The diagnostic value of cyclophilin A and VAP-1 in patients with suspected pulmonary embolism

Pulmoner emboli şüphesi olan hastalarda siklofilin A ve VAP-1'in tanısal değeri

Aynur Sahin[1], Olgun Asik[2], Ozgur Tatli[1], Yunus Karaca[1], Selim Demir[3], Ahmet Mentese[4], Suleyman Caner Karahan[5], Suleyman Turedi[1]

[1]Karadeniz Technical University, Faculty of Medicine, Department of Emergency Medicine, Trabzon, Turkey

[2] University of Health Sciences, Trabzon Kanuni Training and Research Hospital, Department of Emergency Medicine, Trabzon, Turkey

[3]Karadeniz Technical University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Trabzon, Turkey

[4]Karadeniz Technical University, Health Services Vocational School, Medical Laboratory Techniques Department, Trabzon, Turkey

[5]Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Turkey

Received: 06.07.2018 Accepted: 31.08.2018 Doi: 10.21601/ortadogutipdergisi.441377

Abstract

Aim: To investigate the diagnostic value of the biochemical markers cyclophilin A (CYPA) and vascular adhesion protein-1 (VAP-1), associated with endothelial dysfunction, platelet adhesion, and arterial thrombus in pulmonary embolism (PE), in whose pathophysiology platelet activation and aggregation resulting from endothelial damage are known to be involved.

Material and Method: Serum CYPA and VAP-1 levels of 165 patients presenting to the emergency department with suspected acute PE and with presence of PE investigated at spiral computerized tomography angiography were measured in this prospective clinical study. These patients were assigned into two groups, PE (+) and PE (-), based on the computerized tomography results, and their CYPA and VAP-1 levels were then compared.

Results: Comparison of the two groups' measurements revealed a significant difference in terms of VAP-1 levels (p=0.0001), but none for CYPA (p=0.381).

Conclusion: Diagnostic value was determined for serum VAP-1 levels in acute PE, but we couldn't detect any diagnostic value for CYPA in patients with acute PE.

Keywords: Pulmonary embolism, cyclophilin A, VAP-1

Öz

Amaç: Bu çalışmanın amacı endotel hasarı sonucu trombosit aktivasyonu ve kümeleşmesinin patofizyolojisinde rol oynadığı bilinen pulmoner emboli (PE) hastalığında; endotel disfonksiyonu, platelet adezyonu ve arterial trombüs ile ilişkelendirilen siklofilin A (CYPA) ve vasküler adezyon protein- 1 (VAP-1) adlı biyokimyasal belirteçlerin tanısal değerini araştırmaktır.

Gereç ve Yöntem: Prospektif olarak yapılan bu klinik çalışmada acil servise akut PE şüphesi ile başvurarak çalışmaya alınan ve spiral bilgisayarlı tomografi anjiografi ile PE varlığı değerlendirilen 165 hastanın serum CYPA ve VAP-1 düzeyi ölçüldü. Bilgisayarlı tomografi sonuçlarına göre, PE (+) ve PE (-) olarak 2 gruba ayrılan bu hastaların ölçülen CYPA ve VAP-1 düzeyleri birbirleriyle karşılaştırıldı.

Bulgular: Her iki grup ölçümleri birbiriyle karşılaştırıldığında VAP-1 düzeyi bakımından gruplar arası fark istatistiksel anlamlı bulunurken (p=0.0001), CYPA düzeyinin bakımından bu fark istatistiksel olarak anlamlı değildi. (p=0.381).

Sonuç: Akut PE'de serum VAP-1 düzeyinin tanısal olarak kullanılabileceği tespit edilmiştir. Serum CYPA düzeyinin ise akut PE'de tanısal değeri bulunmamaktadır.

Anahtar Kelimeler: Pulmoner emboli, siklofilin A,VAP-1

Introduction

Pulmonary embolism (PE) is a frequently encountered emergency condition. Since early treatment of PE is highly effective, early diagnosis is of very great importance. The latest and current diagnostic and treatment guideline published by the European Society of Cardiology (ESC) in 2014 recommends the use of a diagnostic algorithm that varies depending on the presence of shock findings. Of the current biochemical markers, only D-dimer is included in this algorithm for the purpose of diagnosis. Accordingly, D-dimer values are studied in the evaluation in terms of PE of patients without hypotension and other shock findings if these subjects are identified as low probability using the modified Wells Score as a clinical probability score. If D-dimer is negative, this casts doubt on a diagnosis of PE. Computerized tomography (CT) angiography is recommended in terms of excluding PE in patients with positive D-dimer tests. If the probability of PE based on clinical scoring is strong, direct CT angiography is performed. If patients with hypotension and other findings of shock are stabilized, CT angiography is recommended as the first diagnostic technique. However, if the patient is unstable, or if CT angiography cannot be performed, right ventricular dysfunction evaluation with bedside echocardiography (ECHO) is recommended. If findings of right ventricular loading are present at ECHO, a diagnosis of PE should be considered and treatment administered accordingly [1]. However, since CT angiography the most important component of this algorithm, is not available always and everywhere, problems may arise such as delayed contrast and the need to transport patients in shock outside the emergency department. Although ECHO, another diagnostic method, can be performed at the bedside and is non-invasive, it is user-dependent and has lower sensitivity and specificity than CT angiography [2]. Moreover, as with ECHO, it may not be always be available in all centers.

CYPA is a cytosolic, highly abundant protein with peptidil prolyl cis-trans isomerase activity. CYPA is an intracellular protein released from smooth muscle cells, macrophages and platelets in response to conditions of increased oxidative stress. It has pro-inflammatory effects on endothelial cells and plays an important role in the pathogenesis of inflammatory diseases (such as atherosclerosis) [3]. Additionally, CYPA has been determined to be an important Ca2+ regulator for platelets and to be associated with arterial thrombosis [4]. Furthermore, CYPA causes endothelial cell apoptosis leading to injury and resulting in endothelial dysfunction [5].

VAP-1 is coded by the AOC3 gene and expressed in adipocytes, the endothelium and smooth muscle [6]. VAP-1 possesses enzymatic activity, and is also known as primary amine oxidase or semicarbazide-sensitive amine oxidase. It catalyzes the breakdown of primary amines, and its activity is inhibited by semicarbazide. It is a human endothelial sialoglycoprotein released during the inflammatory process

at the cellular level [7]. VAP-1 activity is directly associated with carotid artery plaque and intima media thickness in the general population, and studies have shown that it may play a role in the pathophysiology of preclinical atherosclerosis [8]. One study determined significant correlation between severity of calcific aortic stenosis and an increase in serum VAP-1 levels, and concluded that VAP-1 can be used for monitoring the severity of aortic stenosis [9]. Plasma VAP-1 protein levels increase with ageing, and this has been linked to arterial stiffness in the elderly [10]. Due to its entry into circulation in ischemic events, VAP-1 has thus been shown to be capable of use as a potential biomarker in early ischemic vasculopathy [11].

Underlying causes implicated in PE include endothelial damage, hypercoagulopathy, and venous stasis. Substances released from endothelial cells and platelets are involved in the primary establishment of hemostasis. Our study was planned around the hypothesis that due to the endothelial damage, thrombosis, and frequently accompanying hypoxia and oxidative stress load in patients with PE, changes may also occur in CYPA and VAP-1 levels. The primary purpose of our study was to determine the diagnostic value of cyclophilin A and VAP-1, which have been linked to endothelial dysfunction, platelet adhesion, and arterial thrombosis in PE, in whose pathophysiology thrombocyte activation and aggregation resulting from endothelial damage are known to be involved. Our secondary aim was to determine the value of CYPA in determining the severity of pulmonary embolism assessed on the basis of ECHO, computerized tomography, and troponin levels. In addition, we also intended to determine whether or not CYPA measured at time of presentation is of any prognostic value in patients with PE by scanning prognosis together with such clinical outcome points as intensive care requirement, intubation and mechanical ventilator requirement, vasopressor support requirement, thrombolytic requirement, and mortality in PE patients in hospital and in the first three months after discharge.

Material and Method

Study design

This research was a prospective, randomized clinical study. Approval for the study was granted by the local ethical committee (No. 2016/173)

Study setting and population

Our study was performed in the Karadeniz Technical University School of Medicine Emergency Department, Turkey, a tertiary center serving approximately 50000 patients a year. All patients aged 18 or over and presenting to the emergency department with suspected PE in the one-year period following receipt of ethical committee approval and consenting to take part were included in the study. Patients with advanced liver failure, kidney failure, or acute mesenteric ischemia at time of presentation, pregnant women, and patients with missing record data were excluded from the study.

Study Protocol

A diagnostic approach compatible with the algorithm set out in the ESC 2014 PE guideline was applied in the case of all patients presenting to the emergency department on suspicion of acute PE. Patients undergoing spiral CT angiography (Siemens Somatom Sensation, Germany) in line with that algorithm and identified as having PE were enrolled in the PE (+) group, and those without PE were enrolled in PE (-) group. The diagnostic values of CYPA and VAP-1 were then investigated.

Laboratory Analysis

Blood specimens collected from subjects together with consent forms were placed into biochemistry tubes containing separator gel and allowed to stand for coagulation for 20 min at room temperature. The tubes were then centrifuged for 10 min at 1800×g. Following centrifugation, the serum part was carefully transferred into small tubes and stored at -80º C until analysis.

VAP-1 and CYPA Measurement

CYPA levels in serum specimens were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Cat No: E-EL-H1934, Wuhan, China) in line with the manufacturer's instructions.VAP-1 levels were also determined using an ELISA kit (Elabscience, Cat No: E-EL-H2259, Wuhan, China). The results were expressed as ng/mL for CYPA, and as pg/mL for VAP-1.

Data Analysis

Statistical analysis was performed on SPSS 23.0 (IBM SPSS, Armonk, NY) statistical software. Normal distribution of data was analyzed using the Shapiro-Wilk test, and the Mann-Whitney U test was used to compare the PE (+) and PE (-) groups. Statistical significance was set at $p < 0.05$.

Results

Two hundred twenty-one patients presenting to the

emergency department during the study period and with suspected PE were evaluated. However, following application of the exclusion criteria, serum VAP-1 and CYPA levels were measured in serum specimens from 165 patients. PE was determined in 40.6% of patients included in the study and undergoing tomography due to suspicion of the condition.

Patients' clinical and demographic characteristics are shown by groups in Table 1. No statistically significant difference was determined between the groups in terms of demographic or clinical characteristics. The mean age of the patients in whom PE was determined was 72.5 years, and women represented 60.6% of patients with suspected PE. The most common presentation symptom was dyspnea, observed in 83% of patients. The least common symptom in patients with confirmed PE was hemoptysis (1.8%).

VAP-1 and CYPA levels measured from serum specimens collected at time of presentation are shown in Table 2. Comparison shows no significant difference between the two groups in terms of CYPA levels (p=0.381), but VAP-1 levels differed significantly (p= 0.0001).

Table 1. Patients' demographic and clinical characteristics

	PE (-)	PE (+)	p value
Age, Median (min-max)	72.5 (18-96)	78 (38-95)	0.69
Sex			
	n (%)	n (%)	
Female	55 (33.3%)	43 (27.3%)	0.194
Male	45 (26.1%)	22 (13.3%)	
Symptom			
	n (%)	n (%)	
Dyspnea	82 (49.7%)	55 (33.3%)	0.834
Chest pain	24 (14.5%)	17 (10.3%)	1.0
Hemoptysis	4 (2.4%)	3 (1.8%)	1.0
Syncope	16 (9.7%)	16 (9.7%)	0.237
DVT symptoms	3 (1.8%)	6 (3.6%)	0.161
Other symptoms	27 (16.4%)	23 (13.9%)	0.391
Clinical signs			
SBP mmHg, Mean±SD	118.57±23.9	117.08±24.4	0.649
DKB mmHg, (Median,min-max)	71.5 (40-140)	72 (40-120)	0.831
Heart beat /min, Mean±SD	101.94±23.3	98.65±21.9	0.320
Respiration rate/min Median (min-max)	22 (10-45)	24 (10-42)	0.257
Body temperature °C, med (min-max)	36.7 (35.9-39.5)	36.8 (36-39.2)	0.697

p values < 0.05 significant according to the Mann-Whitney U test

Table 2. Serum VAP- 1 and CYPA levels in the study groups

	Groups	
	PE(+) n=67	**PE(-) n=98**
VAP-1 *(pg/mL)		
Median	1472a	2257a
IQR	1207-1704	1900-2707
CYPA (ng/mL)		
Median	5.21	5.90
IQR	2.61-20.7	1.52 -17.5

p< 0.05 statistically significant according to the Mann-Whitney U test
* **a** for VAP-1 levels , p=0.0001

Discussion

The biomarker currently used in the diagnostic algorithm for PE is D-dimer. Negative D-dimer excludes a diagnosis of PE, while several pathologies can give rise to positivity. Specific biochemical markers are now needed to assist the use in more limited indications of spiral CTA, the gold standard method in the diagnosis of PE, with its disadvantages of high costs, restricted availability, and potential complications such as contrast nephropathy. This clinical study investigated the diagnostic value in patients presenting to the emergency department with suspicion of PE of serum VAP-1 and CYPA levels, which have previously been linked to thrombosis in previous clinical and experimental studies. In the light of our findings, we conclude that serum CYPA levels are of no diagnostic value in acute PE, while serum VAP-1 levels can be used for diagnostic purposes.

Our first finding is that serum VAP-1 levels in patients with PE identified with spiral CTN angiography were significantly lower than those of the patients without PE. Although previous studies support the idea of an increase in levels in thrombotic events, ours is the first study to show that VAP-1 levels can be used for diagnostic purposes in PE. VAP-1 is a cellular adhesion molecule involved in atherosclerosis and inflammation. Previous studies have linked VAP-1, particularly released by the vascular endothelium, to stroke, obesity, cardiovascular system diseases, and, in addition, inflammatory bowel disease [6,8,12]. One clinical study showed that plasma VAP-1 is an independent risk factor for severity of arterial stiffness. That study cited inflammation, advanced glycation end-products emerging after ischemia, endothelial dysfunction, and increased oxidative stress as mechanisms potentially involved in the mechanisms underlying the relation

between plasma VAP-1 and severity of arterial stiffness, a known predictive risk factor for arterial stenosis, coronary artery disease, and stroke [10]. One experimental study in which an in vivo stroke model was induced in rats determined a positive correlation between plasma VAP-1 levels and myeloperoxidase levels. Those authors concluded that VAP-1 mediates neutrophil activation and migration by being released into plasma within a few hours of acute ischemic events [13]. In an experimentally induced hemorrhagic stroke model in mice, VAP-1 inhibition was shown to reduce adhesion molecule expression by preventing immune cell infiltration, thus causing an anti-inflammatory effect in tissue [14]. However, it is unclear why the anticipated difference should manifest with a low value in our PE patients.

Our second finding is the absence of any difference between the groups in terms of CYPA levels. Several publications have reported that CYPA levels are affected by various pathologies, including coronary artery disease, acute ischemic stroke, and aortic aneurysm [15-17]. The underlying pathogenesis involves the release of reactive oxygen products as a result of thrombosis from endothelial cells in the arterial wall, and these products' increasing the release of CYPA from endothelial cells [18,19]. CYPA release is a mediator that triggers inflammation by increasing leukocyte migration [20]. The results did not support our hypothesis that CYPA may increase in the inflammatory process resulting from acute thrombosis in the pathogenesis of PE, and that it may potentially be used in diagnosis.

In the light of our findings, we conclude that VAP-1 levels measured in patients presenting to the emergency department with suspected PE may be of diagnostic value, while CYPA levels are of no diagnostic significance.

Study Limitations

The first limitation of our study is that since blood was collected from patients with suspected PE at time of presentation, no conclusion can be drawn concerning time-dependent changes in VAP-1 or CYPA levels. The second limitation is that ROC analysis was not performed for VAP-1 levels, which differed significantly between the two groups. The final limitation is that we are unable account for the difference in VAP-1 involving low values in our PE patients.

Declaration of conflicting interests

The author declared no conflicts of interest with respect to the authorship and/or publication of this article.

References

1. Konstantinides S, Torbicki A, Agnelli G, et al. ESC/PCS New Guidelines. Kardiol Pol 2014; 72: 997-1053.
2. Bova C, Greco F, Misuraca G, et al. Diagnostic utility of echocardiography in patients with suspected pulmonary embolism. Am J Emerg Med 2003; 21: 180-3.
3. Seizer P, Fuchs C, Ungern-Sternberg SN, et al. Platelet-bound cyclophilin A in patients with stable coronary artery disease and acute myocardial infarction. Platelets 2016; 27: 155-8. doi:10.3109/09537104.2015.1051466.
4. Elvers M, Herrmann A, Seizer P, et al. Intracellular cyclophilin A is an important Ca(2+) regulator in platelets and critically involved in arterial thrombus formation. Blood 2012; 120: 1317-26. doi:10.1182/blood-2011-12-398438.
5. Tian-Tian Z, Jun-Feng Z, Heng G. Functions of cyclophilin A in atherosclerosis. Exp Clin Cardiol 2013; 18:e118-24.
6. Pannecoeck R, Serruys D, Benmeridja L, et al. Vascular adhesion protein-1: Role in human pathology and application as a biomarker. Crit Rev Clin Lab Sci 2015; 52: 284-300. doi:10.3109/10408363.2015.1050714.
7. Smith DJ, Salmi M, Bono P, Hellman J, Leu T, Jalkanen S. Cloning of vascular adhesion protein 1 reveals a novel multifunctional adhesion molecule. J Exp Med 1998; 188: 17-27.
8. Aalto K, Maksimow M, Juonala M, et al. Soluble vascular adhesion protein-1 correlates with cardiovascular risk factors and early atherosclerotic manifestations. Arterioscler Thromb Vasc Biol 2012; 32: 52332.doi:10.1161/ATVBAHA.111.238030.
9. Altug Cakmak H, Aslan S, Erturk M, et al. Assessment of the Relationship Between Serum Vascular Adhesion Protein-1 (VAP-1) and Severity of Calcific Aortic Valve Stenosis. J Heart Valve Dis 2015; 24: 699-706.
10. Chen DW, Zhao RM, Jin Y, et al. Plasma soluble vascular adhesion protein-1 concentration correlates with arterial stiffness: A cross-sectional study. Arch Gerontol Geriatr 2015; 61: 67-71. doi:10.1016/j.archger.2015.04.007.
11. Airas L, Lindsberg PJ, Karjalainen-Lindsberg ML, et al. Vascular adhesion protein-1 in human ischaemic stroke. Neuropathol Appl Neurobiol 2008; 34: 394-402. doi:10.1111/j.1365-2990.2007.00911.x.

12. Aalto K, Havulinna AS, Jalkanen S, Salomaa V, Salmi M. Soluble vascular adhesion protein-1 predicts incident major adverse cardiovascular events and improves reclassification in a finnish prospective cohort study. Circ Cardiovasc Genet 2014; 7: 529-35. doi:10.1161/CIRCGENETICS.113.000543.

13. Sun P, Sole M, Unzeta M. Involvement of SSAO/VAP-1 in oxygen-glucose deprivation-mediated damage using the endothelial hSSAO/VAP-1-expressing cells as experimental model of cerebral ischemia. Cerebrovasc Dis 2014; 37: 171-80. doi:10.1159/000357660.

14. Ma Q, Manaenko A, Khatibi NH, Vascular adhesion protein-1 inhibition provides antiinflammatory protection after an intracerebral hemorrhagic stroke in mice. J Cereb Blood Flow Metab 2011; 31: 881-93. doi:10.1038/jcbfm.2010.167.

15. Karaca Y, Tatli O, Aksut N, et al. Diagnostic Value of Cyclophilin A in Acute Ischemic Stroke. Eurasian J Emerg Med 2017; 16: 54-6. doi:10.5152/eajem.2017.35119.

16. Nigro P, Satoh K, O'Dell MR, et al. Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. J Exp Med 2011; 208: 53-66. doi:10.1084/jem.20101174.

17. Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X et al. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nat Med. 2009; 15: 649-56. doi:10.1038/nm.1958.

18. Jin ZG, Melaragno MG, Liao DF, et al. Cyclophilin A is a secreted growth factor induced by oxidative stress. Circ Res 2000; 87: 789-96.

19. Liao DF, Jin ZG, Baas AS, et al. Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells. J Biol Chem. 2000;275(1):189-96.

20. Damsker JM, Bukrinsky MI, Constant SL. Preferential chemotaxis of activated human CD4+ T cells by extracellular cyclophilin A. J Leukoc Biol 2007; 82: 613-8. doi:10.1189/jlb.0506317.

Corresponding Author: Aynur Sahin, Karadeniz Technical University, Faculty of Medicine, Department of Emergency Medicine, Farabi Street, No: 66, 61080, Merkez, Ortahisar, Trabzon, Turkey

E-mail: dr-aynursahin@hotmail.com