Could VEGF-D level have a role in clinical risk scoring, estimation of thrombus burden, and treatment in acute pulmonary thromboembolism?

Abstract

Objective: Pulmonary embolism (PE) is usually a complication of deep vein thrombosis and is an important cause of mortality and morbidity. Vascular endothelial growth factor D (VEGF-D) is a secretory protein that plays a role in the remodeling of blood vessels and the lymphatic system. This study aimed to determine the relationship between VEGF-D level and clinical risk scoring in patients with PE.

Methods: The study included 117 patients admitted for PE that were divided into 4 groups: high-risk patients (n=35), high-intermediate-risk patients (n=30), low-intermediate-risk patients (n=24), and low-risk patients (n=28). Plasma VEGF-D was measured from peripheral venous blood samples (5 cc) using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Pulmonary Artery Obstruction Index (PAOI) was calculated from CT angiography imaging.

Results: VEGF-D levels in the low-risk PE group differed significantly from those in the high-intermediate and high-risk groups (p=0.001 for both) but not from that in the low-intermediate-risk PE group (p=0.155). There was no significant difference in troponin-I and NT-proBNP levels between the high-intermediate-risk and high-risk PE patients, whereas VEGF-D levels differed significantly (p=0.134, p=0.146, p=0.016). VEGF-D level was moderately correlated with mean pulmonary artery pressure and PAOI (r=0.481, p=0.01; r=0.404, p=0.01). In ROC curve analysis, a cut-off of 370.1 pg/ml for VEGF-D had 91.4% sensitivity and 67.4% specificity in the differentiation of high-intermediate-risk and high-risk PE patients.

Conclusion: This study showed that plasma VEGF-D level was more reliable than troponin-I and NT-proBNP in clinical risk scoring and demonstrating thrombus burden. VEGF-D can be used as a biomarker in clinical risk scoring and estimation of thrombus burden in patients with acute PE.

Keywords: Pulmonary embolism, clinical severity, VEGF-D

What's already known about this topic?

Right ventricular dilatation results in the release of certain cytokines to ensure oxygenation, neovascularization, and rapid thrombus organization. The most important of these cytokines is vascular endothelial growth factor (VEGF). VEGF causes endothelial proliferation and neovascularization, and prevents reperfusion injury by increasing glutathione peroxidase activity. VEGF-D, a member of the VEGF family, is a secretory protein that induces the restructuring of blood and lymphatic vessels. This property has made VEGF-D a target in the treatment of lymphangioleiomyomatosis and hyperoxic pulmonary edema. It was also determined that mutations in the gene encoding VEGF-D resulted in pulmonary vasculopathy.

What does this article add?

VEGF-D level was superior to other cardiac biomarkers in terms of clinical risk scoring and demonstrating thrombus burden. Therefore, in the future VEGF-D may serve as a guiding biomarker in the diagnosis and treatment of acute PE.

Introduction

Pulmonary embolism (PE) is usually an early complication of deep vein thrombosis (DVT) and can cause high morbidity and mortality if not detected and treated early. Diagnosis of PE begins with clinical suspicion. Therefore, risk factors must also be considered along with the initial symptoms. PE should be suspected in patients who present with dyspnea, tachycardia, and normal chest x-ray with no other conditions that could explain this presentation [1,2].

Patients with submassive (intermediate-risk) PE are commonly subdivided into higher and lower risk groups using the biomarkers troponin, brain natriuretic peptide (BNP), and NT-proBNP. PE leads to dilation of the right ventricle and increases its myocardial oxygen demand. Reduced circulation causes microinfarcts in the right ventricle, which triggers the local release of troponin. High troponin levels were shown in meta-analyses to be strongly associated with complicated clinical course and mortality. This has facilitated the differentiation of submassive PE patients into low-intermediate and high-intermediate-risk groups [3].

Right ventricular dilatation results in the release of certain cytokines to ensure oxygenation, neovascularization, and rapid thrombus organization. The most important of these cytokines is vascular endothelial growth factor (VEGF) [4]. VEGF causes endothelial proliferation and neovascularization, and prevents reperfusion injury by increasing glutathione peroxidase activity. VEGF-D, a member of the VEGF family, is a secretory protein that induces the restructuring of blood and lymphatic vessels. This property has made VEGF-D a target in the treatment of lymphangioleiomyomatosis and hyperoxic pulmonary edema. It was also determined that mutations in the gene encoding VEGF-D resulted in pulmonary vasculopathy [5,6].

The cardiac markers actively used in PE clinical scoring and monitoring can sometimes be inadequate for making critical treatment decisions in intermediate-risk patients. In our study, we aimed to determine the relationship between VEGF-D, which has an important role in pulmonary vascular remodeling and angiogenesis, and the clinical course and prognosis of PE.

Materials and Methods

Study Population

A total of 117 patients admitted to the chest diseases ward due to PE between October 2019 and January 2020 were included in our study. Patients with a history of myocardial infarction and associated treatment within the past 3 months, severe hypoxic respiratory failure, PE with concomitant intracranial hemorrhage, diagnosed malignancy, lymphangioleiomyomatosis, and known history of right heart dilation were not included.

Diagnostic Evaluations

All patients with clinical suspicion of PE (major risk factors, clinical signs) underwent chest x-ray, electrocardiogram (ECG), and echocardiographic (ECHO) evaluation. In cases with high clinical suspicion, CT angiography with a 16-slice multi-detector CT device was performed to confirm the diagnosis. The patients were assessed using the Pulmonary Embolism Severity Index (PESI) in accordance with the 2019 European Society of Cardiology guidelines for the management of PE. This was followed by scoring for acute PE severity and clinical risk of early mortality [3]. Clinical and laboratory evaluations including physical examination, blood pressure and heart rate measurement, oxygen saturation (pO_2), and complete blood count were performed before and after thrombolytic therapy. ECG examination was performed before thrombolytic therapy only.

Definitions

High-risk PE was defined as sustained hypotension (systolic arterial pressure <90 mmHg or ≥40 mmHg fall in systolic arterial pressure for at least 15 min) and cardiogenic shock (with clinical signs such as altered level of consciousness, oliguria, or cool and clammy extremities). Patients with right ventricular dilation and troponin I elevation without hypotension or signs of shock were classified as high-intermediate-risk PE, while those with right ventricular dilatation but no troponin I elevation were classified as low-intermediate-risk PE. Patients without signs of right heart dilation who had negative cardiac biomarkers and exhibited no signs of hypotensive shock were considered low-risk PE.

Pharmacological Regimen

High-risk PE patients under 65 years of age were given full-dose alteplase (10 mg bolus followed by 90 mg infusion over 2 hours), while those over 65 years of age received half-dose alteplase. Immediately after thrombolytic therapy, enoxaparin sodium was initiated

at 12-hour intervals. Patients with low- and intermediate-risk PE received enoxaparin sodium therapy at 12-hour intervals. Two patients in the high-intermediate-risk PE patient group developed hypotension and shock in the first 72 hours and were administered thrombolytic therapy as per the high-risk PE protocol.

Pulmonary Artery Obstruction Index (PAOI)

To calculate the PAOI from CT angiography imaging, the pulmonary arteries in each lung are divided into 10 segmental branches (3 upper lobe, 2 middle lobe and lingula, 5 lower lobe). Depending on thrombus location, thrombi detected in the proximal pulmonary arteries (main, lobar) are given 1 point for each segmental artery distal of the thrombus, resulting in a score equal to the total number of distal segmental arteries. If there is no thrombus in the proximal pulmonary arteries, isolated thrombi in proximal branches are scored as 1 point. Based on the degree of obstruction, the presence of filling defect accompanied by contrast agent is considered partial obstruction, whereas a thrombus that completely filled the artery with no contrast agent in distal pulmonary vessels is considered a totally occlusive thrombus (total obstruction). Partial obstruction is scored as 1 and total obstruction as 2 points. The PAOI is calculated by multiplying the number of segmental arteries distal to the thrombus (*n;* min. 1, max. 20) by degree of obstruction (*d*: 1 or 2), divided by the maximal total score (40) and expressed as a percentage:

$$\text{CT PAIO} = \Sigma(n \times d) / 40 \times 100.$$

Measurement of biochemical markers

After 15 minutes of semi-supine rest, blood samples were obtained from an antecubital vein into tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. Troponin I concentrations were measured by chemiluminescent immunoassay using an Immulite 2500 (Siemens Medical Solutions, Erlangen, Germany). VEGF-D and NT-proBNP were measured by enzyme-linked immunosorbent assay (Elabscience human ELISA kit, UK).

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 20.0; SPSS Inc., Chicago, IL, USA). Pearson chi-square test was used for between-group comparisons of parametric data and Mann–Whitney U test was used for between-group comparisons of non-normally distributed numerical data. Demographic data and laboratory parameters were evaluated using one-way ANOVA with Tukey's test as a post hoc analysis. Pearson

correlation analysis was used to detect correlations between VEGF-D level and NT-proBNP, troponin-I, mean pulmonary artery pressure (PAP), and PAOI. Receiver operating characteristic (ROC) curve analysis was used to calculate the sensitivity and specificity of VEGF-D level in the differentiation of high-risk and high-intermediate-risk patients.

Results

The mean age of patients included in the study was 65.2±13.1 years. There were 62 men and 55 women in the study group. The patients' demographic data, laboratory results, ECHO findings, length of hospital stay, and PAOI values according to clinical scoring groups are shown in Table 1.

VEGF-D level was moderately correlated with mean PAP and PAOI (r=0.481, p=0.01; r=0.404, p=0.01). VEGF-D was also weakly correlated with NR-proBNP, troponin-I, and age (r=0.94, p=0.01; r=0.286, p=0.01; r=0.226, p=0.01) and negatively correlated with pO_2 (r=-0.258, p=0.05). A moderate positive correlation was detected between troponin I and NT-proBNP (r=0.525, p=0.01). Weak correlations were observed between troponin I and PAOI (r=0.222, p=0.05) and pO_2 (r=-0.334, p=0.01) (Figure 1).

Area under the ROC curve for the differentiation of high-risk and high-intermediate-risk PE patients was 0.721 (95% CI: 0.563–0.88). At a cut-off value of 370.1 pg/ml, VEGF-D had a sensitivity of 91.4% and specificity of 67% (Figure 2).

Discussion

In our study, a significant correlation was observed between VEGF-D level and clinical risk scoring in PE. VEGF-D was also significantly correlated with PAOI, troponin I, NT-proBNP, and pO_2, with the strongest correlation between VEGF-D and PAOI. Using a VEGF-D cut-off value of 370.1 pg/ml, this parameter had 91.4% sensitivity and 67% specificity in distinguishing high-risk and high-intermediate-risk patients.

The clinical presentations of PE are classified as high-, intermediate-, and low-risk [7]. In acute intermediate-risk PE patients, transthoracic ECHO is the most frequently used method to evaluate right ventricular dysfunction. Right ventricular dysfunction is defined using parameters such as right ventricular dilation, hypokinesis of the right ventricular wall,

paradoxical movement of the interventricular septum, tricuspid valve insufficiency, increased pulmonary artery diameter, and higher ratio of right ventricular diastolic end diameter to left ventricular diastolic diameter [8,9].

Cardiac troponin T and I are enzymes specific to the cardiac muscles. Right ventricular dilation that occurs as a result of acute right heart failure due to high-risk PE increases right ventricle oxygen demand. Troponin, BNP, and NT-proBNP measurements may be useful in distinguishing high- and intermediate-risk patients from low-risk patients for prognostic evaluation. They can also be used to further stratify intermediate-risk patients into high-intermediate and low-intermediate-risk subgroups. However, it must be kept in mind that these markers are also elevated in conditions such as acute exacerbation of COPD, sepsis, acute kidney failure, trauma, rhabdomyolysis, and congestive heart failure [3,10]. Although cardiac biomarkers have an important place in clinical risk scoring, the absence of a definite cut-off value for the differentiation of high-risk and high-intermediate-risk patients necessitates clinical observation for thrombolytic therapy in the high-intermediate-risk group. However, the time spent in clinical follow-up sometimes works to the patient's detriment, with hypoxic respiratory failure and sudden hemodynamic collapse resulting in a precipitous death. This led to the need to investigate alternative biomarkers in clinical scoring.

VEGF-D is a secretory glycoprotein responsible for the remodeling of blood and lymphatic vessels by binding to its receptors on the endothelium. It stimulates angiogenesis by binding to VEGFR-2 and 3 in the endothelium. Due to the presence of receptors in many parts of the body, VEGF-D has found a place in many areas, from pulmonary indications to cancer. VEGF-D level has been adopted as a biomarker in studies and treatment evaluation in chronic thromboembolic pulmonary hypertension and especially lymphangioleiomyomatosis. In addition, in animal studies, injecting adenovirus that expresses mature VEGF-D into the myocardial tissue of the pig heart was found to increase transmural angiogenesis [5,11]. This was evaluated as guiding data in the treatment of angina pectoris. Hypoxia is one of the main factors that induce VEGF synthesis. It promotes the proliferation of the endothelial progenitor cells that contribute to hypoxic angiogenesis [4]. The vascular stasis and subsequent hypoxic environment following thrombus formation cause an increase in VEGF level. VEGF-A and D play an important role in promoting rapid thrombus organization as well as in thrombolysis. One of the potential mechanisms proposed for VEGFs' antithrombotic activity is that it causes an increase in nitric oxide (NO) and prostacyclin levels. NO inhibits platelet aggregation by increasing intracellular soluble guanyl cyclase level [12]. Another mechanism

may be that VEGF promotes the expression and activity of the serine proteases fibrinoclase, urokinase, and tissue plasminogen activator. These enzymes catalyze the conversion of plasminogen to plasmin, which is a major enzyme in thrombolysis [13,14].

In the present study, we also observed increased VEGF-D level in correlation with greater thrombus burden and lower pO_2. Comparisons of troponin I, NT-proBNP and VEGF-D levels among the clinical scoring groups revealed no significant differences in any of the parameters except between the high-risk and high-intermediate-risk groups. VEGF-D levels differed significantly between the high-risk and high-intermediate-risk groups independent of the other two parameters. If this finding can be confirmed in more extensive studies, we believe that VEGF-D may guide early thrombolytic therapy in the high-intermediate-risk patient group, who receive thrombolytic therapy based on clinical observation and sometimes die during this period due to hypoxic respiratory failure. Hypoxia is the leading known factor in VEGF-D synthesis. However, the fact that VEGF-D level was more strongly correlated with thrombus burden than hypoxia in our study may indicate that thrombus burden also plays an important role in its synthesis.

Although VEGF-D had high sensitivity in ROC curve analysis of the differentiation between high-risk PE patients and high-intermediate-risk PE patients, specificity was low. We attribute this mainly to the decrease in patient number that occured when we excluded patients with diseases associated with VEGF-D elevation to avoid their potential effect on the results of the study.

In conclusion, based on the data obtained in this study, VEGF-D level was superior to other cardiac biomarkers in terms of clinical risk scoring and demonstrating thrombus burden. Therefore, in the future VEGF-D may serve as a guiding biomarker in the diagnosis and treatment of acute PE.

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Figure legends

Figure 1. Correlation between NT-proBNP, troponin-I, mean PAP, PAOI and plasma VEGF-D

Figure 2. Receiver operating characteristic (ROC) curve analysis for VEGF-D level in the differentiation of patients with high-risk and high-intermediate-risk PE.