



## Diagnostic value of serum apelin-13 in patients with pulmonary thromboembolism

Apelin-13 in patients with PTA

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### Abstract

**Aim:** We aimed to investigate whether serum apelin-13 value could be a novel biomarker in the diagnosis of pulmonary thromboembolism (PTE). **Material and Method:** The study included 142 patients, 82 of which were diagnosed with PTE and 60 were control cases. Serum apelin-13 level was measured using venous blood samples with the ELISA kit. **Results:** The serum apelin level was  $2219.4 \pm 65.2$  ng/mL in the PTE group and  $1234.7 \pm 35.5$  ng/mL in the control group ( $p < 0.05$ ). Serum apelin-13 was  $2495.8 \pm 738.0$  ng/mL in the DVT (+) group and  $2118.0 \pm 496.8$  ng/mL in the DVT (-) group ( $p = 0.009$ ). The best cut-off value for apelin-13 in the control and PTE groups was determined as 1579 ng/mL. Sensitivity was 92.7% and specificity was 96.7% (95% confidence interval: 0.939 to 0.995, area under ROC curve: 0.979,  $p < 0.001$ ). **Discussion:** This study showed that serum apelin-13 value can be used as new diagnostic biomarker in patients with PTE. Apelin-13 value also elevates in patients with DVT (+). These results suggest that apelin-13 value may be used as a novel biomarker in the patients with acute PTE and DVT (+) in future practice.

### Keywords

Apelin-13; Pulmonary Thromboembolism; Deep Vein Thrombosis; Adipokines

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## Introduction

Adipokines are found extensively in the adipose tissue, gastrointestinal tract, brain, cardiovascular system, liver, kidneys and lungs, and involve many systemic processes [1-4]. In 1998 Tatemoto et al. isolated apelin, an adipokine from bovine stomach tissue [1]. The most important source of apelin in the bloodstream is the lungs [3]. Apelins are classified by amino acid number. The active form of apelin peptides is separated from the C-terminal of preapelin of 77 amino acids. The fragments 36, 17 and 13 of this peptide are the final bioactive product of apelin, defined as apelin-13 that is more resistant to enzymatic and most potent myocardial protective apelin [5,6,7]. The biochemical differences between the apelin peptides of different lengths and the pharmacokinetic properties of these peptides are still unknown. Congestive heart failure, pulmonary hypertension, and hypoxia have been reported to increase the level of plasma apelin [3,8,9,10,11].

Pulmonary embolism is a clinical condition that occurs when the pulmonary vascular bed is obstructed by thrombus and the diagnosis is made with computed tomography angiography (CTA). PTE is quite mortal when the duration of diagnosis and treatment is delayed. Generally, the thrombus that descends from the deep femoral veins passes through the right ventricle to the pulmonary arteries and plugs in at a point appropriate for its diameter. Clinically, dyspnea, chest pain, tachycardia and hypoxaemia are common symptoms of PTE. In recent studies, D-dimer, C-reactive protein, brain natriuretic peptide (BNP), NT-proBNP were used as serological biomarkers in the diagnosis and severity of pulmonary embolism. In this study, we aimed to show the diagnostic value of serum apelin-13 level in PTE.

## Material and Method

We have analyzed 142 patients who applied to the Recep Tayyip Erdoğan University Medical Faculty Pulmonology Clinic, Rize, Turkey between 2015 and 2016. Eighty-two PTE diagnosed patients and 60 healthy control cases without complaints referred to the chest clinic for routine health checks were included in the study. Ethics committee approval for this study was taken from the Ethics Committee of Recep Tayyip Erdogan University Faculty of Medicine (confirmation no: 38). PTE diagnosis was made by CTA. Echocardiography and both lower extrem-

ity venous Doppler ultrasonography were performed on all patients. Patients with deep vein thrombosis signed as DVT8 (+), patients without deep vein thrombosis signed as DVT (-). Routine biochemical tests, troponin-I and D-dimer analysis were performed.

Patients were classified as massive sub-massive and non-massive according to the clinical table of PTE. Acute right ventricular failure accompanied by hypotension-shock or cardiopulmonary arrest was accepted as a massive PTE. Findings of right ventricular dysfunction (dilation and hypokinesia) detected on echocardiography in response to normal systemic blood pressure were considered to be sub-massive PTE. Patients with normal systemic blood pressures and those with normal right ventricular function were considered non-massive PTE.

The clinical risk score was calculated according to Wells criteria (low clinical probability: <2.0 points, moderate clinical probability: 2.0-6.0 points, high clinical probability: > 6.0 points).

### Serum Apelin-13 measurement

Venous blood samples (10 ml) were taken from the patients to analyze serum apelin-13 within one hour after PTE diagnosis with CTA. Venous blood sample (10 ml) was also taken from 60 healthy control group patients. Blood samples were centrifuged at 3000 rpm for about 10-15 minutes in half an hour, after which the serum fraction was removed and stored at -80°C in Eppendorf tubes. The level of apelin-13 level was measured using the Human Apelin-13 ELISA Kit CSB-E13072h (made in China) by enzyme-linked immunosorbent assay (ELISA).

### Exclusion criteria

Acute coronary syndrome, congestive heart failure, malignancy, pregnancy, acute cerebrovascular event, acute infection, renal insufficiency, and chronic liver disease were excluded.

### Statistical analysis

The IBM-SPSS program (SPSS version 20) is used for statistical analysis. Normal distribution was tested with Kolmogorov-Smirnov test. Continuous variables were given as mean  $\pm$  standard deviation, median, minimum and maximum levels, whereas categorical variables as frequency (n) and percent (%). Mann Whitney-U test was used for comparison of two groups, Kruskal-Wallis tests were used for comparison of multiple groups.

Table 1. Demographic and Laboratory Features of PTE and Control Group

	PTE Group n%,mean ( $\pm$ SD)	Control Group n%,mean ( $\pm$ SD)	DVT(+) n%,mean ( $\pm$ SD)	DVT(-) n%,mean ( $\pm$ SD)	p value
Number (n)	82 (57.7%)	60 (42.3%)	22(26.8)	60(73.2)	
Age (year)	71.0 $\pm$ 14.9	66.2 $\pm$ 8.1	76.0 $\pm$ 12.8	69.1 $\pm$ 15.3	
Sex (female/male)	54/28 (65.8/34.2)	34/26(56/44)	16/6(72.7/27.3)	38/22(63.3/36.6)	p>0.05
Apelin-13 (ng/mL)	2219.4 $\pm$ 65.2	1234.7 $\pm$ 35.5	2495.8 $\pm$ 738.0	2118.0 $\pm$ 496.8	p* <0.001 p+=0.009
Serum D-dimer( ng/mL)	8197.3 $\pm$ 8799.5	-	13350.4 $\pm$ 2046.0	6307.8 $\pm$ 999.9	p=0.001
Troponin-I (pg/mL)	0.36 $\pm$ 0.11	-	-	-	
RDW (%)	14.5 $\pm$ 3.5	-	-	-	
Albumin (g/dL)	3.5 $\pm$ 0.5	-	-	-	
Protein (g/dL)	6.8 $\pm$ 0.8	-	-	-	
Creatinin (mg/dL)	0.9 $\pm$ 0.3	-	-	-	
ECHO. PAP (mm/Hg)	41.8 $\pm$ 14.6	-	-	-	

PTE: Pulmonary Thromboembolism; DVT(+):Patients with deep vein thrombosis; DVT(-):Patients without deep vein thrombosis; ECHO: Echocardiography; PAP: Pulmonary Arterial Pressure; p: P value; p\*: PTE group vs Control group; p+: DVT(+) group vs DVT(-) group

The relationship between variables was examined by Spearman correlation analysis method. ROC-curve analysis was performed to show the efficacy of serum apelin-13 in distinguishing PTE patients from control groups. Specificity, sensitivity, positive and negative predictive values were also calculated. A value of  $p < 0.05$  was considered statistically significant.

**Results**

A total of 142 patients (82.7%) with PTE and 60 (42.3%) healthy controls were included in the study. Demographic and laboratory features of PTE and control group were shown in Table 1. The mean age of the PTE group was  $71.0 \pm 14.9$  and the female/male ratio was 54/28 (65.8%/34.2%). The number of patients with DVT (+) was 22 (26.8%) and DVT (-) was 60 (73.2%). The serum apelin level was  $2219.4 \pm 65.2$  ng/mL in the PTE group and  $1234.7 \pm 35.5$  ng/mL in the control group (Figure 1). There was a significant difference between apelin-13 values between two groups ( $p < 0.001$ ). Serum apelin-13 was  $2495.8 \pm 738.0$  ng/mL in the DVT (+) group and  $2118.0 \pm 496.8$  ng/mL in the DVT (-) group. There was a significant difference between apelin-13 values between the two groups ( $p = 0.009$ ). There was no correlation between apelin-13 and D-dimer, troponin-I, creatinine, protein, albumin and pulmonary artery pressures ( $p > 0.05$  for all).

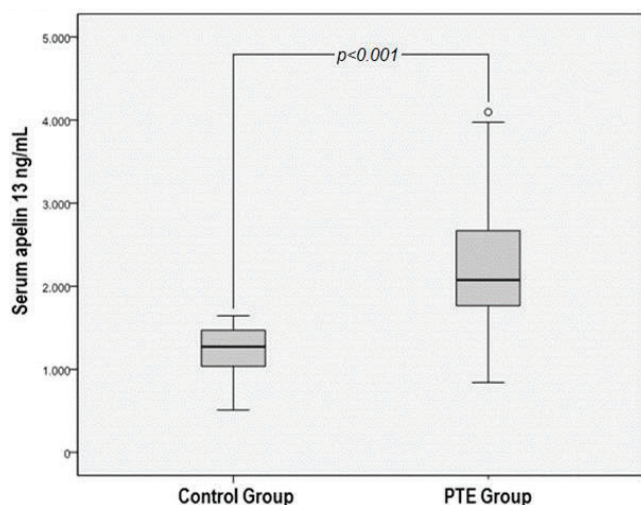


Figure 1. Apelin-13 Levels Between PTE and Control Groups

**Discussion**

This is the first study showing that the level of serum apelin-13 is statistically higher in patients with PTE and DVT (+) than in the control group.

The number of serological biomarkers used in PTE identification is small. D-dimer is a non-invasive biomarker commonly used in the diagnosis of PTE showing endogenic fibrinolytic activation [12]. The sensitivity of D-dimer is high but specificity is low [13]. Without high clinical probability, D-dimer negativity ( $< 500$  ng/mL) is sufficient to exclude PTE. D-dimer can be positive in surgical procedures, trauma, kidney diseases, malignancies, severe infections, pregnancy and autoimmune diseases [14]. Because of this reason, new biomarkers are needed in PTE diagnosis.

Apelin is an adipokine, involved in many metabolic processes as biomarkers. Apelin expression is high in pulmonary vascular

endothelial tissue [4]. It is also known that the highest apelin mRNA expression is in the pulmonary tissue [4]. The effect of apelin on vasomotor tone is variable and may have different effects on the arterial vascular pressure in different pathophysiological conditions [15]. Apelin creates an antagonistic effect on vasoconstrictors by inhibiting reactive oxygen species and increasing NO synthesis [16,17].

In the acute phase of PTE, many vasoactive substances are released from vascular endothelial cells. Recent studies have shown that hypoxia increases apelin expression in adipose tissue cells, endothelial cells, and murine lung tissue [7,18,19]. In an animal model study, apelin expression in the bronchial epithelium was reported to be upregulated in the early stage of acute PTE. Apelin has a potent vasodilator effect on vascular tissue via NO-dependent chemical mechanism [2,20,21]. In hypoxic conditions, vasoconstriction may also occur in vascular smooth muscle cells after apelin-induced chemical phosphorylation [22]. The mechanism of affecting the arterial pressure is still unclear. It is known that the level of blood apelin increases significantly in heart failure and pulmonary hypertension [23]. All these results suggest that the level of blood apelin-13 may increase when the pulmonary artery vascular bed is obstructed by thrombosis, as is the PTE.

In this study, we revealed that the serum apelin-13 level was significantly higher in cases with PTE and DVT (+) than in DVT (-) patients and the control group. This result suggests that apelin-13 is produced from endothelial cells, which play an important role in the fibrotic system in PTE. Serum apelin-13 was significantly higher in the massive PTE group, which was associated with more occlusion of the vascular bed with thrombus and right ventricular failure. But there was no statistically significant difference between groups (massive, sub-massive and non-massive) ( $p > 0.05$ ). According to Wells scoring, serum apelin-13 level was higher in the high clinically probable group than in the moderate and low clinically probable group, but there was no statistically significant difference between the groups ( $p > 0.05$ ) (Table 2).

The best cut-off value for apelin-13 in the patients and control groups was determined as 1579 ng/mL by the receiver operating characteristics (ROC) curve. Sensitivity was 92.7% and specificity was 96.7% (95% confidence interval: 0.939 to 0.995, area under ROC curve: 0.979,  $p < 0.001$ ) (Figure 2). Recently, Sen et al. found that serum apelin-13 level was significantly higher in the PTE group than in the control group ( $p < 0.001$ ) [24]. This result is similar to our study results. Unlike the study of Sen et al.,

Table 2. PTE Clinical Tables. Clinical Probabilities According to Wells Score and Apelin-13 Value

	n(%)	Serum Apelin-13 (ng/ml). mean ( $\pm$ SD)	P value
<b>PTE Clinic Table</b>			
Massive	13(15.8)	2280.8 $\pm$ 663.0	$p > 0.05$
Submassive	54(65.8)	2246.5 $\pm$ 591.4	
Non massive	15(18.2)	2156.0 $\pm$ 432.9	
<b>Wells Score</b>			
Low Clinical Probability	5(6.0)	1840.2 $\pm$ 144.3	$p > 0.05$
Moderate Clinical Probability	31(37.8)	2196.8 $\pm$ 592.0	
High Clinical Probability	46(56.2)	2275.8 $\pm$ 611.3	

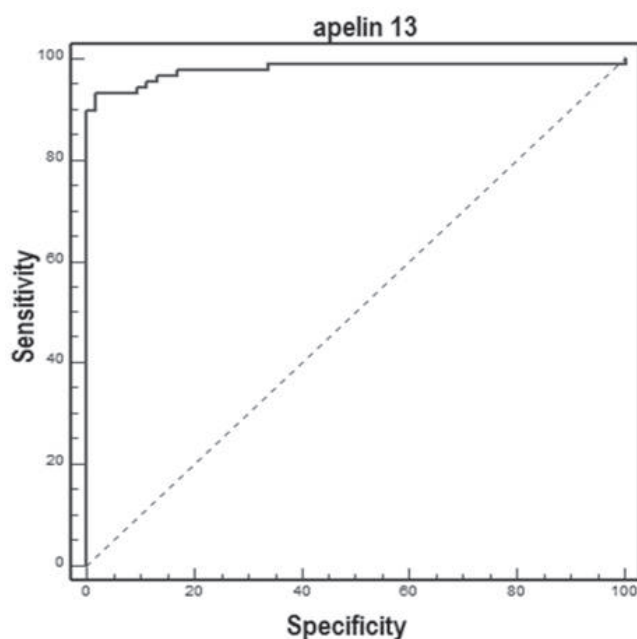


Figure 2. Roc Curve for PTE and Control Groups (95% confidence interval: 0.939 to 0.995, area under ROC curve: 0.979,  $p < 0.001$ )

a high level of apelin-13 level in DVT (+) patients has emerged as a new result. Only 25% of patients with signs and symptoms of DVT can be confirmed by diagnostic tests. In order to confirm the diagnosis, it is necessary to perform clinical risk scoring together with D-dimer or ultrasonography. In our study, both apelin-13 and D-dimer levels were higher in the DVT (+) patient group. This result suggests that there may be more thrombus burden in the presence of DVT with PTE.

In the present study, there are a few limiting factors. First, the study was performed on a relatively small patient and control group. We believe that studies with larger patient populations will reveal the relationship between PTE and apelin-13 more clearly. The second factor is that the study was single-centered. In conclusion, this is the first study showing the diagnostic value of serum apelin-13 level in patients with PTE and DVT (+). We believe that these results and apelin-13 value will contribute to clinical practice in diagnosis of PTE in future.

#### Scientific Responsibility Statement

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

#### Animal and human rights statement

*All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.*

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#### Conflict of interest

*None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.*

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