

Original Article

Plasma follistatin-like protein 1 is correlated with disease severity in patients with acute pulmonary embolism and predicts short-term mortality

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Abstract: We investigated the plasma levels of follistatin-like protein 1 (FSTL1) in patients with acute pulmonary embolism (PE) and whether it could predict short-term mortality. A prospective observational cohort study was conducted in patients with acute PE (n = 220). FSTL1 was measured in plasma samples using enzyme-linked immunosorbent assay. Plasma FSTL1 levels were significantly increased in patients with acute PE, and positively correlated with disease severity (rs = 0.7171). Sensitivity and specificity rates for high-risk PE at a specific FSTL1 cutoff point (23 ng/ml) were 79.3% and 92.9%. Multivariate Cox regression analysis showed that FSTL1 was independently associated with 30-day mortality rate. Addition of FSTL1 to the PE severity index (PESI) scoring system significantly improved the predictive value for 30-day mortality. These results indicate that the plasma level of FSTL1 is correlated with disease severity in patients with acute PE and predicts short-term mortality.

Keywords: Pulmonary embolism, follistatin-like protein 1, biomarker, disease severity, mortality

Introduction

Acute pulmonary embolism (PE) is a potentially life-threatening cardiovascular emergency. It can cause severe right ventricular failure and lead to fatal hemodynamic deterioration by occluding the pulmonary arterial bed. Immediate assessment for the severity of acute PE is important, as it helps in the decision of the initial emergency management [1]. It is known that the severity of PE could be understood as an estimate of PE-related early mortality risk, and some risk markers, including clinical markers, right ventricle (RV) dysfunction markers and myocardial injury markers, allow stratifying patients into high-risk PE, intermediate-risk PE, and low-risk PE. Although these risk markers are useful in the evaluation of disease severity, they still have some limitations in the prediction of short-term mortality, especially for patients who were stratified into intermediate-risk PE [2, 3].

Follistatin-like protein 1 (FSTL1) is a 308-amino acid glycoprotein induced in animal models and patients with chronic inflammatory and cardiovascular diseases [4, 5]. Recently, FSTL1, which is secreted by cells of the mesenchymal lineage, has been found to play an important role in myocardial maintenance and repair in response to harmful stimuli [6, 7]. Cardiac expression levels of FSTL1 are up-regulated in mouse models of myocardial infarction or pressure overload hypertrophy [8]. They are also increased in patients with advanced heart failure, and return to normal following left ventricular assist device implantation [9]. Based on these findings, we speculated that the plasma level of FSTL1 might be correlated with the severity of cardiovascular disorders in acute PE, and could predict short-term mortality. In this study, we investigated the plasma levels of FSTL1 in patients with acute PE, and observed whether it could be a potential biomarker for predicting short-time mortality.

Materials & methods

Study design and subjects

This prospective observational cohort study was conducted at Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine in China. The study complied with the principles outlined in the Declaration of Helsinki and was approved by the medical ethics committee at the Shanghai Jiao Tong University. From January 2009 to December 2015, all adult patients admitted to hospital due to acute PE were recruited. Acute PE was defined as a partial or complete filling defect centrally within the pulmonary artery detected through CT pulmonary angiography. The exclusion criteria were as follows: (1) the patients had chronic thromboembolic pulmonary hypertension; (2) the patients had severe cardiac diseases, including heart failure and acute coronary syndrome, and autoimmune disorders before the onset of acute PE; (3) the patients were concurrently involved in other clinical trials at the time of recruitment into this study. Control subjects were selected from angina patients in whom pulmonary embolism was excluded. During that time, acute PE was diagnosed in 243 consecutive patients. However, 23 patients were dis-enrolled from the study because of meeting exclusion criteria or being lost to follow-up. Thus, finally investigated group included 220 patients. A total of 173 controls, age and gender-matched, were included in the study. All patients gave their written informed consent.

Data collection

At admission, we collected clinical characteristics on factors such as age, sex, time of admission from acute presentation, symptoms, vital signs, mental status, arterial oxyhemoglobin saturation level, medical history, CT pulmonary angiography findings and transthoracic echocardiography results. We calculated the PE severity index (PESI) for each patient. The total point score of PESI for a given patient was obtained by summing the patient's age in years and the points for each predictor (male sex, 10 points; history of cancer, 30 points; history of heart failure, 10 points; history of chronic lung disease, 10 points; pulse in years and the points for each predictor (male sex, 10 points; history of cancer, 30 points; pulse < 30 breaths/min, 20 points; temperature < 36°C, 20 points; altered

mental status, 60 points; arterial oxyhemoglobin saturation level < 90%, 20 points). The score corresponds with the following risk classes: 65 or less, class I; 66 to 85, class II; 86 to 105, class III; 106 to 125, class IV; and more than 125, class V. Patients in risk classes III through V were defined as being at high risk [10].

FSTL1 enzyme-linked immunosorbent assay and other laboratory tests

Plasma samples were obtained from patients at the time of diagnosis, and at 2 weeks after acute presentation. Briefly, peripheral venous blood was drawn into vacutainer tubes containing 1.8 mg K₂EDTA per ml blood. The vacutainer tubes were inverted carefully, and centrifuged immediately at 1300 RCF for 10 minutes at room temperature. The plasma was aspirated carefully and pooled in a centrifuge tube. Plasma samples were aliquoted into cryovials and stored at -80°C until use. Plasma FSTL1 levels were measured using a standard enzyme-linked immunosorbent assay kit (Cloud-Clone Corp., Houston, TX, USA) with a 13.9 pg/ml detection limit of sensitivity. The intra-assay coefficient of variation (CV) was below 10% and inter-assay CV below 12%. As twenty-six of the 220 included patients died within 2 weeks after acute presentation, the plasma FSTL1 levels at the time point of 2 weeks were not analyzed in these patients. Control plasma samples were obtained from healthy individuals in health examination. All analyses and calibrations were performed in duplicate. Optical densities were determined using an ELISA plate reader (Multiskan Ex Primary EIA V. 2.3, Thermo, Vantaa, Finland) at 450 nm. CurveExpert 1.4 software (Daniel G. Hyams, Hixson, TN, USA) was used to analyze all materials and depict the standard curve.

The plasma levels of D-dimer, cardiac troponin I (cTnI) and brain natriuretic peptide (BNP) were measured at the time of diagnosis. D-dimer was determined by an immunoturbidimetric assay (INNOVANCE® D-Dimer assay; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), cTnI by a chemiluminescent immunoenzymatic assay (Access® AccuTnI®, Beckman Coulter, Fullerton, CA, USA), and BNP by a radioimmunoassay (Peninsula Laboratories, Inc, Belmont, CA, USA). All assays were performed according to manufacturer's instructions.

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Table 1. Characteristic features of patients included in the study

Partient characteristics	PE			Control (n = 173)
	High-risk (n = 56)	Intermediate-risk (n = 92)	Low-risk (n = 72)	
Median age (SD), in years	74.4 (9.6)	72.1 (7.8)	69.4 (7.8)	71.2 (6.8)
Median weight (SD), in kg	64.6 (7.0)	66.0 (6.0)	62.8 (6.7)	61.3 (8.9)
Male Sex %	64.3	67.3	61.1	65.8
Shock or hypertention due to PE %	100	0	0	0
Arterial oxyhemoglobin saturation (SaO ₂) < 90%	57.1	19.6	0	0
RV dysfunction %	100	52.2	0	0
D-dimer (SD) in µg/ml	12.7 (4.3)	10.8 (3.1)	5.6 (3.7)	0.4 (0.2)
cTnl ≥ 0.04 ng/ml %	71.4	45.7	0	0
BNP ≥ 100 pg/ml %	100	78.3	5.6	0
Thrombolytic therapy %	78.6 [†]	43.5	0	0
30-day morality %	46.4	5.4	0	0

[†]Twelve patients with high-risk PE did not accept thrombolytic therapy because of recent major surgery, gastrointestinal bleeding, and advanced liver disease. PE: Pulmonary embolism; SD: Standard deviation; RV: Right ventricle; cTnl: Cardiac troponin I; BNP: Brain natriuretic peptide.

Specialists' assessment of disease severity and screening for thrombolytic therapy

The disease severity of acute PE was assessed by pulmonologists according to the European Society of Cardiology (ESC) guidelines [1]. High-risk PE was diagnosed in the presence of shock or hypotension, which was defined as systolic blood pressure of < 90 mmHg. Non-high-risk PE was further stratified into intermediate- and low-risk PE. Intermediate-risk PE was diagnosed when at least one right ventricle dysfunction (RVD) marker (including RV dilatation, hypokinesis or pressure overload on echocardiography, and BNP elevation) or cTnl was positive. Low-risk PE was diagnosed when all checked RVD markers and cTnl were found negative.

Follow-up and study outcomes

All of the included patients were followed up 30 days, or until death. The survival status was obtained in all patients using the hospital electronic patient records system. The immediate and underlying causes of death, according to the International Statistical Classification of Diseases V.10 (ICD-10), were obtained from death certificates. We chose 30-day all-cause mortality as a primary short-term outcome. All-cause mortality was defined as death from any cause, which included PE-related death and death attributed to other documented cause. We defined PE-related death as a fatal event

occurring in the hours after clinical deterioration due to PE, or if death could not be attributed to a documented cause and PE could not be confidently ruled out.

Statistical analysis

Statistical analysis was performed with SAS version 8.0 statistical software (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA). The data of normal distribution were presented as the mean ± standard deviation. The *t*-test was used to compare quantitative data between PE patients and controls, and a χ^2 test was used to compare frequencies. We used analysis of variance for repeated measures with Bonferroni's post-hoc contrasts to compare FSTL1 levels among different groups. Spearman correlation coefficient was used to evaluate the correlation of FSTL1 plasma levels with disease severity, D-dimer, cTnl, BNP and patient PESI score. The high-risk PE patient group and no-high-risk PE patient group were combined to calculate sensitivity and specificity rates for high-risk PE development with FSTL1 concentration cutoff point, and a receiver operating characteristic (ROC) curve was constructed. The univariate survival analysis was based on the Kaplan-Meier method with log-rank tests. A multivariate Cox regression model was used to analyze the influence of various factors on 30-day mortality. To assess the added value of FSTL1 as a prognostic fac-

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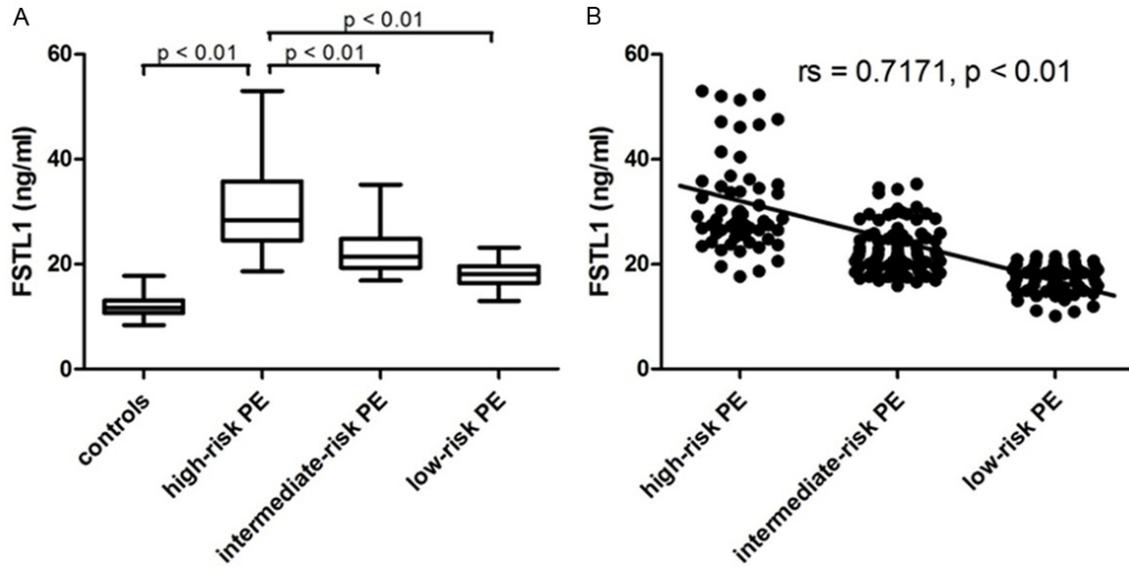


Figure 1. Plasma FSTL1 concentrations in patients with different risk levels at the time of diagnosis (A). The correlation between FSTL1 and disease severity at diagnosis was calculated using spearman correlation coefficient (B). Whiskers of the boxplot mark the 5th and 95th percentiles, the box 25th percentile, median and 75th percentile. PE: Pulmonary embolism; FSTL1: Follistatin-like protein 1.

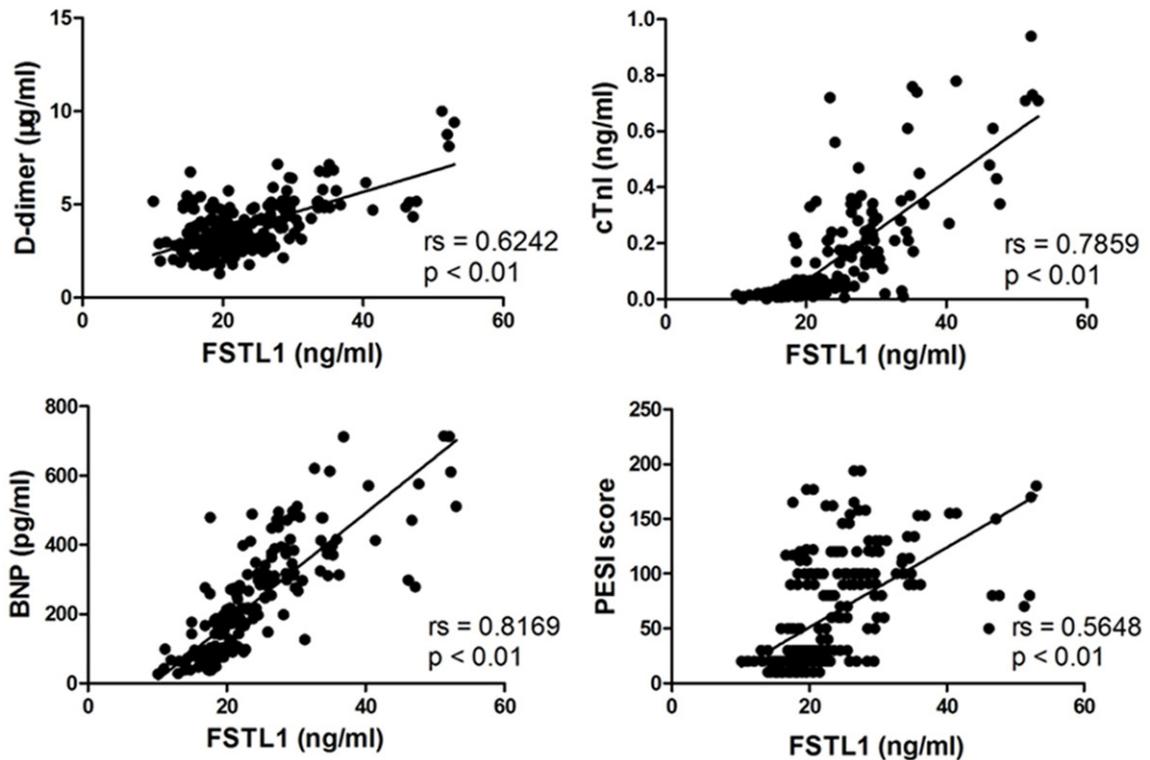


Figure 2. Correlation of serum FSTL1 levels with D-dimer, cTnI, BNP and patient PESI score in acute PE population. Correlation coefficients (rs) from the Spearman rank-order test are displayed. Each point represents an individual value. FSTL1: Follistatin-like protein 1; BNP: Brain natriuretic peptide; cTnI: Cardiac troponin I; PESI: Pulmonary embolism severity index; PE: Pulmonary embolism.

tor of 30-day mortality, multivariate analysis of PESI scores and FSTL1 levels was conducted

using logistic regression model. Based on the model for risk prediction, the ROC curve was

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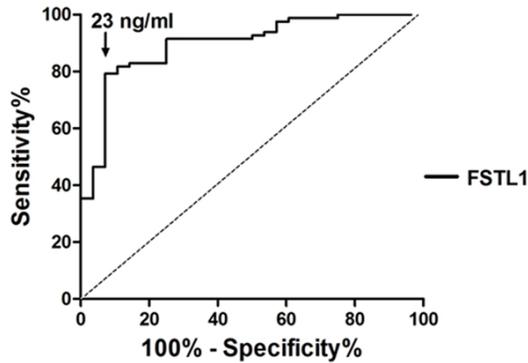


Figure 3. Receiver operator curve analysis for FSTL1 levels at no-high-risk PE and high-risk PE. The area under the curve was 0.8968 (95% CI, 0.8302-0.9634). The sensitivity rate of 79.3% and specificity rate of 92.9% corresponded to 23 ng/ml. FSTL1: Follistatin-like protein 1; CI: Confidence interval.

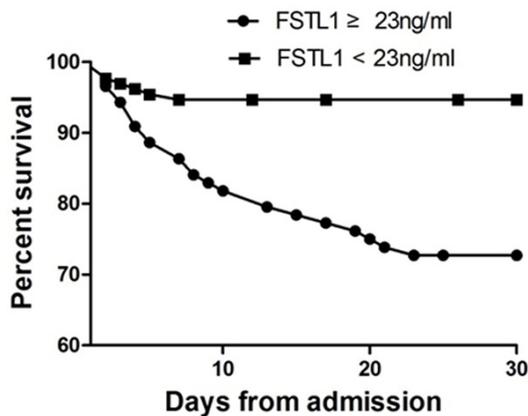


Figure 4. Kaplan Meier survival analysis according to FSTL1 levels. FSTL1: Follistatin-like protein 1.

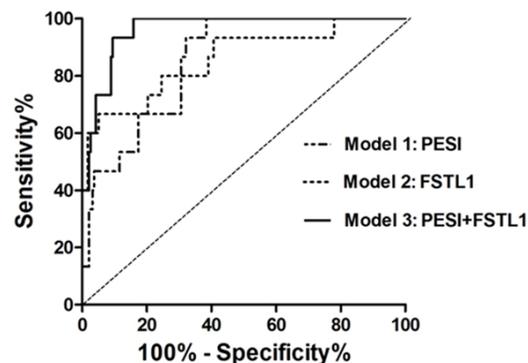


Figure 5. Receiver operating characteristic curves for the 30-day mortality. The curves are based on logistic regression models for risk prediction, incorporating the following: Model 1, PESI (AUC = 0.8523; 95% CI, 0.7686-0.9360); Model 2, FSTL1 (AUC = 0.8682; 95% CI, 0.7502-0.9762); Model 3, PESI + FSTL1 (AUC = 0.9439; 95% CI, 0.9021-0.9806), P =

0.0006 versus Model 1, P = 0.0238 versus Model 2. PESI: Pulmonary embolism severity index; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; FSTL1: Follistatin-like protein 1.

constructed. We used the area under the curve (AUC) to quantify the ability of the model to distinguish between high- and low-risk subjects. To compare the performance of PESI scores and PESI + FSTL1 model in the prediction of 30-day mortality risk, we calculated the difference between 2 AUCs with the exact binomial confidence interval for the AUCs. The improved discriminative and predictive values of the PESI + FSTL1 model were examined by calculating the net reclassification improvement (NRI), as described elsewhere [11]. A p value < 0.05 was considered to denote statistically significant differences.

Results

Patients' characteristics and clinical course

Among these 220 patients, the mean age was 71.8 ± 8.4 years and mean body weight was 65.2 ± 6.5 kg. The majority of patients (64%) were admitted to intensive care units and the others were hospitalized in general respiratory departments. Sixty-three percent were admitted from home, 29% were referred from another hospital or a department other than respiratory medicine, and 8% were diagnosed with PE while hospitalized in a respiratory ward for some other reason. Clinical presentations were as follows: dyspnea (68%), chest pain (39%), tachycardia (27%), cough (20%), syncope (18%). The time interval between acute presentation and admission was 6.8 ± 3.6 hours. The Baseline characteristics were shown in **Table 1**. There were no significant differences in the distribution of age, weight, or sex among groups.

FSTL1 was increased in acute PE and correlated with disease severity

The plasma FSTL1 levels in acute PE were significantly elevated compared with those in control subjects (23.4 ± 7.5 ng/ml versus 12.0 ± 1.7 ng/ml, $P < 0.0001$) at the time of diagnosis. Plasma FSTL1 levels declined at 2 weeks (16.8 ± 3.1 ng/ml), but were still significantly higher than those in the control subjects ($P < 0.0001$). At the time of diagnosis, patients with high-risk PE had significantly higher levels of FSTL1 than

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Table 2. Reclassification of 30-day survivors and non-survivors based on PESI + FSTL1 model

PESI	PESI + FSTL1			Total
	< 3.5%	3.5%-10%	> 10%	
Non-survivors				
< 3.5%	2	3	3	8
3.5%-10%	0	5	2	7
> 10%	0	2	14	16
Total	2	10	19	31
Survivors				
< 3.5%	135	0	0	135
3.5%-10%	32	14	2	48
> 10%	4	2	0	6
Total	171	16	2	189

Reclassification tables for survivors or non-survivors of acute PE were constructed using PE risk categories of 30-day mortality. Classification improved using the model with PESI + FSTL1. For the 31 non-survivors, 2 subjects (cell in *italics*) were reclassified down and 8 subjects (cells in bold) were reclassified up. For the 189 survivors, 38 subjects (cells in *italics*) were reclassified down and 2 subjects (cells in bold) were reclassified up. PESI: Pulmonary embolism severity index; FSTL1: Follistatin-like protein 1.

patients with intermediate-risk PE, and patients with low-risk PE (**Figure 1A**). There was a good correlation between FSTL1 levels and disease severity (**Figure 1B**). FSTL1 levels also showed positive correlation with D-dimer, cTnl, BNP and patient PESI scores (**Figure 2**).

FSTL1 level had high sensitivity and specificity rates as a biomarker for high-risk PE development at diagnosis. The receiver operating curve analysis for FSTL1 levels at no-high-risk PE and high-risk PE had an area under the curve of 0.8968 (95% CI, 0.8302-0.9634; **Figure 3**). With the receiver operating curve, a threshold of 23 ng/ml yielded a sensitivity rate of 79.3%, with a specificity rate of 92.9%.

Survival analysis

Of the 220 included patients diagnosed with acute PE, 31 (14.1%) died within 30 days of hospitalization, and 189 (85.9%) survived at least 30 days after hospital admission. Causes of death were considered to be related to PE in 27 patients (87.1%); cancer (n = 3), major bleeding (n = 1). **Figure 4** shows Kaplan-Meier survival analysis according to FSTL1 level. 30-day mortality increased with increasing FSTL1 lev-

els (log rank test $\chi^2 = 25.251$, $P < 0.0001$). After the multivariate Cox regression analysis, PESI scores ($P = 0.0014$, relative risk: 1.56, 95% CI: 1.13-2.01) and FSTL1 level ($P = 0.0025$, relative risk: 1.39, 95% CI: 1.14-1.71) were found to be independent prognostic factors for 30-day mortality.

Addition of FSTL1 to the PESI scoring system improves the predictive value for 30-day mortality

The ROC analysis showed similar predictive performance between PESI and FSTL1 (AUC = 0.8523 and 0.8682, respectively; $Z = 0.22$, $P = 0.8291$). However, when combining the results of PESI and FSTL1, the PESI + FSTL1 model had a stronger predictive performance than PESI or FSTL1 alone ($Z = 3.44$, $P = 0.0006$; $Z = 2.26$, $P = 0.0238$), with an AUC of 0.9439 (95% CI, 0.9021-0.9806) for all 220 patients studied (**Figure 5**). Patients were reclassified using the PESI + FSTL1 model (**Table 2**). The classification was significantly improved with the NRI estimated at 38.4% ($Z = 3.58$, $P = 0.0003$).

Discussion

High-risk PE is a major life-threatening emergency, with the short-term mortality > 15%. Efforts to define clinical or serological risk factors for the development of high-risk PE have been described for the last 10 years. Our findings reported here indicated that plasma FSTL1 was significantly elevated in patients with high-risk PE, and predicted the risks of 30-day mortality, suggesting that FSTL1 could be a potential biomarker that helps identifying patients who are at high risks of early mortality.

FSTL1, also known as TSC-36, is a secreted glycoprotein that belongs to the follistatin family of proteins [12]. Follistatin family members bind to transforming growth factor- β superfamily proteins and inhibit their functions [13]. Although the mechanism by which FSTL1 correlated with the disease severity of acute PE is speculative, possible relation with myocardial injury during heart failure is worth consideration. FSTL1 is a cardiac-secreted factor that functions as an antiapoptotic protein during myocardial injury [14], and it shares structural similarity with secreted protein acidic, rich in cysteine (SPARC), a matricellular protein involved in cell-extracellular matrix interac-

tions, which mediates early extracellular matrix remodeling following myocardial ischemia [15, 16]. In addition, myocardial injury in heart failure increases the expression of follistatin-related genes including those encoding extracellular matrix constituents and adhesion molecules [9]. Thus, we speculated that plasma FSTL1 level might reflect the severity of myocardial injury in acute PE, and could help predicting the risks of hemodynamic instability.

In this study, we evaluated the significance of FSTL1 in predicting 30-day mortality. Multivariate Cox regression analysis indicated FSTL1 was an independent prognostic factor. Although the ROC analysis of FSTL1 did not show stronger predictive performance than PESI, which was the previously established PE prognostic index for 30-day mortality [17], when FSTL1 was added to PESI, a statistically significant increase in AUC was observed. The NRI achieved by the PESI + FSTL1 model, which could be presented as weighted differences of their components that corresponded to improvements in sensitivity and specificity, was statistically significant. These results indicated that when combined with PESI, FSTL1 has great value in predicting the risks of 30-day mortality in patients with acute PE.

Although many biomarkers, such as cTnI, BNP and D-Dimer, have been used to predict the disease severity in acute PE, there are still some limitations for their application in clinical practice. A recent study has clarified the kinetics of cTnI in acute PE, and highlights the situations in which an early cTnI can be false negative [18]. Although the increase of the BNP biomarker is significantly associated with early recurrent venous thromboembolism, this relationship appears insufficient to guide the initiation of thrombolytic therapy [19, 20]. The main limitation of D-dimer tests is relatively low specificity especially in hospitalized population, in elder patients, with cancer and in pregnancy [21]. Our findings in this study showed that plasma FSTL1 was a potential new biomarker for prediction of disease severity and had positive correlation with these biomarkers. Further studies are needed to compare the prognostic values of FSTL1 with those of existing biomarkers and assess the validity of plasma FSTL1 levels for disease severity prediction in clinical practice.

So far there is considerable debate regarding the use of thrombolytic therapy in patients with

intermediate-risk PE. Although thrombolytic therapy rapidly relieves dyspnea, reduces pulmonary arterial pressure and revascularizes the embolized blood vessels, as the occurrence of bleeding is relatively higher, the benefit and risk of thrombolytic therapy should be assessed sufficiently prior to the administration of thrombolytic medication [22]. Here, our results suggested that the plasma FSTL1 concentration > 23 ng/ml was an indication for patients who had high risks of clinical deterioration and might be a potential marker for the implementation of thrombolytic therapy. The value of FSTL1 in screening intermediate-risk PE patients for thrombolytic therapy is needed to be assessed in future studies.

Limitations to our study included the relatively small numbers of patients in the acute PE group and there were no white or black patients in the study. Additionally, to avoid the influence of other cardiovascular diseases and chronic inflammatory disorders on the plasma FSTL1 level, we excluded those patients who had severe cardiac diseases or autoimmune disorders before the onset of acute PE. Therefore, to provide further validation of our findings, additional studies are needed to investigate the prognostic value of FSTL1 in a larger sample and assess the influence of other cardiovascular diseases and inflammatory disorders on the value of FSTL1 in predicting short-term mortality.

In conclusion, our findings indicated that plasma FSTL1 level was correlated with the disease severity in patients with acute PE and predicted the risks of 30-day mortality. The plasma FSTL1 level at the time of diagnosis may be helpful for screening patients who are at high risks of early mortality.

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Disclosure of conflict of interest

None.

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