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Investigation of the Relationship of SCUBE-1 and VAP-1 Levels on Diagnosis, Prognosis and Clinical Results in Patients with Pulmonary Thromboembolism Diagnosis in Emergency Department

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Abstract

Aim: Acute pulmonary thromboembolism (APT) is a life-threatening disease. The aim of this study is to investigate the relationship between the diagnosis and prognosis of alternative biomarkers such as signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1) and vascular adhesion protein-1 (VAP-1) in the diagnosis of clinically suspected acute APT.

Materials and Methods: Patients diagnosed as APT in emergency department were included in the study. Patients with acute ischemic disease, liver failure, renal failure, pregnancy, active malignancy and/or history of known APT were excluded from the study. A control group was formed from healthy volunteers at similar age and sex. SCUBE-1 and VAP-1 levels were studied from serum samples taken from the patient and control groups.

Results: Serum SCUBE-1 levels were 7.60 (6.22-71.05) ng/mL in the patient group and 23.79 (5.08-118.28) ng/mL in the control group ($p<0.001$). Serum VAP-1 levels were 1.07 (0.20-24.36) ng/mL in the patient group and 9.31 (0.21-25.98) ng/mL in the control group ($p<0.001$). Both serum levels of SCUBE-1 and VAP-1 were significantly lower in the patient group. There was no correlation between both biomarkers with Wells rules, revised Genova score, Pulmonary Embolism Severity index (PESI) sPESI and early mortality risk.

Conclusion: Serum SCUBE-1 and VAP-1 levels were found to be useful in the diagnosis of APT. However, both biomarkers are not successful in predicting prognosis. In the light of these data; it can be said that studies with larger patient subgroups are needed in order to enter into clinical use in terms of diagnosis and prognosis of serum levels of SCUBE-1 and VAP-1 in patients with APT.

Keywords: Acute pulmonary thromboembolism, emergency department, SCUBE-1, VAP-1

Introduction

Acute pulmonary thromboembolism (APT) is a major health problem that significantly threatens human life (1). The disease often presents with non-specific clinical symptoms, and diagnosis relies heavily on clinical suspicion. Definitive diagnosis is achieved through ventilation/perfusion (V/Q) scintigraphy or thorax computed tomography angiography (CTA) (2). However, these methods require radiation and contrast exposure, which may not be feasible in all patients. Among laboratory tests, D-dimer is widely used to exclude acute pulmonary thrombosis rather than confirm it, due to its low specificity (3). Given these limitations, there is a strong clinical need for alternative, non-invasive, and more specific diagnostic biomarkers.

The non-specificity of the clinical picture, the fact that the diagnosis depends on the experience of the physician, and the

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necessity of exposure to radiation and contrast material for the definitive diagnosis have revealed the necessity of alternative diagnostics for APT. Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1) is a cell surface glycoprotein stored in platelet alpha-granules and released upon platelet activation. It also originates from endothelial cells under inflammatory or hypoxic conditions, highlighting its relevance in acute vascular events (4,5). SCUBE-1 contributes to thrombus formation and has been studied in diseases such as myocardial infarction and ischemic stroke. Vascular adhesion protein-1 (VAP-1) also known as amine oxidase copper-containing 3, is a transmembrane glycoprotein with dual functionality as an adhesion molecule and an enzyme. It mediates leukocyte adhesion, rolling, and transmigration during inflammation. A soluble form is also released into the circulation, suggesting systemic effects in inflammatory states (6-8).

These two biomarkers were selected for this study due to their direct roles in the pathophysiology of APT: SCUBE-1 in platelet aggregation and thrombogenesis, and VAP-1 in endothelial inflammation and immune cell recruitment. SCUBE-1, by reflecting acute platelet activity, may offer advantages over D-dimer in terms of specificity. Although VAP-1 has primarily been evaluated in chronic inflammatory diseases, its endothelial involvement in acute inflammation makes it a candidate for further study in acute thromboembolic events (6,8,9).

However, the temporal kinetics of VAP-1 as a relatively novel biomarker such as its rise, peak, and normalization times during acute inflammatory conditions remain unclear, which may limit its early diagnostic utility (10). The aim of this study was to investigate the serum levels of SCUBE-1 and VAP-1 in patients diagnosed with APT in the emergency department and to evaluate their potential diagnostic and prognostic value.

Materials and Methods

Study Design and Selection of Patient and Control Group

The study was prospectively planned. Patients who presented to the emergency department of our hospital between 01.01.2019 and 30.06.2019 and were diagnosed with APT by contrast-enhanced CTA were included in the study. A group of healthy volunteers, matched for gender, age, and exclusion criteria, was formed for comparison with the patients with APT included in the study. Demographic data, clinical probability scores (Wells' criteria, revised Geneva score), simplified Pulmonary Embolism Severity index (sPESI), and early mortality risk score (in 30 days) of the patients were recorded.

In the power analysis (G*Power 3.1.9.7 package program), a minimum (min) sample size of 16 was determined for each of the control and patient groups for 99% power with an effect size of 1.62 at a 95% significance level.

Inclusion criteria for the study were defined as being older than 18 years of age, presenting to the emergency department, and being diagnosed with acute APT by contrast-enhanced CTA. Exclusion criteria of the study: acute ischemic disease, liver or advanced heart failure, disease, pregnancy, active malignancy, hematological disease, and a known history of APT.

Determination of Serum SCUBE-1 and Serum VAP-1 Levels

5 mL of blood taken from the peripheral veins of the patients was placed in a biochemistry tube and centrifuged at 3000 × g (core NF800, REF: Z10.NF 800). After centrifugation, the serum part of the blood was separated, placed in an eppendorf tube, and stored in an ultra-deep freezer (NUAIRE, Serial No: 9394248) at -80 °C until the study day. Before starting the study, the ELISA kits kept at 2-8 °C, and the samples kept in an ultra-deep freezer at minus 80 °C were brought to room temperature. Serum SCUBE-1 and VAP-1 levels in the samples were determined using the Human Scube1 ELISA kit (SunRed, Cat: 201-12-5378) and the Human SVAP-1 ELISA kit (SunRed, Cat. No: 201-12-2134). While the sensitivity for the Human Scube1 ELISA kit is 0.852 ng/mL, the measurement range is 1-300 ng/mL; the sensitivity for the Human SVAP-1 ELISA kit is 0.185 ng/mL, and the measurement range is 0.2-60 ng/mL.

Statistical Analysis

Data were analyzed with IBM SPSS v23. The normality of quantitative data was analyzed using the Shapiro-Wilk test. Mann-Whitney U test and Kruskal-Wallis tests were used to compare non-parametric data. receiver operating characteristic (ROC) analysis was performed to obtain the cut-off value. The area under the curve, as a result of the ROC analysis, was presented with a 95% confidence interval. Sensitivity, specificity, positive and negative likelihood ratios, positive and negative predictive value, and correct classification rate were calculated for the diagnostic test evaluation data. The relationship between the data was examined with the Spearman's correlation test. The chi-square test was used to analyze categorical data. Results were presented as median (min to maximum), frequency (n), and percentage (%). The significance level was accepted as $p<0.05$. Our study received ethics approval from the Ondokuz Mayıs University Clinical Research Ethics Committee (decision number: OMÜ KAEK 2019/26, date: 14.05.2019). The Clinical trial number obtained for the study is NCT06525051, and it was assigned on 2024-12-10.

Results

The study included 44 patients with acute pulmonary embolism and 44 control patients with similar age, gender, and exclusion criteria, making a total of 88 patients. Demographic characteristics of the patients are shown in Table 1.

Table 1. Characteristic properties of the patients

		Patient	Control
Female*		24 (54.5)	24 (54.5)
Age**		68.5 (22-84)	68.5 (22-84)
BMI**		29.0 (18-51)	23.3 (20-33)
Symptom*			
	Dyspnea	32 (72.7)	
	Chest pain	22 (50.0)	
	Leg swelling	17 (38.6)	
	Cough	10 (22.7)	
	Back pain	9 (20.5)	
	Altered mental status	9 (20.5)	
	Flank pain	7 (15.9)	
	Palpitation	7 (15.9)	
Vital signs**			
	Systolic blood pressure (mmHg)	120 (80-160)	
	Diastolic blood pressure (mmHg)	70 (50-100)	
	Fever (oC)	36.4 (35.0-37.6)	
	Pulse (bpm/minute)	91 (54-145)	
	Respiratory rate (/minute)	22 (13-42)	
	O_2 saturation (%)	94.5 (70-100)	
Komorbidities*			
	Hypertension	14 (31.8)	
	Coronery artery disease	8 (18.2)	
	Diabetes mellitus	6 (13.6)	
	COPD	3 (6.8)	
	Cerebrovascular disease	3 (6.8)	
Wells' criteria**		4.5 (0-9)	
	PE likely*	31 (70.4)	
Revized geneva score**		6 (0-13)	
	PE likely*	24 (54.5)	
Right ventricular dysfunction*		29 (65.9)	
sPESI score**		1 (0-3)	
	0 point*	19 (43.2)	
	≥1 point*	25 (56.8)	

BMI: Body mass index, COPD: Chronic obstructive pulmonary disease, PE: Pulmonary embolism, sPESI: Simplified Pulmonary Embolism Severity index, *n (%), **median (minimum-maximum)

Serum SCUBE-1 level was found to be significantly lower in the patient group compared to the control group (p<0.001). When the cut-off was taken at 9.00 ng/mL, the sensitivity was calculated as 75.0%, the specificity as 75.0% [area under the curve (AUC): 0.742, p<0.001].

Serum VAP-1 level was also found to be significantly lower in the patient group compared to the control group (p<0.001). When the cut-off was taken at 3.50 ng/mL, the sensitivity was calculated as 72.7% and the specificity as 77.3% [AUC: 0.737 (0.629-0.845), p<0.001]. Values for serum SCUBE-1 and VAP-1 are shown in Table 2, and ROC analysis is shown in Figure 1.

There was no significant difference between clinical probability scores and serum SCUBE-1 and VAP-1 levels (p>0.05) (Table 3). No significant difference was found between serum SCUBE-1 and serum VAP-1 for the sPESI and 30-day early mortality risk classification (p>0.05).

Table 2. Comparison of signal peptide CUB-EGF domain-containing protein-1 and vascular adhesion proteini-1 serum parameters among the groups

	SCUBE-1 (ng/mL)	VAP-1 (ng/mL)
Patient	7.60 (6.22-71.05)	1.07 (0.20-24.36)
Control	23.79 (5.08-118.28)	9.31 (0.21-25.98)
p	**<0.001**	**<0.001**
Cut-off	9.00 ng/mL	3.5 ng/mL
Sensitivity	75.00 (59.66-86.81)	72.73 (57.21-85.04)
Spesifisity	75.00 (59.66-86.81)	77.27 (62.16-88.53)
AUC (95% CI)	0.742 (0.663-0.851)	0.737 (0.629-0.845)
p	**<0.001**	**<0.001**

SCUBE-1: Such as signal peptide-CUB-EGF domain-containing protein 1, VAP-1: Vascular adhesion protein-1, AUC: Area under the curve, CI: Confidence interval

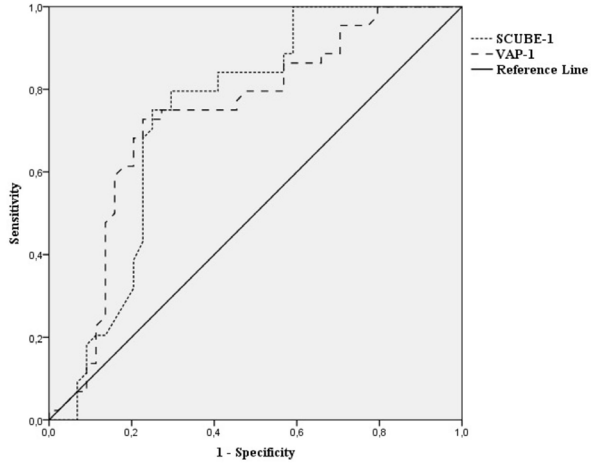

Figure 1. Receiver operating characteristics curve analysis of serum SCUBE-1 and serum VAP-1 values

SCUBE-1: Such as signal peptide-CUB-EGF domain-containing protein 1, VAP-1: Vascular adhesion protein-1

Table 3. Comparison of signal peptide CUB-EGF domain-containing protein-1 and vascular adhesion proteini-1 serum parameters among clinical probability scores and predictive mortality risk scores			
		SCUBE-1 (ng/mL)*	**VAP-1 (ng/mL)***
Wells' criteria			
	PE unlikely (n=13)	7.60 (6.22-71.05)	1.05 (0.20-24.36)
	PE likely (n=31)	7.60 (6.22-67.75)	1.13 (0.23-24.29)
	p	0.949	0.616
Revized geneva score			
	PE unlikely (n=20)	7.75 (6.22-68.59)	0.98 (0.20-24.36)
	PE likely (n=24)	7.45 (6.22-71.05)	1.15 (0.23-24.36)
	p	0.604	0.596
sPESI			
	0 point (n=19)	7.75 (6.22-68.59)	1.09 (0.20-23.34)
	≥1 point (n=25)	7.60 (6.22-71.05)	0.90 (0.23-24.36)
	p	0.577	0.943
Early mortality risk			
	Low risk (n=8)	8.66 (6.22-68.59)	5.29 (0.49-23.34)
	Intermediate-low risk (n=21)	7.60 (6.22-56.16)	1.05 (0.20-22.15)
	Intermediate-high risk (n=9)	7.23 (6.81-8.48)	0.65 (0.23-2.39)
	High risk (n=6)	7.72 (6.60-71.05)	3.37 (0.68-24.36)
	p	0.688	0.070

SCUBE-1: Such as signal peptide-CUB-EGF domain-containing protein 1, VAP-1: Vascular adhesion protein-1, PE: Pulmonary embolism, sPESI: Simplified Pulmonary Embolism Severity index, *median (minimum-maximum)

Table 4. Relationship between serum SCUBE-1, serum VAP-1 and demographic properties and laboratuvary values				
	SCUBE-1		**VAP-1**	
	r	**p**	**r**	**p-value**
Age	-0.012	0.910	-0.097	0.370
Body mass index	**-0.404**	**<0.001**	**-0.308**	**0.004**
Leukocyte	-0.128	0.409	0.156	0.313
Hemoglobin	-0.192	0.211	**-0.325**	**0.031**
Platelet	0.194	0.208	**0.471**	**0.001**
Lymphocyte	0.221	0.150	**0.425**	**0.004**
Neutrophil	-0.168	0.276	-0.030	0.845
D-dimer	-0.191	0.215	-0.273	0.073
Troponin I	-0.052	0.735	-0.166	0.280
Creatine	**-0.488**	**0.001**	**-0.329**	**0.029**
pH	0.125	0.420	0.077	0.621

r: Spearman's rho correlation, SCUBE-1: Such as signal peptide-CUB-EGF domain-containing protein 1, VAP-1: Vascular adhesion protein-1

The relationship between serum SCUBE-1 and serum VAP-1 levels and demographic characteristics of the patients was evaluated with the Spearman's rho correlation test. A moderately negative correlation was found between the patients' body mass index (BMI) and serum SCUBE-1 levels, and a positive correlation with VAP-1 levels (for SCUBE-1 r=-0.397, p<0.001; for VAP-1 r=0.337, p=0.001). The relationship between serum SCUBE-1 and VAP-1 levels and the characteristics of the patients is shown in Table 4.

Discussion

APT is one of the most common cardiovascular diseases threatening human life (11). Clinical probability scores have been developed for preliminary diagnosis. Definitive diagnosis includes CTA or V/Q scintigraphy. This means exposure to radiation, contrast material, or radioactive material for patients who are suspected of having a particular diagnosis. The most important factor leading to the diagnosis is the physician's prediction and experience. Today, the number of biomarkers that can be used as an aid in diagnosis is very small. The most commonly used D-dimer is used to exclude the diagnosis because it is elevated in many diseases (3). Although clinical studies are ongoing for new biomarkers, there is no new biomarker that has been used yet. The lack of biomarkers, coupled with clinical suspicion, contributes to the burden of radiation and contrast agents in patients. Therefore, new biomarkers are needed to diagnose APT. If validated in future studies, SCUBE-1 and VAP-1 could potentially aid in early decision-making and reduce reliance on contrast-enhanced imaging, thus lowering exposure to radiation and nephrotoxic agents in select patient populations.

SCUBE-1 was first studied by Dai et al. (5) in acute myocardial infarction and ischemic stroke, and it was emphasized that it could be an indicator of thrombus. In the literature, there are studies showing that serum SCUBE-1 level increases with advanced age and decreases with obesity (12). SCUBE-1 has been studied several times in acute and chronic conditions. There are studies showing that serum levels may either increase or decrease in cases of acute thrombosis (4,13,14). There are two articles in the literature examining the relationship between APT and SCUBE-1. The first of these is a preliminary study, a study conducted with a few patients and control groups. This study consists of 11 patient groups and 23 volunteer groups (13). Another study was conducted with two groups: patients with suspected APT who applied to the emergency department, and a control group of those not considered to have APT, who also applied to the emergency department (14). The fact that each patient participates in this study and has a disease that requires admission to the emergency department precludes objective comparisons between groups. Serum SCUBE-1 levels were significantly higher in the APT group in both studies. Our study has the potential to be a reference point, as it includes the largest number of patients and control groups in this area. In addition, the control group selected in our study consisted of healthy volunteers of similar age and gender, who did not apply to the hospital. Contrary to the other two studies, the serum SCUBE-1 level in our study was found to be significantly higher among the healthy volunteers included in the study group. This contrasts with prior studies and may stem from methodological differences, such as timing of sample collection, control group selection, and the potential consumption of biomarkers in acute thrombotic processes.

Serum SCUBE-1 level starts to rise 6 hours after activation in patients with acute platelet activation (5). This could indicate that, in acute presentations, there may be a delay in the measurable elevation of or a consumption effect due to active thrombus formation, contributing to the unexpectedly low levels. This may explain why this biomarker is not elevated enough in the serum of patients presenting with sudden dyspnea and chest pain, in this acute period. The body mass indices of patients with APT were found to be high, and high body mass indies may cause low serum SCUBE-1 levels. It is known that smoking may cause a decrease in serum SCUBE-1 level (5). The control group was composed of healthy non-smoker volunteers, whereas the patient group did not have a similar distinction applied.

Serum VAP-1 levels are biomarkers that have been studied mostly in chronic conditions (15-17). The available data on VAP-1 is limited. For example, in an acute ischemic condition, information such as when the serum level will start to rise, when it will peak, when it will begin to decline, or in which situations it will not be available in the current literature. There is one article in the literature examining the relationship between VAP-1 and APT (18). In this study, patients who underwent CTA with a pre-diagnosis of APT were included, and the serum VAP-1 level was found to be lower in patients with APT than in patients without APT. In our study group, serum VAP-1 level was higher in healthy volunteers than in the patient group. VAP-1 is a biomarker secreted in inflammatory processes; there is no information about when it will increase in acute situations. This limits its interpretability as a diagnostic biomarker in acute conditions such as APT. Further research is needed to elucidate the temporal dynamics of VAP-1 levels in acute inflammation. VAP-1 has been studied mostly in chronic diseases in the literature, and it has been found to be higher in chronic conditions (16,17).

There was no statistically significant difference between serum SCUBE-1 and serum VAP-1 levels and pulmonary embolism clinical probability scores and clinical risk classifications. Likewise, when the risk of early mortality, which predicts 30-day

mortality, and serum levels are compared, there is no statistically significant difference, although the serum levels decrease as the risk increases. Although these biomarkers were helpful in making the diagnosis, they were insufficient to evaluate the prognosis. Furthermore, no statistically significant correlation was found between these biomarkers and clinical scores or 30-day mortality. This limits their prognostic utility in current clinical practice.

Thrombosis and inflammation are now recognized as interrelated processes rather than isolated events. SCUBE-1, a molecule stored in platelet alpha-granules, plays an active role in platelet adhesion, aggregation, and thrombus formation. Its release during acute vascular injury links it directly to the coagulation cascade. Elevated SCUBE-1 levels have been reported in myocardial infarction and ischemic stroke, both of which involve platelet-rich thrombi (4,5). In contrast, VAP-1 is predominantly involved in endothelial activation and leukocyte trafficking. It facilitates the adhesion and transmigration of inflammatory cells across the vascular wall, a key step in the inflammatory response that may exacerbate thrombus formation (6-9). Therefore, the measurement of SCUBE-1 and VAP-1 together may reflect complementary aspects of the thrombo-inflammatory response seen in APT.

Despite this mechanistic relevance, the current study demonstrated lower serum levels of both SCUBE-1 and VAP-1 in APT patients compared to healthy controls. This unexpected finding may be explained by biomarker consumption during acute thrombus formation or by delayed systemic release, particularly in the very early stages of presentation. Furthermore, the lack of temporal kinetic data especially for VAP-1 limits our ability to determine the optimal time window for measurement (10). As such, while these biomarkers show biological plausibility, their clinical utility as early diagnostic markers for APT remains to be validated in larger prospective studies. In summary, SCUBE-1 and VAP-1 reflect key processes in thromboinflammation and may contribute to a more nuanced understanding of APT pathophysiology.

In a study on rats, the serum SCUBE-1 level was found to be low in obese rats (12). In our study, a low negative correlation was found between serum SCUBE-1 and VAP-1 levels with BMI. This situation was identified by our first study on humans. In addition, it has been previously shown that the serum SCUBE-1 level increases with advanced age (5). However, in our study, neither SCUBE-1 nor VAP-1 levels were found to be significantly associated with age.

Study Limitations

In biomarker studies with APT, there are many exclusion criteria to control confounding variables. This demonstrates that multiple studies, including subgroups, are required to adapt the results to all patients with APT. Additionally, while our study was adequately powered, the relatively small sample size (n=44 per group) remains a limitation for the generalizability of the findings. Larger multi-center studies are warranted.

Conclusion

In our study, serum SCUBE-1 and serum VAP-1 levels were found to be significantly lower in patients diagnosed with APT compared to healthy controls. These findings suggest that both biomarkers may reflect thromboinflammatory processes associated with APT. Although they showed potential diagnostic value, their prognostic utility appeared limited. Given the dynamic nature of biomarker expression in acute events, further prospective, multicenter studies are needed to clarify the optimal timing, clinical applicability, and prognostic significance of SCUBE-1 and VAP-1 measurements in APT management.

Ethics

Ethics Committee Approval: Our study received ethics approval from the Ondokuz Mayıs University Clinical Research Ethics Committee (decision number: OMÜ KAEK 2019/26, date: 14.05.2019).

Informed Consent: The study was prospectively planned.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.A., H.U.A., Ö.K.T., Concept: İ.A., H.U.A., Ö.K.T., Design: İ.A., H.U.A., Ö.K.T., Data Collection or Processing: İ.A., H.U.A., Analysis or Interpretation: İ.A., H.U.A., Ö.K.T., Literature Search: İ.A., H.U.A., Writing: İ.A., H.U.A., Ö.K.T.

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