

Research Article

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Neutrophil gelatinase-associated lipocalin as a potential biomarker for pulmonary thromboembolism

Pulmoner Tromboembolinin Potansiyel Bir Biyobelirteci Olarak Nötrofil Jelatinaz-İlişkili Lipokalin (NGAL)

<https://doi.org/10.1515/tjb-2018-0308>

Received July 25, 2018; accepted February 28, 2019; previously published online October 12, 2019

Abstract

Objective: Pulmonary thromboembolism (PTE) is a clinical condition that can be lethal unless promptly diagnosed and treated. The objective was to evaluate the significance of serum neutrophil gelatinase-associated lipocalin (NGAL) in the diagnosis of PTE.

Materials and methods: In this study, 60 patients hospitalized for acute PTE between May 2015 and December 2016 were enrolled. PTE was diagnosed using spiral computed tomography angiography of the thorax. Cardiac enzyme levels, arterial blood gas, and echocardiography

measurements were performed. Whole blood samples were drawn to measure serum NGAL before treatment.

Results: The PTE group comprised 34 women and 26 men, and the healthy control group included 22 women and 18 men. The mean ages of the patient and control groups were 70.3 ± 14.4 years and 69.0 ± 10.2 years, respectively. Serum NGAL was significantly higher in the patients than in the controls (88.6 ± 33.6 vs. 31.7 ± 10.0 ng/mL, $p < 0.001$, respectively). The optimal NGAL cut-off value was >50 ng/mL, the sensitivity was 100%, specificity was 98.3%, the negative predictive value was 100%, and the positive predictive value was 68%.

Conclusion: Serum NGAL is a new biomarker with high sensitivity and specificity to detect, diagnose, and exclude PTE.

Keywords: Neutrophil gelatinase-associated lipocalin; Pulmonary thromboembolism; Biomarker; Inflammation; Diagnosis.

ÖZ

Amaç: Pulmoner tromboembolizm (PTE), hızlı tanı konulup tedavi edilmediğinde öldürücü olabilen klinik bir durumdur. Bu çalışmada amacımız PTE tanısında serum nötrofil jelatinaz ilişkili lipokalinin (NGAL) önemini değerlendirmektir.

Gereç ve Yöntem: Bu çalışmaya Mayıs 2015 – Aralık 2016 tarihleri arasında akut PTE nedeniyle hospitalize edilen 60 hasta alındı. PTE tanısı toraks spiral bilgisayarlı tomografisi ile kondu. Kardiyak enzim düzeyi, arter kan gazı ve ekokardiyografi ölçümleri yapıldı. Tedavi öncesi serum NGAL düzeyini ölçmek için tam kan örnekleri alındı.

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Bulgular: PTE grubunun 34'ü kadın, 26'sı erkek, sağlıklı kontrol grubunun 22'si kadın, 18'i erkek hastalardan oluşmaktaydı. PTE grubunun ve kontrol grubunun yaş ortalamaları sırası ile 70.3 ± 14.4 ve 69.0 ± 10.2 idi. Serum NGAL, PTE grubunda kontrol grubundan anlamlı derecede yüksekti (88.6 ± 33.6 'ya karşılık 31.7 ± 10.0 ng/mL, $p < 0.001$). Optimal NGAL kesme değeri >50 ng/mL alındığında, duyarlılık % 100, özgüllük % 98.3, negatif prediktif değer % 100 ve pozitif prediktif değer % 68 saptandı.

Sonuç: Serum NGAL, PTE'yi saptama, tanısını koyma ve dışlama konularında yüksek duyarlılığa ve özgüllüğe sahip yeni bir biyobelirteçtir.

Anahtar kelimeler: nötrofil jelatinaz-ilişkili lipokalin; pulmoner tromboemboli; biyobelirteç; inflamasyon; tanı.

Introduction

Pulmonary thromboembolism (PTE) is a clinical condition that often occurs due to the complete or partial obstruction of pulmonary arteries by dislodged thrombi originating from the venous system of the lower extremities. In most cases, there is a delay in diagnosing PTE due to the non-specificity of clinical findings, which leads to an increase in mortality. PTE was confirmed by contrast-enhanced thorax computed tomography angiography (CTA) in suspected cases. Since PTE is known as a disease that activates acute inflammatory pathways, serum D-dimer is the most frequently used parameter when contrast agent cannot be used [1]. In many studies, serologic biomarkers such as D-dimer, C-reactive protein, brain natriuretic peptide (BNP), N-terminal pro-hormone of brain natriuretic peptide (NT-proBNP), and troponin have been investigated to predict the diagnosis of pulmonary embolism and its severity.

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 or siderocalin, is a 25-kDa glycoprotein synthesized in granules of neutrophils. It has been shown that NGAL is also produced by renal proximal tubule cells, endothelial cells and smooth muscle cells [2, 3]. Although its function is not fully known, it is thought to play a role in the immune response that occurs in various situations in the body. It has been shown that there is an increase in blood NGAL levels in acute and chronic inflammatory diseases, in the case of ischemic diseases such as stroke and myocardial infarction, metabolic diseases such as obesity and type 2 diabetes mellitus, acute and chronic renal failure, heart and kidney transplantation, solid tumors such as lung, colon and breast, and chronic obstructive pulmonary disease [4–6]. It has been shown that NGAL can be a useful biomarker for the early detection and prognosis of acute renal damage associated

with pulmonary embolism [7, 8]. NGAL has also been shown as a potential biomarker in two studies conducted on patients with pulmonary thromboembolism [9, 10]. The purpose of this study was to investigate serum NGAL levels in the diagnosis of PTE since it is thought to be a potentially valuable marker in the evaluation of PTE.

Materials and methods

This study was conducted on patients with PTE diagnosed and hospitalized by the Department of Pulmonary Diseases in Recep Tayyip Erdogan University Faculty of Medicine. The study was approved by the local clinical Ethics Committee. Patients older than 20 years old who had a filling defect at least in their segmental pulmonary artery on spiral thorax CTA between June 2015 and June 2016 were included in the study. The subjects enrolled in the study underwent routine biochemical blood analysis, D-dimer and troponin-T assays. Clinical risk scores were calculated according to the Wells criteria. Simplified pulmonary embolism severity index (sPESI) score was used to assess early mortality risk. Patients with at least one of the following criteria were classified as high-risk: cardiovascular disease, malignancy, arterial systolic blood pressure of 90–100 mm Hg, oxygen saturation of lower than 90%, and a pulse rate higher than 110/min.

Echocardiography (ECHO) was performed by a cardiologist in all patients, and systolic pulmonary artery pressure and right ventricular structure and function were evaluated. Right ventricular dilatation, hypokinesia, paradoxical motion of the interventricular septum, pulmonary artery dilatation, and tricuspid insufficiency were accepted as the findings of right ventricular dysfunction [11]. Deep vein thrombosis (DVT) was investigated using lower extremity venous Doppler ultrasonography. Troponin-T was determined using chemiluminescence on a Siemens AdviaCentaur CP autoanalyzer (Germany) (threshold value: 0–0.06 ng/mL), and D-dimer was measured using time-resolved fluorescence on a AQT90 Flex Radiometer autoanalyzer (Denmark) (threshold value: 0–654 µg).

The control group comprised 40 healthy subjects with similar age and gender characteristics. Demographic characteristics were recorded and 10 mL venous blood samples were taken for serum NGAL measurement.

Exclusion criteria

Patients who were pregnant, and those with acute infectious conditions, advanced heart failure ($EF < 45\%$), malignancy (active or remission), autoimmune diseases, acute

thrombotic disease history (e.g. ischemic cerebrovascular event, acute coronary syndrome), acute or chronic renal failure, and chronic liver disease were excluded from the study.

Ethics approval

This study was approved by local Ethics Committee (Number: 2016/27).

Serum NGAL measurement

Ten milliliters of venous blood was taken using red sterile plain serum collection tubes (BD Vacutainer SST II Advance Serum Separator Tubes, USA) for the measurement of serum NGAL within the first 30 min of admission. The blood sample was centrifuged within 1 h for 10 min at 3000 rpm. Serum was removed by pipetting into Eppendorf tubes and stored at -80°C until required for analysis. Serum NGAL levels were quantitatively measured using an enzyme-linked immunosorbent assay (ELISA) with human lipocalin 2/NGAL antibodies from Biovendor, Research and Diagnostics Products (Karasek, Czech Republic). Calibration of this assay is based on the concentration of the recombinant protein used as the standard; there is no international reference material that can be used for a traceability study.

Statistical analyses

Statistical evaluations were performed using the IBM-SPSS program (SPSS version 21; SPSS Inc., Chicago, IL, USA). Normal distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables are given as mean $\pm$ standard deviation, median, minimum and maximum levels, and categorical variables as frequency (n) and percentage (%). The Mann-Whitney U test was used for the comparison of two groups, and the Kruskal-Wallis test was used for the comparison of multiple groups. Comparisons of categorical variables were made using the χ^2 test. The relationship between variables was examined using Spearman correlation analysis. Receiver operating characteristics (ROC) curve analysis was performed to show the efficacy of serum NGAL in distinguishing patients with PTE from healthy patients. Specificity, sensitivity, positive and negative predictive values were also calculated. A p value of <0.05 was considered statistically significant.

Results

The PTE group included 34 women and 26 men, and the healthy control group consisted of 40 subjects (22 women, 18 men). The mean age of the PTE group and healthy control group was 70.3 ± 14.4 years and 69.0 ± 10.2 years, respectively.

In the prognostic classification performed using the sPESI criteria, 25 patients were found to be in the high-risk group and 35 patients were in the low-risk group. Patients were divided into high, moderate, and low-risk groups based on the presence of shock and hypotension, and right ventricular dysfunction found in ECHO and cardiac markers. Thus, the high-risk (massive), moderate-risk (submassive), low-risk (non-massive) groups comprised 946, and 5 patients, respectively.

Serum NGAL levels were significantly higher in the PTE group than in the control group (88.6 ± 33.6 ng/mL vs. 31.7 ± 10.0 ng/mL, respectively) ($z = -8.436$, $p < 0.001$) (Figure 1). The clinical characteristics of the PTE groups are summarized in Table 1. The relationship between the clinical characteristics of the patients and NGAL levels is shown in Table 2. There was no significant relationship between the clinical characteristics and NGAL.

In this study, there was a positive correlation between D-dimer and troponin ($r = 0.706$, $p < 0.001$) and a negative correlation between D-dimer and fibrinogen ($r = -0.522$, $p < 0.001$). There was no positive or negative correlation between NGAL and any of the other variables. Serum NGAL levels were similar between the massive (median: 81.5, range = 50–151), sub-massive (median: 82.3, range = 50–152) and non-massive (median: 80.7, range = 73–283) groups ($\chi^2 = 0.442$, $p = 0.506$) (Table 2). In addition, serum NGAL levels of DVT-positive subjects were

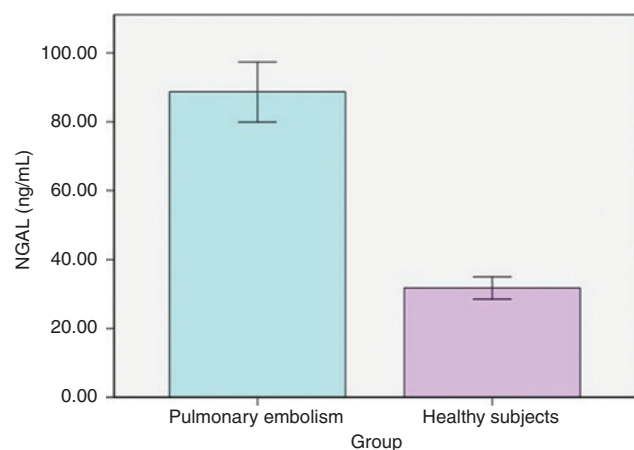


Figure 1: Comparison of NGAL levels in pulmonary embolism and healthy controls.

Table 1: The clinical characteristics, arterial blood gas values, and troponin levels of patients.

Variable	Mean (SD)	Median (min–max)
SBP (mm Hg)	120.3 (22.2)	120 (90–230)
DBP (mm Hg)	74.6 (13.4)	70 (50–110)
Pulse rate (/min)	87.8 (15.9)	86 (55–110)
Respiratory rate (/min)	15.7 (3.2)	15 (12–26)
PO ₂ (mm Hg)	63.9 (15.9)	65 (27–94)
PCO ₂ (mm Hg)	31.3 (6.3)	32 (15–57)
Oxygen saturation (%)	91.2 (7.2)	93 (59–98)
Troponin T (ng/mL)	0.56 (0.73)	0.16 (0–2.5)

SD, Standard deviation; min, minimum; max, maximum; SBP, systolic blood pressure; DBP, diastolic blood pressure; PO₂, partial oxygen pressure; PCO₂, partial carbon dioxide pressure.

not different from those who were DVT-negative (median: 81.8, range=50–283 vs. median: 82.3, range=50–151) ($z = -0.163$, $p = 0.871$) (Table 2).

The specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of different values for serum NGAL were measured and the results are shown in Table 3. The specificity, sensitivity, and PPV and NPV of a 50 ng/dL serum level of NGAL was found as 100%, 98.3%, 100%, and 68%, respectively.

Table 2: NGAL levels of the patients with PTE ($n = 60$) with respect to their clinical characteristics.

	Total	NGAL (ng/mL)	Statistics	
	n (%)	M (min–max)	z or χ^2	p-Value
Gender				
Male	26 (43.3)	70 (10–114)	–1.268	0.205
Female	34 (56.7)	69.4 (10–283)		
RVD on ECHO				
Yes	37 (61.7)	81.5 (50–283)	–0.996	0.319
No	23 (38.3)	84.9 (61–152)		
Mortality risk				
High (massive)	9 (15.0)	81.5 (50–151)	0.442	0.506
Intermediate (sub-massive)	46 (76.7)	82.3 (50–152)		
Low (non-massive)	5 (8.3)	80.7 (73–283)		
DVT presence				
Yes	28 (46.7)	82.3 (50–151)	–0.163	0.871
No	32 (53.3)	81.8 (50–283)		
sPESI				
Low-risk	35 (58.3)	82.7 (56–283)	–0.757	0.449
High-risk	25 (41.7)	81.5 (50–152)		

M, Median; min, minimum; max, maximum; RVD, right ventricle dysfunction; ECHO, echocardiography; DVT, deep vein thrombosis; sPESI, simplified pulmonary embolism severity index.

Table 3: Specificity and sensitivity of NGAL in different levels.

Serum NGAL	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
40 ng/dL	82.5	100	89.5	50
45 ng/dL	95	100	96.7	62
50 ng/dL	100	98.3	100	68
55 ng/dL	100	95	100	70

PPV, Positive predictive value; NPV, negative predictive value.

Discussion

PTE is generally acknowledged as being difficult to diagnose despite the advances in diagnostic methods, thus mortality and morbidity are high in patients whose treatment is delayed. In undiagnosed patients, mortality increases up to 30%, whereas this rate is between 2 and 8% in those who are able to receive early diagnosis and treatment [12]. Rapid diagnosis and treatment initiation is very important in terms of decreasing mortality and morbidity because mortality rates are higher in the early period following PTE, especially in the first 3 months [12, 13].

Although some studies indicated that the serum levels of cardiac markers are found to be elevated in myocardial injury such as cardiac troponin-T (cTn-T), NT-proBNP, heart-type fatty acid-binding protein (H-FABP), and myoglobin (Mb), these markers could also be associated with poor prognosis and early mortality in PTE [14, 15]. There are few useful biomarkers that can be used for diagnosing PTE. D-dimer, which indicates activation of the endogenous fibrinolytic system, is one of the non-invasive blood biomarkers used in the diagnosis of PTE [16]. Although the sensitivity of the D-dimer test is high, its specificity is low [17]. A negative D-Dimer test is highly reliable in PTE exclusion, except in patients with high clinical probability. Test results can be positive in cases of surgical intervention, trauma, kidney diseases, malignancies, severe infections, systemic lupus erythematosus (SLE), and pregnancy [18]. Therefore, new non-invasive biomarkers are needed that can be applied easily, and can be used to determine both the diagnosis and severity of the disease.

In the literature, a few studies have investigated NGAL levels in PTE. The most important result of our study is that serum NGAL levels can be used as an acute inflammation biomarker in patients with PTE. The serum NGAL levels in our study were significantly higher in patients with PTE than in the healthy control group. ROC analysis showed that the best cut-off value was 50 ng/mL for NGAL. The sensitivity at this value was calculated as 100%, and the specificity was 98.3%. In a study conducted

by Saritabak [9], serum NGAL levels were found to be significantly higher in patients with PTE in emergency care. The calculated cut-off value for NGAL was reported as 88 ng/mL, with a sensitivity of 71%, a specificity of 61%, and a PPV and NPV of 59 and 72%, respectively. As a result, it was suggested that NGAL could be used as a tool for diagnosis, but could not replace imaging methods especially spiral thorax CTA [9].

In contrast, Nordenholz examined the diagnostic value of 50 biomarkers including NGAL in PTE and concluded that NGAL levels were not diagnostic for PTE [1]. On the contrary, the results of our study show that serum NGAL levels above 50 ng/mL would support PTE and may be a useful marker for diagnosis. The study by Saritabak revealed higher NGAL levels in patients with PTE who also had pulmonary infarction, on pulmonary CTA, than patients without infarction [9]. In addition, higher NGAL levels were found in patients with massive PTE who were treated with thrombolytic therapy in the emergency department than in patients with non massive PTE. In our study, there was no statistically significant difference between the subgroups because of the low number of patients with massive emboli and thrombolytic therapy, and also no correlation was found between NGAL and any of the other variables. In a study by Kostrubiec et al. it was reported that NGAL levels were valuable in determining 3-month mortality in PTE [8]. In our study, there was no correlation between NGAL and mortality due to the fact that no deaths occurred during the first and third months of follow-up. Previous studies have shown that sPESI is as effective as the standard PESI score in determining the prognosis of pulmonary embolism [19, 20]. In the study by Kostrubiec et al. [8], both the PESI score and right ventricular dysfunction were found to be correlated with NGAL levels. However, there was, no such correlation in our study.

The most useful biomarker used in pulmonary embolism to date is D-dimer as measured using ELISA. It is known that serum levels <500 ng/mL excludes the diagnosis of embolism at a rate of 95–99%; therefore, the sensitivity is high but the specificity is low [12, 16, 17]. D-dimer negativity is used to exclude PTE in outpatients with no comorbidities who have low or moderate clinical probability [12].

In this study, we investigated the diagnostic value of serum NGAL level in pulmonary embolism, and the sensitivity and specificity were found as 100% and 98.3%, respectively, when the cut-off value of NGAL is considered 50 ng/mL. Although the study sample consisted of 60 patients, this finding suggests that NGAL could be used as a valuable biomarker in PTE.

As a result, serum NGAL levels may be helpful in the diagnosis of PTE as a non-invasive, cheap, and easily accessible marker given that there are limited number of diagnostic markers for PTE. Currently available diagnostic methods for PTE are invasive, have high complication rates, and high costs. However, it is not practical to use in emergency conditions because NGAL assessment requires manual applications and procedures, but it could still be analysed with an autoanalyser for suitable patient groups. There is a need for further studies that include patients with massive emboli, covering large number of patients, investigating whether NGAL can be used as a predictor of mortality.

Acknowledgement: None.

Conflict of interest: There are no conflicts of interest to be declared by the authors.

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