

Association of Thrombin-Activatable Fibrinolysis Inhibitor with Acute Pulmonary Embolism

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Abstract

Background Thrombin-activatable fibrinolysis inhibitor (TAFI) inhibits fibrinolysis and high levels may have an association with thrombosis. The aim of the current study was to investigate the association of TAFI antigen levels with pulmonary thromboembolism (PTE).

Patients and Methods A case–control study was conducted with 29 patients with PTE and 17 age- and gender-matched control individuals. Plasma levels of TAFI were measured at the time of diagnosis, then at 3 and 6 months after the event.

Results Initial TAFI levels (%) were higher in patients with PTE than in the control group (190,0 [65,0–250,0] vs 133,0 [83,0–153,0]; $p = 0.003$). TAFI levels significantly decreased at the third and sixth months after initial diagnosis ($p < 0.05$). The percentage reductions in TAFI levels were 12 and 36.8% at 3 and 6 months, respectively. The Odds ratio (OR) of TAFI level for PTE was found to be 1.024 (95% CI: 1.007–1.040; $p = 0.005$). There was no significant correlation of initial TAFI levels with age, gender, smoking status, history of thrombosis, pulmonary artery pressure, and D-dimer levels ($p > 0.05$). In the sixth month of treatment, patients with residual thrombosis were seen to have similar baseline levels and reductions of TAFI as patients without residual thrombosis ($p > 0.05$).

Conclusion The result of this study suggests that high TAFI levels may have a role in the occurrence of PTE without impact on treatment outcome.

Keywords

- ▶ TAFI
- ▶ pulmonary thromboembolism
- ▶ association

Introduction

Pulmonary thromboembolism (PTE) as a severe clinical consequence of venous thromboembolism (VTE) is one of the leading causes of morbidity and mortality.^{1,2} There are many predisposing factors including both acquired characteristics and congenital genetic features of the patients. However, the other underlying major factor may be due to

impaired balance in the coagulation and fibrinolysis system. The role of responsible factors in this complex system has been under investigation for many years, but has not yet been clearly elucidated.

It is well known that hypercoagulability together with hypofibrinolysis increases the risk of VTE.³ It has been previously demonstrated that hypofibrinolysis-associated

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VTE is maintained by thrombin-activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor type 1 (PAI-1).³ TAFI has an important bridging function between coagulation and the fibrinolysis system.² It has also been reported that TAFI accounts for the antifibrinolytic effect that accompanies prothrombin activation.⁴ TAFI circulates as an inactive proenzyme in the bloodstream, and becomes activated during blood clotting. Moreover, TAFI has been demonstrated to play a role in inflammation and tissue repair.^{5,6}

With this current knowledge, it is thought that high TAFI plasma levels may contribute to an increased risk of VTE.⁷ In 2000, van Tilburg et al reported that TAFI levels above the 90th percentile were associated with an increased risk of thrombosis in patients with VTE.⁸ Recently, in 2009, Meltzer et al also demonstrated that increased plasma levels of TAFI and PAI-1 are associated with VTE.⁹ To the best of our knowledge, the only study on this subject showed that TAFI antigen levels in patients with PTE were not associated with the presence of PE overall but maybe related to the extent of PE.¹⁰ The aim of the current study was to investigate the association of TAFI antigen levels with PTE and suggest a rationale for an alternative treatment option that targets TAFI.

Patients and Methods

A total of 29 patients who presented at the Department of Pulmonology of Ankara Etlik Training and Research Hospital and were newly diagnosed with PTE were enrolled in this case-control study. A control group was formed of 17 healthy individuals who did not have diabetes mellitus, hypertension, hyperlipidemia, chronic renal failure, or a history of any cardiovascular disease. Patients younger than 18 years or older than 75 years were excluded from the study. The demographic and clinical characteristics of the patients were recorded. The diagnosis of PTE was confirmed by thoracic CT angiography in patients who presented at the emergency department or pulmonology department with chest pain and shortness of breath and whose D-dimer level was greater than 0.55 (normal limit: 0–0.55). CT angiography was applied to all patients at the time of diagnosis, then in the third and sixth months of treatment. Cardiac functions were assessed with echocardiography for all patients at the time of diagnosis and after 6 months of treatment. D-dimer and TAFI levels were measured at the time of diagnosis, and at 3 and 6 months. TAFI antigen (TAFI-Ag) levels were quantitatively determined using ELISA kits (Imclone; American Diagnostic Inc., United States). The assay was performed according to the manufacturer's instructions. The results of TAFI-Ag were given as a percentage. TAFI levels were expressed as percentage of normal pooled plasma.

Statistical Analysis

Statistical analyses of the study data were made using SPSS Statistics 20 software (IBM, Armonk, New York, United States). Descriptive data were reported as number (*n*) and percentage (%). TAFI (%) data at the time of diagnosis were given as median (Min–Max) values. In the comparison of two unpaired data with measurement values without normal

distribution, the Mann–Whitney U-test (Z-table value) was used. In the comparison of two paired data with measurement values, the Wilcoxon test was used, whereas for more than three variables, the Friedman test was used. A value of $p < 0.05$ was accepted as statistically significant.

Ethical Approval and Informed Consent

All procedures performed in studies involving human participants 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.

Results

Evaluation was made of 29 patients with PTE and 17 healthy individuals. Of the PTE patients, 18 had at least one comorbidity. The most common comorbidities were chronic obstructive pulmonary disease, coronary artery disease, and hypertension. There were 10 patients with thrombosis history. The demographic and disease characteristics of the patients are given in **Table 1**. Warfarin treatment was given to 25 patients and low-molecular-weight heparin was administered to 4. Pulmonary artery pressure (PAP; mm Hg) measured by Echocardiography (ECO) was normal for 14 patients and greater than 40 for 15 patients. After the treatment period, four patients still had PAP greater than 40.

Initial median TAFI levels (%) were higher in patients with PTE than in the control group (190,0 [65,0–250,0] vs 133,0 [83,0–153,0]; $p = 0.003$). TAFI levels significantly decreased at 3 and 6 months after initial diagnosis ($p < 0.05$). The percentage reductions in TAFI levels were 12 and 36.8% at 3 and 6 months, respectively. The levels and comparisons of

Table 1 Demographic and disease characteristics of the patients

	Patients (<i>n</i> = 29)	Control (<i>n</i> = 17)
Age (± SD, y)	53.07 ± 16.64	52.41 ± 6.43
Gender		
Female	12 (41.3%)	9 (52.9%)
Male	17 (58.7%)	8 (47.1%)
Smoking status		
Smoker	13 (44.8%)	
Non-smoker	8 (27.5%)	
Ex-smoker	8 (27.5%)	
History of thrombosis		
Yes	10 (34.4%)	
No	19 (65.6%)	
Site of thrombosis		
Left pulmonary artery	8	
Right pulmonary artery	12	
Bilateral	9	

Table 2 Biochemical parameters of the patients and healthy subjects

	Patients with PTE (n = 29)			Control (4) (n = 17)	p
	At the time of PTE diagnosis (1)	Post-PTE 3 mo (2)	Post-PTE 6 mo (3)		
D-dimer (ng/mL)	850 [344–1,400]	350 [100–900]	60 [20–300]	–	0.000 ^a
TAFI (%)	190 [65–250]	140 [40–250]	100 [40–250]	133 [83–153]	0.000 ^a 0.003 ^b

Abbreviations: PTE, pulmonary thromboembolism; TAFI, thrombin-activatable fibrinolysis inhibitor.

^aDifference between 1 and 2–3, difference between 2 and 3.

^bDifference between 1 and 4.

Table 3 Correlation analysis of the TAFI levels with other parameters at the time of diagnosis

Parameters	TAFI (%)	
	r	p
Age (y)	–0.323	0.088
D-dimer (ng/mL)	–0.100	0.606

Abbreviation: TAFI, thrombin activatable fibrinolysis inhibitor.

the biochemical parameters of the patients and the control group are shown in **Table 2**. The optimal TAFI cutoff value was determined as 145 with 69.0% sensitivity and 76.5% specificity (AUC = 0.764; $p < 0.05$). Logistic regression analysis showed that the initial TAFI levels were significantly correlated with PTE. The OR for TAFI level was found to be 1.024 (95% CI: 1.007–1.040; $p = 0.005$). There was no significant correlation of TAFI levels with age, gender, smoking status, history of thrombosis, PAP, and D-dimer levels ($p > 0.05$; **Tables 3–4**).

Control CT angiography in the 6th month of treatment revealed that 24 patients had no residual thrombosis, and 5

Table 4 Correlation analysis of the TAFI levels with other parameters at the time of diagnosis

	n	TAFI (%)	p
Gender			
Male	17	146 [65–250]	0.135
Female	12	220 [65–250]	
Smoking status			
Smoker	13	190 [65–250]	0.354
Non-smoker	8	220 [65–250]	
Ex-smoker	8	140 [94–250]	
Thrombosis history			
Yes	10	250 [94–250]	0.295
No	19	180 [65–250]	
Echo (PAP)			
Normal	14	220 [65–250]	0.257
Abnormal	15	146 [65–250]	

Abbreviations: Echo, echocardiography; PAP, pulmonary artery pressure; TAFI, thrombin activatable fibrinolysis inhibitor.

patients still had thrombosis. In the comparison of patients with residual thrombosis and patients without residual thrombosis, there was no significant difference between the two groups in respect of age, gender, smoking status, thrombosis history, or TAFI levels both at the time of diagnosis and in the follow-up period ($p > 0.05$; **Table 5**).

Discussion

A variety of studies have demonstrated a role for TAFI/TAFIa in venous and arterial diseases.^{7–9,11,12} It has been identified as a link between coagulation and fibrinolysis.⁵ TAFI levels have been investigated in cardiovascular diseases and have been reported to be related with thrombosis. In a Swedish population, increased TAFI levels were measured in patients with stable angina pectoris and coronary heart disease.¹³ In another case–control study, TAFI levels were assessed in 45 patients with AMI and 42 control individuals, and the TAFI activity levels were found to be higher in patients with AMI

Table 5 Comparison of patients with residual thrombosis and patients without

	Patients without residual thrombosis (n = 22)	Patients with residual thrombosis (n = 7)	p
Age (\pm SD, y)	50.41 \pm 15.25	61.43 \pm 19.24	0.129
Gender			
Female	12 (54.5%)	5 (71.4%)	0.665
Male	10 (45.5%)	2 (28.6%)	
Smoking status			
Yes	10 (45.5%)	3 (42.9%)	0.626
No	12 (54.5%)	4 (57.1%)	
Thrombosis history			
Yes	8 (36.4%)	2 (28.6%)	0.541
No	14 (63.6%)	5 (71.4%)	
TAFI (at the time of diagnosis, %)	220 [65–250]	146 [113–250]	0.161
TAFI (at 6 mo, %)	115 [40–250]	92 [40–250]	0.175

Abbreviation: TAFI, thrombin-activatable fibrinolysis inhibitor.

than in the control group.¹⁴ Another study demonstrated the role of TAFI in human abdominal aortic aneurysm (AAA).¹⁵

However, a few studies have shown no correlation between TAFI and thrombosis. Donmez et al showed that the plasma activated TAFI level was not related with the presence of thrombosis in patients with Behcet's disease.¹⁶ Another study demonstrated that TAFI antigen levels are increased in type 2 diabetic patients, but are not related to diabetic foot ulcer development.¹⁷ A case-control study suggested that TAFI does not play a role in the pathogenesis of the hypercoagulable state in gastric cancer patients.¹⁸

Only one previous study has analyzed the association between TAFI and PTE, in which the TAFI antigen levels did not differ between patients with and without acute PE. It was also demonstrated that TAFI antigen levels did not correlate with age, gender, and D-dimer levels.¹⁰ In this study, it was determined that TAFI levels may be associated with PTE. Similar to the earlier-mentioned study, there was no correlation of TAFI with any other parameter such as age, gender, smoking status, history of thrombosis, PAP, D-dimer, and IgE levels. According to these results, it can be suggested that TAFI may be independently related with PTE. The use of this parameter for diagnostic purposes should be considered and studied by researchers. In contrast, Grosso et al found a positive association between TAFI and age in their study demonstrating increased TAFIa levels in antiphospholipid syndrome patients affected by arterial thrombosis.¹⁹ Another interesting study about pulmonary diseases investigated the role of TAFI in patients with chronic thromboembolic pulmonary hypertension (CTEPH). According to the results of that study, it was indicated that plasma levels of TAFI are elevated in patients with CTEPH and are correlated with resistance to clot lysis in those patients.²⁰

Zidane et al conducted a gene level study investigating the role of TAFI in patients with PTE, and there was seen to be a possible association of the -438 A-allele with a reduced risk of PE.²¹ Since TAFI antigen levels are decreased in the presence of the -438 A allele of the TAFI gene,²² this result supports the significant role of TAFI in PTE.

As TAFI has been demonstrated to be associated with thrombosis, many investigators have studied the inhibitors of TAFI in thrombotic disorders. Many inhibitors have been developed and have been shown to have a profibrinolytic effect.^{2,7,23} Denorme et al studied the inhibition of TAFI and PAI-1 in a mouse model of transient middle cerebral artery occlusion. The inhibition of TAFI or PAI-1 was seen to have significantly decreased cerebral infarct size by 50% at 24 hours after stroke. The results suggested that targeting of PAI-1 and TAFI is protective in an ischemic stroke model.²⁴ An experimental agent (DS-1040) has been recently shown to inhibit the activated form of TAFI but to have no impact on bleeding time.²⁵

With the consideration to analyze the association of TAFI with treatment outcome, control CT angiography and TAFI levels were examined in the sixth month of treatment. The TAFI levels of the five patients with residual thrombosis did not differ from those of the patients without residual thrombosis. This result may suggest that TAFI levels may be important in acute disease period but not for treatment

outcome which is important for the prognosis of survival, life-long anticoagulation, or thrombophilia screening.

The major limitation of this study was small number of patients and control group which make the results only preliminary. Also TAFI levels were not normally distributed reducing the statistical significance. Further large-scale, prospective, randomized clinical trials are needed to determine the hypothesis that whether TAFI is associated with PTE, especially in respect of treatment.

Conclusion

PTE is one of the leading causes of morbidity and mortality worldwide. TAFI has an important bridging function between coagulation and fibrinolysis system, and its role in venous and arterial diseases has been demonstrated in a variety of studies. The result of this study suggested that high TAFI levels may be independently associated with hypercoagulable state of PTE, but not with treatment outcome. Larger, prospective studies are required to clarify the role of TAFI in PTE and its utility in the diagnosis and treatment of PTE.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Rosovsky R, Zhao K, Sista A, Rivera-Lebron B, Kabrhel C. Pulmonary embolism response teams: purpose, evidence for efficacy, and future research directions. *Res Pract Thromb Haemost* 2019;3(03):315–330
- Willemsse JL, Heylen E, Nesheim ME, Hendriks DF. Carboxypeptidase U (TAFIa): a new drug target for fibrinolytic therapy? *J Thromb Haemost* 2009;7(12):1962–1971
- Lisman T. Decreased plasma fibrinolytic potential as a risk for venous and arterial thrombosis. *Semin Thromb Hemost* 2017;43(02):178–184
- Bajzar L, Manuel R, Nesheim ME. Purification and characterization of TAFI, a thrombin-activatable fibrinolysis inhibitor. *J Biol Chem* 1995;270(24):14477–14484
- Marx PF. Thrombin-activatable fibrinolysis inhibitor. *Curr Med Chem* 2004;11(17):2335–2348
- Bavbek N, Ceri M, Akdeniz D, et al. Higher thrombin activatable fibrinolysis inhibitor levels are associated with inflammation in attack-free familial Mediterranean fever patients. *Ren Fail* 2014;36(05):743–747
- Mosnier LO, Buijtenhuijs P, Marx PF, Meijers JC, Bouma BN. Identification of thrombin activatable fibrinolysis inhibitor (TAFI) in human platelets. *Blood* 2003;101(12):4844–4846
- van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. *Blood* 2000;95(09):2855–2859
- Meltzer ME, Lisman T, de Groot PG, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood* 2010;116(01):113–121
- Schroeder V, Kucher N, Kohler HP. Role of thrombin activatable fibrinolysis inhibitor (TAFI) in patients with acute pulmonary embolism. *J Thromb Haemost* 2003;1(03):492–493
- Radu CM, Spiezia L, Campello E, Gavasso S, Woodhams B, Simioni P. Thrombin activatable fibrinolysis inhibitor in cancer patients with and without venous thromboembolism. *Thromb Res* 2013;132(04):484–486

- 12 Libourel EJ, Bank I, Meinardi JR, et al. Co-segregation of thrombophilic disorders in factor V Leiden carriers; the contributions of factor VIII, factor XI, thrombin activatable fibrinolysis inhibitor and lipoprotein(a) to the absolute risk of venous thromboembolism. *Haematologica* 2002;87(10):1068–1073
- 13 Silveira A, Schatteman K, Goossens F, et al. Plasma procarboxypeptidase U in men with symptomatic coronary artery disease. *Thromb Haemost* 2000;84(03):364–368
- 14 Leenaerts D, Bosmans JM, van der Veken P, Sim Y, Lambeir AM, Hendriks D. Plasma levels of carboxypeptidase U (CPU, CPB2 or TAFIa) are elevated in patients with acute myocardial infarction. *J Thromb Haemost* 2015;13(12):2227–2232
- 15 Bridge KI, Bollen L, Zhong J, et al. Thrombin-activatable fibrinolysis inhibitor in human abdominal aortic aneurysm disease. *J Thromb Haemost* 2017;15(11):2218–2225
- 16 Donmez A, Aksu K, Aydin H, et al. The plasma levels of activated thrombin activatable fibrinolysis inhibitor and thrombomodulin in Behçet disease and their association with thrombosis. *Thromb Res* 2010;126(03):207–210
- 17 Erdogan M, Solmaz S, Canataroglu A, et al. Plasma thrombin-activatable fibrinolysis inhibitor (TAFI) antigen levels in diabetic foot ulcers. *Endocrine* 2010;37(03):449–454
- 18 Eser M, Kement M, Balin S, et al. Is there any role of thrombin activatable fibrinolysis inhibitor in the development of a hypercoagulable state in gastric cancer. *World J Surg Oncol* 2012;10:180
- 19 Grosso G, Vikerfors A, Woodhams B, et al. Thrombin activatable fibrinolysis inhibitor (TAFI)—a possible link between coagulation and complement activation in the antiphospholipid syndrome (APS). *Thromb Res* 2017;158(158):168–173
- 20 Yaoita N, Satoh K, Satoh T, et al. Thrombin-activatable fibrinolysis inhibitor in chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2016;36(06):1293–1301
- 21 Zidane M, de Visser MC, ten Wolde M, et al. Frequency of the TAFI-438 G/A and factor XIII A Val34Leu polymorphisms in patients with objectively proven pulmonary embolism. *Thromb Haemost* 2003;90(03):439–445
- 22 Brouwers GJ, Vos HL, Leebeek FW, et al. A novel, possibly functional, single nucleotide polymorphism in the coding region of the thrombin-activatable fibrinolysis inhibitor (TAFI) gene is also associated with TAFI levels. *Blood* 2001;98(06):1992–1993
- 23 Declerck PJ. Thrombin activatable fibrinolysis inhibitor. *Hämostaseologie* 2011;31(03):165–166, 168–173
- 24 Denorme F, Wyseure T, Peeters M, et al. Inhibition of thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 reduces ischemic brain damage in mice. *Stroke* 2016;47(09):2419–2422
- 25 Zhou J, Kochan J, Yin O, et al. A first-in-human study of DS-1040, an inhibitor of the activated form of thrombin-activatable fibrinolysis inhibitor, in healthy subjects. *J Thromb Haemost* 2017;15(05):961–971