

The diagnostic significance of signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein-1 levels in pulmonary embolism

Nigar Dirican, Ali Duman¹, Gülcan Sağlam², Akif Arslan³, Onder Ozturk, Sule Atalay, Ahmet Bircan, Ahmet Akkaya, Munire Cakir

Departments of Chest Diseases and ³Cardiology, Faculty of Medicine, Suleyman Demirel University, Isparta, ¹Department of Emergency Medicine, Faculty of Medicine, Adnan Menderes University, Aydın, ²Department of Biochemistry, Medical Park Hospital, Usak, Turkey

Address for correspondence:

Dr. Nigar Dirican,
Department of Chest Diseases, Faculty of Medicine, Suleyman Demirel University, 32260 Isparta, Turkey.
E-mail: nigardirican@yahoo.com

Submission: 06-07-2016
Accepted: 30-07-2016

Access this article online

Quick Response Code:



Website:

www.thoracicmedicine.org

DOI:

10.4103/1817-1737.191876

Abstract:

BACKGROUND: Pulmonary embolism (PE) is a common and potentially life-threatening disorder. Patients with PE often have nonspecific symptoms, and the diagnosis is often delayed.

AIM: The aim of our study was to investigate the role of signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1 (SCUBE1) used in the diagnosis of PE.

METHODS: The study was designed prospectively. A total of 57 patients who were admitted to emergency service with clinically suspected PE were included in the study. The patients diagnosed with PE were defined as PE group ($n = 32$), and the patients with undetectable embolism on computerized tomographic pulmonary angiography were defined as non-PE group ($n = 25$). Twenty-five age- and sex-matched healthy cases were chosen for the study. Routine biochemical analysis, complete blood count, D-dimer, SCUBE1, and arterial blood gas analysis were performed early after admission.

RESULTS: Mean SCUBE1 levels were higher in the PE group (0.90 ng/mL) than in the non-PE (0.38 ng/mL) and control groups (0.47 ng/mL) ($P < 0.01$). A cutoff point of 0.49 ng/mL for SCUBE1 indicated 100% sensitivity and 64% specificity in patients with PE. Mean D-dimer levels were not different between PE and non-PE groups ($P = 0.591$). A multivariable logistic regression analysis (with dichotomous PE groups as the response variable; age, gender, chest pain, syncope, diabetes mellitus, chronic obstructive pulmonary disease, hypertension, D-dimer, neutrophil-lymphocytes ratio, and SCUBE1 variables as predictors) showed that the significant and independent predictors of PE diagnosis were SCUBE1 and chest pain.

CONCLUSION: This study suggests that serum SCUBE1 measurement might be used as a diagnostic biomarker in PE.

Key words:

Diagnosis, pulmonary embolism, signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1

Acute pulmonary embolism (PE) is a relatively common and potentially life-threatening vascular disease. Its clinical symptoms and signs are nonspecific. Acute dyspnea, chest pain, syncope, and palpitations are the most frequent presenting symptoms of PE, representing more than 96% of PE presentations.^[1] Unfortunately, most cardiopulmonary diseases share at least one of these symptoms, making the differential diagnosis a daily clinical challenge. Diagnostic tests are necessary to establish the presence or absence of PE.^[2] Undiagnosed PE is fatal if left untreated in about 30% of patients.^[3]

Today, there is no specific biomarker for the diagnosis of PE. D-dimer has been mentioned, but positive predictive value of elevated D-dimer levels is low. Advanced age, the presence

of infection, pregnancy, active malignancy, recent surgical interventions, liver failure, and rheumatoid arthritis may result in false-positive

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Dirican N, Duman A, Sağlam G, Arslan A, Ozturk O, Atalay S, *et al*. The diagnostic significance of signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein-1 levels in pulmonary embolism. *Ann Thorac Med* 2016;11:277-82.

plasma D-dimer measurement. Anticoagulation therapy, the presence of small clots, isolated small pulmonary infarcts, and existing symptoms persisting for more than 5 days may lead to false-negative plasma D-dimer measurement results.^[4] Some prediction rules have been developed, such as Wells and Geneva scores that have been used in daily practice. However, today, despite all these methods, many patients with PE cannot be diagnosed easily. Therefore, we need new biomarkers that may help in the diagnosis.

The new biochemical marker, signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1 (SCUBE1), is a member of the SCUBE family and secreted cell surface protein expressed during early embryogenesis. It forms part of the epidermal growth factor (EGF) superfamily and consists of several domain structures, such as cysteine-rich and EGF-like repeats and CUB domain.^[5] The protein is found in platelet and endothelial cells.^[6] Studies suggested that the platelet is probably the principal source of the SCUBE1 expressed in the vascular system. These proteins are stored within the α -granules of inactive platelets, translocated to the platelet surface upon activation by thrombin, and incorporated into thrombus.^[7] Furthermore, SCUBE1 accumulation has been determined immunohistochemically in the subendothelial matrix of advanced atherosclerotic lesions in humans. Several studies have shown that SCUBE1 is a helpful biomarker in identifying acute thrombotic diseases, including acute coronary syndrome (ACS) and acute ischemic stroke (AIS).^[8] Turkmen *et al.* investigated the diagnostic value of SCUBE1 in patients with PE and healthy people. They reported high SCUBE1 levels in patients with PE.^[9]

Based on these previous results, we decided to conduct a new study. The aim of the present study was to compare the SCUBE1 levels in patients with PE, in patients with suspected PE, and healthy people and also to investigate the levels of SCUBE1 in other diseases which remain in the differential diagnosis of PE.

Methods

The study was designed prospectively. Data were collected at the Chest Disease Clinic of Suleyman Demirel University and Emergency Department of Adnan Menderes University. The local ethics committee approved the present study.

Study population

A total of 57 patients who were admitted to emergency service with clinically suspected PE were included in this prospective study. Definitive diagnosis of pulmonary thromboembolism was made by showing a filling defect of PTE on spiral computerized tomographic (CT) pulmonary angiography according to the predefined standard protocol.^[10] CT pulmonary angiography was applied to all patients. The patients diagnosed with PE were defined as PE group ($n = 32$), and the patients with undetectable embolism on CT pulmonary angiography were defined as non-PE group ($n = 25$). Twenty-five consecutive sex- and age-matched healthy individuals without relevant current status and medical history were included in the study. Patients with ACS, acute myocardial infarction, hypertensive crises, acute ischemic cerebrovascular disease, peripheral artery disease, advanced liver and kidney failure, idiopathic

cardiomyopathy, liver disease, chronic infection, autoimmune disease, and malignancy were excluded from the study. The exclusion criteria in the control group were the same as those in the patient groups.

Study design

The demographic, clinical, and laboratory characteristics of the patient groups were taken from the patients' histories and results of physical examinations. Routine biochemical analysis, complete blood count, D-dimer, and arterial blood gas analysis were performed early after admission. Wells and Geneva scores to assess the risk of PE were made. Echocardiographic examinations in patients with PE were performed by a cardiologist, and pulmonary arterial pressures were measured. In patients, plasma D-dimer examinations were performed using the automatic coagulation analyzer and the immune turbidimetry method, with reference values of 69–243 ng/mL.

Measurement of signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1

The serum was centrifuged at 4000 rpm for 20 min in sterile conditions. Sera were stored in clean and dry microcentrifuge tubes at -80°C before analysis. Obtaining the results did not exceed 6 months. Patient serum and a standard solution were pipetted into human SCUBE1 antibody-coated wells. Biotin-conjugated anti-SCUBE1 antibody was added to each well. After incubation at 37°C temperature for 2 h, the wells were washed three times with 350 μL of wash solution. Next, Streptavidin-HRP solution was added and allowed to incubate at 37°C temperature for an hour, and then the washing procedure was repeated. Chromogen solution was added, and incubation was carried out in the dark. Concentration was calculated according to the standard absorbance curve after absorbance was read at 450 nm by an enzyme-linked immunosorbent assay (ELISA) plate reader. Human SCUBE1 ELISA kit (Elabsience Biotechnology Co., Ltd., China, Catalog no: E-EL-H5405, Lot: AK0015NOV30024) was used with BIOTEK semiautomatic ELISA reader. The results were expressed as nanogram/milliliter. The analysis of SCUBE1 concentrations takes 6 h to complete.

Statistical analysis

All statistical analyses were performed using the SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to determine whether or not the parameters were normally distributed. Continuous variables were expressed as mean \pm standard deviation or median values. Categorical variables were expressed as numbers and percentages. The Chi-square test was used to compare the proportions in different groups. The Student's *t*-test or Mann–Whitney U-test was used to compare the two independent groups according to their distribution state. The Kruskal–Wallis test was used to compare more than two independent groups for cases with nonnormally distributed variables. In cases where the Kruskal–Wallis test yielded a statistical significance, a *post hoc* analysis was performed to identify the groups that showed differences using a Bonferroni-corrected Mann–Whitney U-test. The area under the receiver operating characteristics (ROC) curve was used to calculate the discriminative ability of SCUBE1 to determine patients with PE. Sensitivity, specificity, negative predictive

value, and positive predictive value were calculated according to ROC curves for SCUBE1. Logistic regression models were constructed for the disability as outcome. $P < 0.05$ was considered statistically significant.

Results

Thirty-two patients were diagnosed with PE, 16 were females and 16 were males, and the mean age was 61.1 years. Non-PE group comprised patients with pneumonia ($n = 14$), chronic obstructive pulmonary disease (COPD) exacerbations ($n = 5$), and congestive heart failure ($n = 6$). Among groups there were no significant differences in age and gender. Baseline characteristics of patient groups and healthy controls are shown in Table 1. Chest pain and syncope were significantly higher

Table 1: Demographic and clinical characteristics of pulmonary embolism, nonpulmonary embolism, and control groups

	n (%) or mean±SD			P
	PE (n=32)	Non-PE (n=25)	Control (n=25)	
Age (years)	61.1±17.1	63.2±15.2	62.0±14.6	0.365
Sex				
Male	16 (50)	12 (48)	15 (60)	0.739
Female	16 (50)	13 (52)	10 (40)	
Symptom				
Dyspnea	27 (84.4)	21 (84)	-	0.969
Chest pain	21 (65.6)	4 (16)	-	<0.001
Fever	2 (6.3)	9 (36)	-	0.005
Hemoptysis	5 (15.6)	2 (8)	-	0.384
Syncope	6 (18.8)	0	-	0.022
Coexisting condition				
Hypertension	7 (21.9)	7 (28)	-	0.594
Diabetes	2 (6.3)	6 (24)	-	0.056
COPD	1 (3.1)	5 (20)	-	0.039
Asthma	3 (9.4)	1 (4)	-	0.431
Sign				
Heart rate (beats/min)	89±13.8	111±15.6***	84±12	<0.001
Systolic BP (mmHg)	118±21	121±18.8	115±10.3	0.460
Diastolic BP (mmHg)	72±10.5	71±10.4	73±7.7	0.770
Echocardiography				
Systolic PAP (mmHg)	40.6±3.5			
Wells score	4.8±1.1	2.3±1.2	-	<0.001
Geneva score	5.5±1.99	5.32±1.80	-	0.869
sPESI				
Low risk	8 (25.8)	-	-	
High risk	23 (74.2)			
ABG				
pH	7.44±0.03	7.44±0.06	-	0.765
PaO ₂ (mmHg)	60.3±12.8	61.6±13.6	-	0.579
PaCO ₂ (mmHg)	32.0±5.4	29.7±6.9	-	0.049
O ₂ saturation	88.1±7.4	87.8±9.2	-	0.766

* $P < 0.001$ versus control group, ** $P < 0.001$ versus PE group. Wells score clinical probability - 0-1: Low, 2-6: Intermediate, >7: High. Geneva score clinical probability - 0-3: Low, 4-10: Intermediate, >11: High. PAP = Pulmonary artery pressure, sPESI = Simplified pulmonary embolism severity index, ABG = Arterial blood gas, COPD = Chronic obstructive pulmonary disease, PE = Pulmonary embolism, SD = Standard deviation, BP=Blood pressure

in the PE group when compared to non-PE group, whereas fever was significantly higher in the non-PE group when compared to PE group. However, there was no difference for other symptoms. Heart rate was higher in the non-PE groups compared to the PE and control groups ($P < 0.001$). Arterial blood gas values were similar among the two patients' groups. In addition, the Wells score used in the diagnosis of PE was found as 4.8 ± 1.1 in the patient group with PE whereas it was 2.3 ± 1.2 in the non-PE group ($P < 0.001$). Systolic pulmonary artery pressure on echocardiography was found to be 40.6 ± 3.5 mmHg in patients with PE. Nine patients in PE group were administered thrombolytic treatment.

Routine laboratory tests such as LDH, AST, and ALT were in normal range in all groups. Neutrophil (median 6.8, 11.6, $3.8 \times 10^3/\text{mm}^3$, respectively; $P < 0.001$), neutrophil-lymphocytes ratio (NLR) (median 4.4, 8.1, 1.6, respectively; $P < 0.001$), and C-reactive protein (CRP) (median 39.5, 83, 3.4, respectively; $P < 0.001$) levels were higher in PE and non-PE groups than in the control group. Platelet counts were comparable among the groups [Table 2].

Mean D-dimer levels were not different between PE and non-PE groups ($P = 0.591$). Mean SCUBE1 levels were higher in the PE group (0.90 ng/mL) than in the non-PE (0.38 ng/mL) and control groups (0.47 ng/mL) [$P < 0.01$, Table 2 and Figure 1]. When the SCUBE1 results were analyzed using the ROC curve analysis, the optimum diagnostic cutoff point for PE was 0.49 ng/mL and the area under the curve was 0.791 (95% confidence interval [CI] 0.696–0.886); the sensitivity, specificity, positive predictive value, and negative predictive value were 100%, 64%, 56%, and 100%, respectively. The power of the test was found to be 70% [Figure 2].

A multivariable logistic regression analysis (with dichotomous PE groups as the response variable; age, gender, chest pain, syncope, diabetes mellitus, COPD, hypertension, D-dimer, NLR, and SCUBE1 variables as predictors) showed that the significant and independent predictors of PE diagnosis were SCUBE1 (odds ratio [OR]:

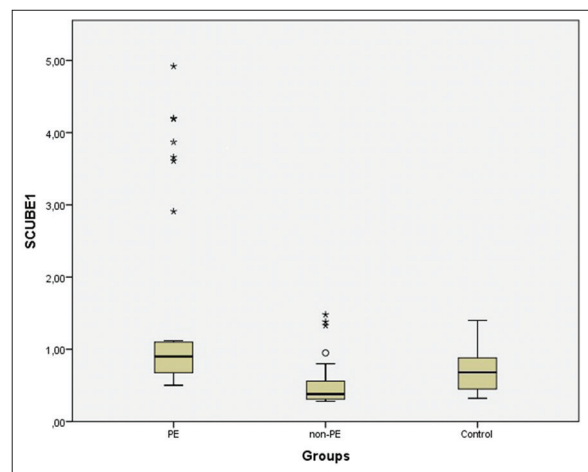


Figure 1: SCUBE1 levels in PE, non-PE, and control groups. Horizontal lines represent the median of SCUBE1 levels for PE, non-PE, and control groups as 0.90, 0.38, and 0.47 ng/mL, respectively. SCUBE1: Signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1

Table 2: Comparison of signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1 and laboratory parameters among the groups

	Minimum–maximum			P
	PE (n=32)	Non-PE (i=25)	Control (n=25)	
SCUBE1 (ng/mL)	0.90*, ^ε (0.50-4.92)	0.38 (0.28-1.48)	0.47 (0.32-1.40)	<0.001
SCUBE1				
High (≥0.49)	32 (100)	8 (32)		<0.001
Low (<0.49)	0	17 (68)		
D-dimer (ng/mL)	2465 (114-21000)	1866 (552-7200)	-	0.591
Platelets (10 ³ /mm ³)	250 (167-568)	284 (105-602)	259 (170-352)	0.110
MPV (fl)	9.3* (8.0-11.8)	9 (7.4-10.3)	8.1 (5.9-11.8)	0.001
Hemoglobin (g/dl)	13.0 ^ε (10.5-18.4)	11.5 ^ε (7.0-17.0)	13.4 (10.1-18.5)	0.001
RDW (fl)	14.0 (12.9-19.3)	15.0 (12.7-17.0)	13.8 (12.4-17.9)	0.168
Neutrophils (×10 ³ /mL)	6.8 ^ε (3-19.7)	11.6 ^ε (2.2-17.5)	3.8 (1.4-10.5)	<0.001
Lymphocytes (×10 ³ /mL)	1.7 ^{ε,ε} (0.3-3.8)	0.875* (0.16-1.8)	2.7 (1.4-3.3)	<0.001
NLR	4.4* (0.69-24.0)	8.1* (1.98-30.6)	1.6 (0.70-5.14)	<0.001
CRP	39.5* (3.2-156)	83* (3-2000)	3.4 (3.1-3.8)	<0.001

P values represent the comparisons among three groups. *P < 0.01 versus control, ^εP < 0.05 versus control group, ^εP < 0.05 versus non-PE group. MPV = Mean platelet volume, RDW = Red cell distribution width, NLR = Neutrophil-lymphocyte ratio, AST = Aspartate transaminase, ALT = Alanine transaminase, LDH = Lactate dehydrogenase, CRP = C-reactive protein, PE = Pulmonary embolism, SCUBE1 = Signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1

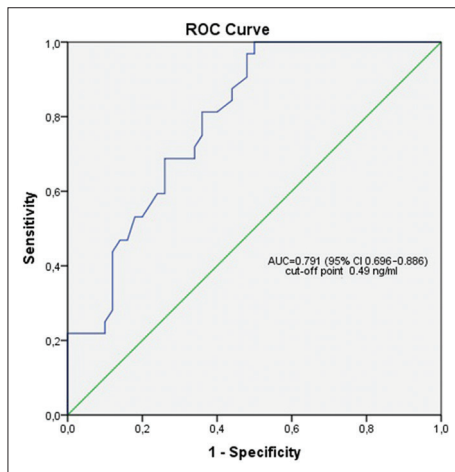


Figure 2: Receiver operating characteristics curve analysis of plasma SCUBE1 values in pulmonary embolism patients. The optimum diagnostic cutoff point of SCUBE1 was 0.49 ng/mL; the area under the curve was 0.791 (95% confidence interval: 0.945–1.000); the sensitivity, specificity, positive predictive value, and negative predictive value were 100%, 64%, 56%, and 100%, respectively.

SCUBE1: Signal peptide-complement C1r/C1s, Uegf, and Bmp1-epidermal growth factor domain-containing protein 1

3.07, 95% CI: 1.26–8.53, $P = 0.039$) and chest pain (OR: 1.45, 95% CI: 1.01–2.25, $P = 0.011$).

Discussion

In the present study, we aimed to investigate the diagnostic value of SCUBE1, considered a new platelet activation marker, in patients with PE. We have shown that SCUBE1 was significantly higher in PE patients compared with non-PE patients and control cases. Moreover, SCUBE1 was associated with high sensitivity (100%) and moderate specificity (64%).

Patients with PE often have nonspecific symptoms and signs. Therefore, there are difficulties in the diagnosis of PE. Among patients with acute PE, the most prevalent symptoms include

dyspnea at rest or on exertion, pleuritic chest pain, hemoptysis, fever, and syncope.^[11,12] None of these are, however, specific for the presence of PE and all may be caused by more prevalent cardiopulmonary disorders, such as COPD exacerbation, acute heart failure, and pneumonia.^[13] This study revealed that chest pain and syncope symptoms were significantly more frequent in PE group when compared to non-PE group. Therefore, these symptoms should be evaluated attentively while making a differential diagnosis of PE.

Furthermore, laboratory findings of PE are nonspecific. Some proven efficacy biomarkers such as D-dimer, B-type natriuretic peptide, and troponin I are used in the diagnosis and risk stratification of PE.^[14-16] D-dimer is recommended as an initial test in all PE guidelines and is the most useful laboratory parameter for emergency departments. For high-sensitive D-dimer assays, such as the ELISA or the latex quantitative assay, the sensitivity for acute PE is over 95%.^[17] A negative D-dimer test result reliably excludes PE diagnosis in patients with low and intermediate clinical probability of PE. However, negative D-dimer should not be predictive in excluding the diagnosis in cases with high clinical suspicion, and further examinations should be performed.^[18,19] The false-negative rate of high-sensitive D-dimer tests in patients with the likely clinical probability has been reported to be as high as 9.3%.^[20] The main drawback of D-dimer testing is its low specificity, which is approximately 35–40% for high-sensitive assays. This is because D-dimer levels are elevated in numerous other conditions, including heart failure, pneumonia, sepsis, kidney failure, and cancer.^[21] Further, it has been well established that D-dimer levels increase with age, leading to a lower specificity in the elderly.^[22,23] In our study, mean D-dimer levels were not different between PE and non-PE groups. A cutoff point of 243 ng/mL for D-dimer revealed 93% of sensitivity and 23% of specificity in patients with PE.

Nowadays, new biomarkers that can be used in the diagnosis of PE are being investigated. In the study of Mirshahi *et al.*, the role

of soluble fibrin and D-dimer was investigated in the diagnosis of PE. The sensitivity of D-dimer was 94% and specificity of D-dimer was 54% for PE. Sensitivity and specificity of soluble fibrin were 94% and 95% for PE, respectively.^[24] Tissue plasminogen activator (tPA) in the diagnosis of PE was also investigated in another study by Flores *et al.* Sensitivity of tPA was 95% and specificity of tPA was 36% for diagnosing PE.^[25]

SCUBE1 is stored in platelet α -granules and replaced on the cell surface with platelet stimulation and activation. Surface-exposed platelet SCUBE1 mediates platelet-platelet agglutination under thrombotic conditions. High levels of SCUBE1 have been determined in human platelets. Upon platelet stimulation, SCUBE1 was translocated to platelet surface, cleaved, and then released into the plasma.^[6] It has been investigated in various diseases. Dai *et al.* evaluated the diagnostic value of SCUBE1 in ACS and AIS and showed high SCUBE1 levels in both, but not in chronic coronary artery disease patients. These results have indicated that SCUBE1 can be used in acute thrombotic disease.^[8] In another experimental study, it was suggested that SCUBE1 can be used as a biomarker in the early diagnosis of AIS. Another finding from this experimental study is the strong correlation between atrophic neuron percentages and SCUBE1 levels at histopathological examination of the brain in ischemia of thrombotic source.^[26]

The only study measuring SCUBE1 levels in PE was the study of Turkmen *et al.* The researchers showed that SCUBE1 levels were significantly higher in patients with PE than healthy control group. SCUBE1 revealed 91% specificity and 82% sensitivity in patients with PE.^[9] In our study, SCUBE1 levels were higher in the PE group than in the non-PE and control groups. A cutoff point of 0.49 ng/mL for SCUBE1 indicated 100% sensitivity and 64% specificity in patients with PE. These findings may have an important role in the diagnosis of PE.

Several limitations of this study should be considered. First, the results were based on a relatively small sample size that had limited statistical power to detect small differences. A lack of short- and long-term follow-up of the patients was a second limitation. Finally, because of a single measurement on admission, the changes in SCUBE1 levels in response to treatment could not be evaluated. Furthermore, we were not assessed false-positive effect of the drugs.

Conclusion

This study revealed that serum SCUBE1 concentrations are elevated in patients with PE, and its sensitivity and specificity are higher than D-dimer for the studied patient population. Therefore, SCUBE1 might be used as a diagnostic biomarker in PE patients. However, since it is still a new marker, further investigations are needed to support the diagnostic value of SCUBE1 in PE.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Miniati M, Prediletto R, Formichi B, Marini C, Di Ricco G, Tonelli L, *et al.* Accuracy of clinical assessment in the diagnosis of pulmonary embolism. *Am J Respir Crit Care Med* 1999;159:864-71.
2. Hull RD. Diagnosing pulmonary embolism with improved certainty and simplicity. *JAMA* 2006;295:213-5.
3. Carson JL, Kelley MA, Duff A, Weg JG, Fulkerson WJ, Palevsky HI, *et al.* The clinical course of pulmonary embolism. *N Engl J Med* 1992;326:1240-5.
4. Kabrhel C, Mark Courtney D, Camargo CA Jr., Plewa MC, Nordenholz KE, Moore CL, *et al.* Factors associated with positive D-dimer results in patients evaluated for pulmonary embolism. *Acad Emerg Med* 2010;17:589-97.
5. Zhuang J, Deane JA, Yang RB, Li J, Ricardo SD. SCUBE1, a novel developmental gene involved in renal regeneration and repair. *Nephrol Dial Transplant* 2010;25:1421-8.
6. Tu CF, Yan YT, Wu SY, Djoko B, Tsai MT, Cheng CJ, *et al.* Domain and functional analysis of a novel platelet-endothelial cell surface protein, SCUBE1. *J Biol Chem* 2008;283:12478-88.
7. Tu CF, Su YH, Huang YN, Tsai MT, Li LT, Chen YL, *et al.* Localization and characterization of a novel secreted protein SCUBE1 in human platelets. *Cardiovasc Res* 2006;71:486-95.
8. Dai DF, Thajeb P, Tu CF, Chiang FT, Chen CH, Yang RB, *et al.* Plasma concentration of SCUBE1, a novel platelet protein, is elevated in patients with acute coronary syndrome and ischemic stroke. *J Am Coll Cardiol* 2008;51:2173-80.
9. Turkmen S, Sahin A, Gunaydin M, Sahin S, Mentese A, Turedi S, *et al.* The value of signal peptide-CUB-EGF domain-containing protein-1 (SCUBE1) in the diagnosis of pulmonary embolism: A preliminary study. *Acad Emerg Med* 2015;22:922-6.
10. Torbicki A, Perrier A, Konstantinides S, Agnelli G, Galiè N, Pruszczyk P, *et al.* Guidelines on the diagnosis and management of acute pulmonary embolism: The task force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). *Eur Heart J* 2008;29:2276-315.
11. Stein PD, Beemath A, Matta F, Weg JG, Yusen RD, Hales CA, *et al.* Clinical characteristics of patients with acute pulmonary embolism: Data from PIOPED II. *Am J Med* 2007;120:871-9.
12. Miniati M, Cenci C, Monti S, Poli D. Clinical presentation of acute pulmonary embolism: Survey of 800 cases. *PLoS One* 2012;7:e30891.
13. Squizzato A, Luciani D, Rubboli A, Di Gennaro L, Landolfi R, De Luca C, *et al.* Differential diagnosis of pulmonary embolism in outpatients with non-specific cardiopulmonary symptoms. *Intern Emerg Med* 2013;8:695-702.
14. Kucher N, Printzen G, Doernhoefer T, Windecker S, Meier B, Hess OM. Low pro-brain natriuretic peptide levels predict benign clinical outcome in acute pulmonary embolism. *Circulation* 2003;107:1576-8.
15. Cetiner MA, Sayin MR, Yildirim N, Karabag T, Aydin M. The association of eccentricity indexes with cardiac biomarkers in normotensive acute pulmonary embolism patients: An observational study. *Anadolu Kardiyol Derg* 2013;13:108-14.
16. Hakemi EU, Alyousef T, Dang G, Hakmei J, Doukky R. The prognostic value of undetectable highly sensitive cardiac troponin I in patients with acute pulmonary embolism. *Chest* 2015;147:685-94.
17. Stein PD, Hull RD, Patel KC, Olson RE, Ghali WA, Brant R, *et al.* D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism: A systematic review. *Ann Intern Med* 2004;140:589-602.
18. Quinn DA, Fogel RB, Smith CD, Laposata M, Taylor Thompson B, Johnson SM, *et al.* D-dimers in the diagnosis of pulmonary embolism. *Am J Respir Crit Care Med* 1999;159(5 Pt 1):1445-9.

19. Mavromatis BH, Kessler CM. D-dimer testing: The role of the clinical laboratory in the diagnosis of pulmonary embolism. *J Clin Pathol* 2001;54:664-8.
20. Gibson NS, Sohne M, Gerdes VE, Nijkeuter M, Buller HR. The importance of clinical probability assessment in interpreting a normal d-dimer in patients with suspected pulmonary embolism. *Chest* 2008;134:789-93.
21. Righini M, Perrier A, De Moerloose P, Bounameaux H. D-Dimer for venous thromboembolism diagnosis: 20 years later. *J Thromb Haemost* 2008;6:1059-71.
22. Masotti L, Ceccarelli E, Cappelli R, Forconi S. Plasma D-dimer levels in elderly patients with suspected pulmonary embolism. *Thromb Res* 2000;98:577-9.
23. Haase C, Joergensen M, Ellervik C, Joergensen MK, Bathum L. Age- and sex-dependent reference intervals for D-dimer: Evidence for a marked increase by age. *Thromb Res* 2013;132:676-80.
24. Mirshahi S, Soria C, Kouchakji B, Kierzek G, Borg JY, Varin R, *et al.* New combinational assay using soluble fibrin and D-dimer determinations: A promising strategy for identifying patients with suspected venous thromboembolism. *PLoS One* 2014;9:e92379.
25. Flores J, García-Avello A, Alonso E, Ruíz A, Navarrete O, Alvarez C, *et al.* Tissue plasminogen activator as a novel diagnostic aid in acute pulmonary embolism. *Vasa* 2014;43:450-8.
26. Turkmen S, Eryigit U, Karaca Y, Mentese A, Sumer UA, Yulug E, *et al.* Diagnostic value of plasma signal peptide-Cub-Egf domain-containing protein-1 (SCUBE-1) in an experimental model of acute ischemic stroke. *Am J Emerg Med* 2015;33:262-5.