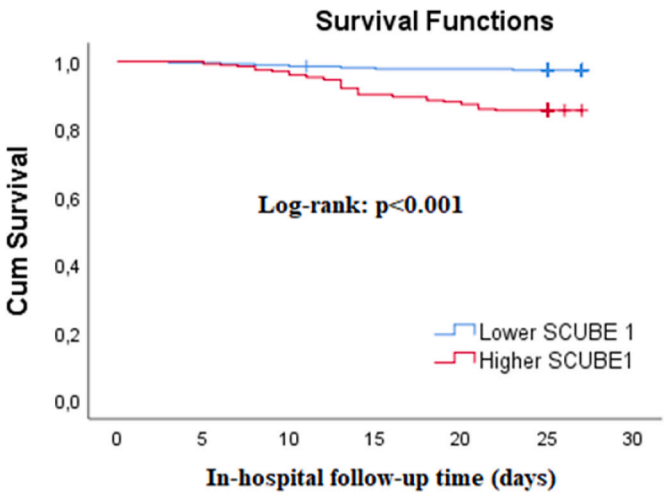


Fig. 4. Kaplan-Meier survival curve of in-hospital all-cause mortality according to SCUBE1 level.

Table 5
Severity of disease, thrombotic complications and in-hospital mortality rates according to plasma SCUBE 1 levels.

In-hospital follow-up	Lower SCUBE1 (≤2.30 ng/mL) (n: 276)	Higher SCUBE1 (>2.30 ng/mL) (n: 277)	p value
Severity of disease, severe cases, n (%)	24 (8.6)	52 (18.7)	0.001
Thrombotic complications, n (%)	2 (0.7)	28 (10.1)	<0.001
In-hospital mortality, n (%)	7 (2.5)	40 (14.4)	<0.001

Table 6
Cox Regression Models to Estimate the Effects of SCUBE 1 Levels on in-hospital mortality.

	In-hospital mortality	
	Hazard ratio (95 % CI)	p-Value
Model 1	5.296 (3.731–7.517)	<0.001
Model 2	5.296 (3.731–7.517)	<0.001
Model 3	5.296 (3.731–7.517)	<0.001
Model 4	4.226 (2.623–5.807)	<0.001
Model 5	2.692 (1.879–3.858)	<0.001

Model 1: Unadjusted. Model 2: adjusted for age and sex. Model 3: further adjusted for smoking, history of diabetes mellitus, hypertension, dyslipidemia. Model 4: further adjusted for white blood cell, lymphocyte, neutrophil, phosphorus and platelet. Model 5: further adjusted for D-dimer and fibrinogen. CI: confidence interval.

this process, both microvascular and macrovascular thrombotic complications occur in the arterial, venous and capillary vascular bed [21]. In studies, it has been shown that besides endothelial damage in vasculopathy caused by COVID-19 virus, vasculitis with direct effect and disruption of coagulation hemostasis between endothelium and platelet play a role in the precoagulative state in the disease process [18]. COVID-19 disease causes hypercoagulation, platelet activation and endothelial dysfunction with acutely increased inflammatory process [22]. In COVID-19 patients presenting with thrombotic complications, increased fibrinogen and D-dimer levels are usually observed initially, whereas minor changes in prothrombin time and platelet count are detected [17,23]. In critically severe patients, decreased fibrinogen and thrombocytopenia can be observed in the late stages of the disease [24].

SCUBE1 is highly expressed in both vascular endothelial cells and platelets [25,26]. This glycoprotein is secreted and surface-exposed on activated platelets, promotes platelet-platelet interaction and promotes platelet-matrix adhesion and agglutination and therefore, increased plasma level of SCUBE1 is used as a biomarker of platelet activation in acute thrombotic diseases [26,27]. Clinical studies have shown significantly higher plasma SCUBE1 levels in acute thrombotic complication processes such as acute coronary syndrome, acute ischemic stroke, high thrombus load in patients presenting with STEMI, and the no-reflow phenomenon after stent implantation [28–30]. In the experimental study by Wu et al., they showed that plasma SCUBE1 is not only a biomarker in acute thrombotic processes, but also plays an active role in the thrombosis process [8]. In addition, in a study by Heit et al., SCUBE1 gene polymorphism was found to be associated with venous thromboembolism [31]. In a study by Topcu et al., higher serum SCUBE1 levels were found in breast cancer patients known to cause a procoagulant state compared to the control group [32]. Various circulating inflammatory coagulation biomarkers directly involved in coagulation such as fibrinogen, D-dimer, P-selectin and von Willebrand Factor (VWF) have been shown to take an active role in the procoagulant process in the COVID-19 disease [33]. An imbalance between procoagulant and anticoagulant factors in the disease process causes thrombotic complications during the disease [34]. Our findings suggest that SCUBE1 also plays an active role in the prothrombotic process during the course of COVID-19 disease.

In COVID 19 patients, in accordance with our study, thrombotic complications are more common in severe cases, and mortality is higher in patients with thrombotic complications [34]. In our study, high SCUBE1 levels in patients with severe course requiring ICU follow-up and those with thrombotic complications make us think that SCUBE1 may play an active role especially in severe cases with thrombotic complications. In this context, SCUBE1 may be a therapeutic target in patients with COVID-19.

4.1. Limitations

Our study had some limitations. First of all, this study was an observational study and the number of patients was relatively small. Secondly, outpatient follow-up patients with COVID were not included in the study. Third, SCUBE1 measurements were based on a single plasma sample only. Serial follow-ups of SCUBE1 in the course of the disease were not performed.

5. Conclusions

SCUBE 1 levels were higher than in the control group, and higher SCUBE1 levels were associated with disease severity, in-hospital mortality, and thrombotic complications. Based on these findings, it can be said that SCUBE1 can indicate disease severity, in-hospital mortality and hypercoagulability. Therefore, high SCUBE1 levels measured in the early stages of the disease may predict more intensive medical therapy to prevent disease progression and more intensive antithrombotic therapy to prevent thrombotic complications. In this respect, it may be a useful biomarker and therapeutic target in patients with COVID-19. Further studies are needed to confirm these findings.

Declaration of competing interest

The authors report no conflict of interest or financial support.

References

[1] R. Ochani, A. Asad, F. Yasmin, S. Shaikh, H. Khalid, S. Batra, et al., COVID-19 pandemic: from origins to outcomes. A comprehensive review of viral pathogenesis, clinical manifestations, diagnostic evaluation, and management, Infez Med. 29 (2021) 20–36.
[2] M. Iwasaki, J. Saito, H. Zhao, A. Sakamoto, K. Hirota, D. Ma, Inflammation triggered by SARS-CoV-2 and ACE2 augment drives multiple organ failure of severe

COVID-19: molecular mechanisms and implications, Inflammation 44 (2021) 13–34.
[3] J.E. Gómez-Mesa, S. Galindo-Coral, M.C. Montes, A.J. Muñoz Martin, Thrombosis and coagulopathy in COVID-19, Curr. Probl. Cardiol. 46 (3) (2021 Mar), 100742.
[4] A. Birnhuber, E. Fließer, G. Gorkiewicz, M. Zacharias, B. Seeliger, S. David, et al., Between inflammation and thrombosis: endothelial cells in COVID-19, Eur. Respir. J. 58 (2021) 2100377.
[5] M. Ackermann, S.E. Verleden, M. Kuehnel, A. Haverich, T. Welte, F. Laenger, A. Vanstapel, C. Werlein, H. Stark, A. Tzankov, W.W. Li, V.W. Li, S.J. Mentzer, D. Jonigk, Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19, N. Engl. J. Med. 383 (2) (2020 Jul 9) 120–128.
[6] S. Zhang, Y. Liu, X. Wang, L. Yang, H. Li, Y. Wang, M. Liu, X. Zhao, Y. Xie, Y. Yang, S. Zhang, Z. Fan, J. Dong, Z. Yuan, Z. Ding, Y. Zhang, L. Hu, SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19, J. Hematol. Oncol. 13 (1) (2020 Sep 4) 120.
[7] W.J. Liao, M.Y. Wu, C.C. Peng, Y.C. Tung, R.B. Yang, Epidermal growth factor-like repeats of SCUBE1 derived from platelets are critical for thrombus formation, Cardiovasc. Res. 116 (1) (2020 Jan 1) 193–201.
[8] M.Y. Wu, Y.C. Lin, W.J. Liao, C.F. Tu, M.H. Chen, S.R. Roffler, R.B. Yang, Inhibition of the plasma SCUBE1, a novel platelet adhesive protein, protects mice against thrombosis, Arterioscler. Thromb. Vasc. Biol. 34 (7) (2014 Jul) 1390–1398.
[9] D.F. Dai, P. Thajeb, C.F. Tu, F.T. Chiang, C.H. Chen, R.B. Yang, J.J. Chen, Plasma concentration of SCUBE1, a novel platelet protein, is elevated in patients with acute coronary syndrome and ischemic stroke, J. Am. Coll. Cardiol. 51 (22) (2008 Jun 3) 2173–2180.
[10] C.F. Tu, Y.T. Yan, S.Y. Wu, B. Djoko, M.T. Tsai, C.J. Cheng, R.B. Yang, Domain and functional analysis of a novel platelet-endothelial cell surface protein, SCUBE1, J. Biol. Chem. 283 (18) (2008 May 2) 12478–12488.
[11] R.B. Yang, C.K. Ng, S.M. Wasserman, S.D. Colman, S. Shenoy, F. Mehraban, L. G. Komuves, J.E. Tomlinson, J.N. Topper, Identification of a novel family of cell-surface proteins expressed in human vascular endothelium, J. Biol. Chem. 277 (48) (2002 Nov 29) 46364–46373.
[12] C.F. Tu, Y.H. Su, Y.N. Huang, M.T. Tsai, L.T. Li, Y.L. Chen, C.J. Cheng, D.F. Dai, R. B. Yang, Localization and characterization of a novel secreted protein SCUBE1 in human platelets, Cardiovasc. Res. 71 (3) (2006 Aug 1) 486–495.
[13] W.J. Liao, K.C. Tsao, R.B. Yang, Electrostatics and N-glycan-mediated membrane tethering of SCUBE1 is critical for promoting bone morphogenetic protein signalling, Biochem. J. 473 (5) (2016 Mar 1) 661–672.
[14] Y.H. Jin, Q.Y. Zhan, Z.Y. Peng, X.Q. Ren, X.T. Yin, L. Cai, et al., Evidence-Based Medicine Chapter of China International Exchange and Promotive Association for Medical and Health Care (CPAM); Chinese Research Hospital Association (CRHA). Chemoprophylaxis, diagnosis, treatments, and discharge management of COVID-19: An evidence-based clinical practice guideline (updated version), Mil Med Res. 7 (1) (2020) 41.
[15] Coronavirus Disease, (COVID-19) Treatment Guidelines [Internet], National Institutes of Health (US), Bethesda (MD), 2019, 2021 Apr 21–2022 Aug 8.
[16] M. Levi, Diagnosis and treatment of disseminated intravascular coagulation, Int. J. Lab. Hematol. 36 (3) (2014 Jun) 228–236.
[17] B. Ibanez, S. James, S. Agewall, M.J. Antunes, C. Bucciarelli-Ducci, H. Bueno, et al., ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC), Eur Heart J. 39 (2) (2018 Jan 7) 119–177.
[18] M. Roffi, C. Patrono, J.P. Collet, C. Mueller, M. Valgimigli, F. Andreotti, et al., ESC scientific document group. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC), Eur Heart J. 37 (3) (2016 Jan 14) 267–315.
[19] S.V. Konstantinides, G. Meyer, C. Becattini, H. Bueno, G.J. Geersing, V.P. Harjola, et al., The task force for the diagnosis and management of acute pulmonary embolism of the european Society of Cardiology (ESC). 2019 ESC guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS): the task force for the diagnosis and management of acute pulmonary embolism of the european Society of Cardiology (ESC), Eur. Respir. J. 9;54(3):1901647 (2019 Oct).
[20] M.B. Streiff, G. Agnelli, J.M. Connors, M. Crowther, S. Eichinger, R. Lopes, et al., Guidance for the treatment of deep vein thrombosis and pulmonary embolism, J. Thromb. Thrombolysis 41 (1) (2016 Jan) 32–67.
[21] M.H. Rodallec, A. Krainik, A. Feydy, A. Hélias, J.M. Colombani, M.C. Jullès, V. Marteau, M. Zins, Cerebral venous thrombosis and multidetector CT angiography: tips and tricks, Radiographics. 26 (Suppl 1) (2006 Oct).
[22] J.E. Gómez-Mesa, S. Galindo-Coral, M.C. Montes, A.J. Muñoz Martin, Thrombosis and coagulopathy in COVID-19, Curr. Probl. Cardiol. 46 (3) (2021 Mar), 100742.
[23] G.D. Wool, J.L. Miller, The impact of COVID-19 disease on platelets and coagulation, Pathobiology 88 (1) (2021) 15–27.
[24] T. Iba, J.M. Connors, J.H. Levy, The coagulopathy, endotheliopathy, and vasculitis of COVID-19, Inflamm. Res. 69 (12) (2020 Dec) 1181–1189.
[25] R.C. Becker, COVID-19-associated vasculitis and vasculopathy, J. Thromb. Thrombolysis 50 (3) (2020 Oct) 499–511.
[26] M. Ackermann, S.E. Verleden, M. Kuehnel, A. Haverich, T. Welte, F. Laenger, A. Vanstapel, C. Werlein, H. Stark, A. Tzankov, W.W. Li, V.W. Li, S.J. Mentzer, D. Jonigk, Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19, N. Engl. J. Med. 383 (2) (2020 Jul 9) 120–128.
[27] R. Flaumenhaft, K. Enjyoji, A.A. Schmaier, Vasculopathy in COVID-19, Blood 140 (3) (2022 Jul 21) 222–235.
[28] M. Levi, J. Thachil, T. Iba, J.H. Levy, Coagulation abnormalities and thrombosis in patients with COVID-19, Lancet Haematol. 7 (6) (2020) e438–e440440.
[29] I. Eljilany, A.N. Elzouki, D-dimer, fibrinogen, and IL-6 in COVID-19 patients with suspected venous thromboembolism: a narrative review, Vasc. Health Risk Manag. 13 (16) (2020 Nov) 455–462.
[30] C. Barale, E. Melchionda, A. Morotti, I. Russo, Prothrombotic phenotype in COVID-19: focus on platelets, Int. J. Mol. Sci. 22 (24) (2021 Dec 20) 13638.
[31] R.B. Yang, C.K. Ng, S.M. Wasserman, S.D. Colman, S. Shenoy, F. Mehraban, L. G. Komuves, J.E. Tomlinson, J.N. Topper, Identification of a novel family of cell-surface proteins expressed in human vascular endothelium, J. Biol. Chem. 277 (48) (2002 Nov 29) 46364–46373.
[32] C.F. Tu, Y.H. Su, Y.N. Huang, M.T. Tsai, L.T. Li, Y.L. Chen, C.J. Cheng, D.F. Dai, R. B. Yang, Localization and characterization of a novel secreted protein SCUBE1 in human platelets, Cardiovasc. Res. 71 (3) (2006 Aug 1) 486–495.
[33] C.F. Tu, Y.T. Yan, S.Y. Wu, B. Djoko, M.T. Tsai, C.J. Cheng, R.B. Yang, Domain and functional analysis of a novel platelet-endothelial cell surface protein, SCUBE1, J. Biol. Chem. 283 (18) (2008 May 2) 12478–12488.
[34] D.F. Dai, P. Thajeb, C.F. Tu, F.T. Chiang, C.H. Chen, R.B. Yang, J.J. Chen, Plasma concentration of SCUBE1, a novel platelet protein, is elevated in patients with acute coronary syndrome and ischemic stroke, J. Am. Coll. Cardiol. 51 (22) (2008 Jun 3) 2173–2180.