Original contribution

Thrombin-Activatable Fibrinolysis Inhibitor Levels in Patients with Non–Small-Cell Lung Cancer

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Abstract

BACKGROUND: An increased incidence of thromboembolic events has been described in patients with cancer. Cancer cells are attributed with producing procoagulant substances such as cysteine protease and tissue factor to activate factor X and factor VII, respectively. However, there are limited data on the pathogenesis behind this hypercoagulability state, and the thrombin generation, fibrinolytic system, and coagulation inhibition system during cancer are largely obscure. In this study, we investigated the changes of different steps of coagulation pathway in patients with non–small-cell lung cancer (NSCLC) and compared the data with those of healthy controls. PATIENTS AND METHODS: Forty-four patients with NSCLC and 36 age-matched controls were recruited for this study. Prothrombin fragment 1 + 2 (F 1 + 2) were used as a marker of thrombin generation; thrombin-activatable fibrinolysis inhibitor (TAFI) immunologic activity level was measured for inhibition of the fibrinolytic system, and tissue factor pathway inhibitor (TFPI) activity was assessed for the coagulation inhibition system. In the patient group, the relationships between TAFI activity levels and patient parameters (age, sex, body mass index [BMI], histopathology, and stage) were evaluated. RESULTS: The TAFI activity, F 1 + 2 levels, and TFPI activity were significantly higher in patients with lung cancer than in subjects in the control group ($P < .05$; $P < .0001$; and $P < .0001$; respectively). However, there were no statistically significant associations between TAFI activity levels and patient age, sex, BMI, histopathology, or stage of disease ($P > .05$). CONCLUSION: In this study, it was clearly shown that patients with lung cancer have hypercoagulable states and that the pathogenesis of thrombotic events in these patients is multifactorial. Increased TFPI is a reflection of thrombin activity in this patient group. Confirmatory studies with larger patient groups should be performed in this population.

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Key words: Carboxypeptidase U, Plasminogen activator inhibitor-1, Prothrombin fragment, Tissue factor pathway inhibitor, Venous thromboembolism

Introduction

Thromboembolism is a well-recognized complication of malignant disease. Several studies have demonstrated the tendency of clotting and fibrinolytic disorders to develop in patients with malignancies.[1,2] Clinical manifestations vary from venous thromboembolism (VTE), which is typically associated with primary solid tumor growth, to disseminated intravascular coagulation, more commonly observed in patients with hematologic malignancies and in those with widespread metastatic cancer.[3,4] Recently, a new potent inhibitor of fibrinolysis, the thrombin-activatable fibrinolysis inhibitor (TAFI), or carboxypeptidase U, has been isolated and characterized from human plasma. Thrombin-activatable fibrinolysis inhibitor is a 58-kD glycoprotein synthesized by the liver that can be activated by thrombin-, thrombin-thrombomodulin complex–, plasmin- or trypsin-catalyzed proteolysis to carboxypeptidase B–like enzymes that inhibit fibrinolysis.[5] It is well known that C-terminal lysines on cell-surface proteins and partially degraded fibrin enhance fibrinolysis by providing binding sites for plasminogen. Once bound, it adopts a more activatable conformation. Activated TAFI inhibits activation of plasminogen to plasmin by removing these C-terminal lysine

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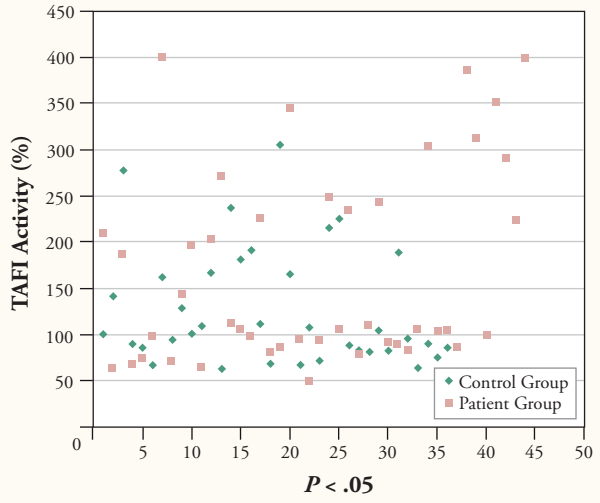
Table 1 Levels of Tissue Factor Pathway Inhibitor, Thrombin-Activatable Fibrinolysis Inhibitor, and Prothrombin Fragment 1 + 2

Parameter	Control Group	Patient Group	*P* Value
TFPI Activity (%)	127.63 ± 63.9	167.95 ± 105.4	.05
TAFI Activity (U/mL)	2.56 ± 0.7	3.84 ± 0.8	.0001
F 1 + 2 (nmol/L)	1.29 ± 0.4	2.12 ± 1.1	.0001

Figure 1 Thrombin-Activatable Fibrinolysis Inhibitor Activity in Patient and Control Groups



residues. In addition, activated TAFI might also directly inactivate plasmin, further impairing fibrinolysis.[6]

Despite studies of the relationship between TAFI and thromboembolic and coronary artery diseases or various metabolic syndromes, studies on TAFI and neoplastic disease association are limited.[7-10] The aims of our study are to compare the level and activity of TAFI in patients with non–small-cell lung cancer (NSCLC) with healthy controls and to evaluate the relationship of TAFI with other demographic and histopathologic parameters.

Patients and Methods

The study included 44 patients who presented with the diagnosis of NSCLC in the Outpatient Medical Oncology Clinic in the Department of Internal Medicine at the Dr. Lutfi Kirdar Research and Treatment Center, prospectively. The study excluded patients who had a history of secondary malignancy except for nonmelanoma skin cancer, a history of thrombosis-related disease, diabetes mellitus, chronic renal failure, or similar chronic metabolic disease. The control group consisted of 36 healthy subjects with similar demographic characteristics to the patient group. The institutional ethical committee approved the study protocol. All patients gave informed consent before study entry.

A 10-mL blood sample was drawn from patients to determine the immunologic activity levels of TAFI, tissue factor pathway

Figure 2 Tissue Factor Pathway Inhibitor Activity in Patient and Control Groups

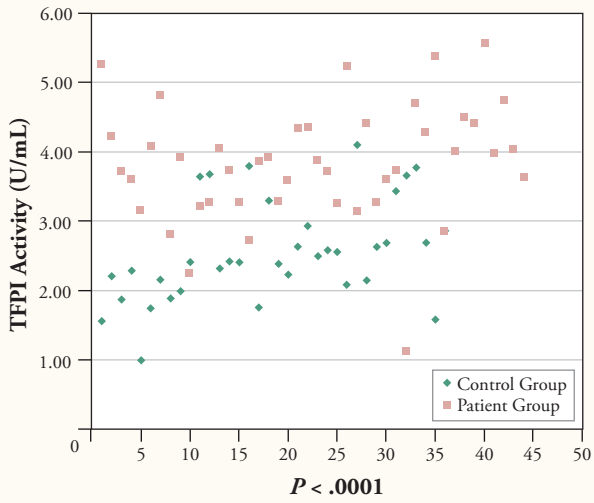
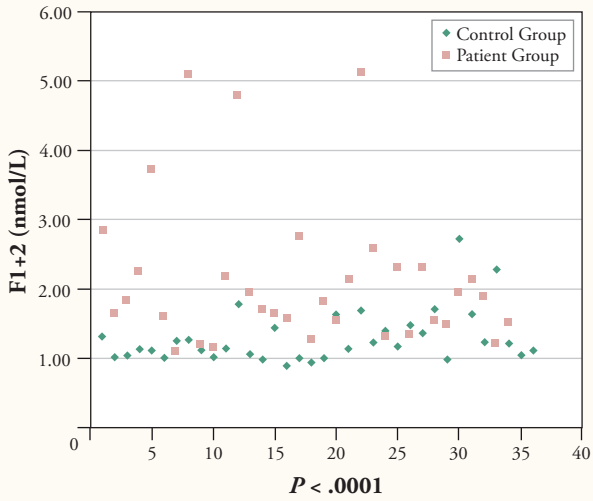


Figure 3 Prothrombin Fragment 1 + 2 in Patient and Control Groups



inhibitor (TFPI) activity, and prothrombin fragment 1 + 2 (F 1 + 2). The sera was centrifuged and stored at −20°C until the time of analysis. In addition, data on patient age, sex, body mass index (BMI), histopathologic diagnosis, and stage of disease were recorded. The same parameters were also recorded for the control group. Subsequently, we assessed the difference in the levels of TAFI, TFPI, and F 1 + 2 between the patient and control groups. Moreover, we investigated the relation of TAFI, TFPI, and F 1 + 2 levels with age, sex, and BMI in the patient group.

Thrombin-activatable fibrinolysis inhibitor functional activity was assayed using the Actichrome® TAFI Activity kit. TAFI levels were determined by incubating the plasma with TAFI activation reagent, after which an activating stop reagent was added to halt the activation step. Next, the TAFI developer containing substrate was added to diluted (1:25) plasma samples, and enzymatic

Table 2 Levels of Tissue Factor Pathway Inhibition, Thrombin-Activatable Fibrinolysis Inhibitor, and Prothrombin Fragment 1 + 2 by Patient Sex, Age, Body Mass Index, Histopathology, and Stage

Variable	Tissue Factor Pathway Inhibition (U/mL)	*P* Value	Thrombin-Activatable Fibrinolysis Inhibitor (%)	*P* Value	Prothrombin Fragment 1 + 2 (nm/L)	*P* Value
Sex						
Female	2.92	.116	132	.628	1.69	.566
Male	3.88		169.6		2.14	
Age						
> 60 Years	3.88	.952	150.9	.310	1.8	.456
≤ 60 Years	3.81		179.7		2.37	
Body Mass Index						
> 24	3.83	.795	163	.652	2.05	.289
≤ 24	3.81		177.2		2.27	
Histopathology						
Adenocarcinoma	3.72	.426	184	.783	1.92	.209
Squamous cell carcinoma	3.81		167.8		1.92	
Non–small-cell lung cancer, not otherwise specified	3.98		154.3		2.58	
Stage						
III	3.79	1	177.6	.557	1.82	.026
IV	3.89		157.3		2.33	

reaction was initiated. The reaction was stopped by the addition of sulfuric acid and read at 490 nmol/L. Plasma that was not activated (not incubated with the TAFI activation reagent) was assayed in parallel as a control. The difference in absorbance between the activated and nonactivated plasma was calculated as an amount of TAFI activity.

Tissue factor pathway inhibition activity was measured using the Actichrome® TFPI Activity assay. Prothrombin fragment 1 + 2 was measured with a sandwich enzyme–linked immunosorbent assay method (Enzygnost F 1 + 2 micro).

The Student *t* test and the parametric Mann-Whitney *U* and Kruskal-Wallis tests were used to compare the levels of TAFI, TFPI, and F 1 + 2 and assess their relation to the demographic and clinical characteristics between the patient and control groups.

Results

There was no difference in studied marker levels between patients with different histologic tumor types, namely NSCLC (not otherwise specified), squamous, or adenocarcinoma. Although the values for TFPI, TAFI, and F 1 + 2 were higher in male patients than in female patients, these differences were not statistically significant. The TAFI, TFPI, and prothrombin fragment levels for the patient and control groups are shown in Table 1. There were statistically significant differences for all 3 parameters between the patient and control groups ($P < .05$; $P < .0001$; and $P < .0001$, respectively; Figures 1-3).

In the patient group, we investigated the relation between the TAFI, TFPI, and F 1 + 2 levels and patient age, BMI, histopathologic diagnosis, and stage of disease. Although the relationship between TAFI and TFPI values and patient and tumor characteristics was not significant, we found statistically significant associations between F 1 + 2 and the stage of the cancer. (In more advanced disease, higher F 1 + 2 values were measured; $P = .026$). The results are summarized (with probability values) in Table 2.

Discussion

Venous thromboembolism is the second most common cause of death in patients with cancer and also points toward unfavorable prognosis. In about 10% of patients with idiopathic VTE, there is an underlying malignancy. However, the efficacy of extensive tumor screening in these patients is not yet established. Numerous plasmatic and cellular components contribute to the phenomenon of hypercoagulability in patients with cancer, including cancer procoagulant and activation of coagulation with high levels of coagulation factors.[11] Low fibrinolytic function has also been reported to be an important cause of vascular thrombosis in patients with cancer.[12]

A thrombus occurs when there is an imbalance between the prothrombotic and antithrombotic factors. This imbalance results from the stimulation of thrombogenesis or a disorder in anticoagulant of fibrinolytic systems. Many studies of patients with cancer indicate high levels of procoagulant protein and a decreased concentration of functional anticoagulant protein.[13-15] The main cause of the thrombotic events in patients with cancer is reduced fibrinolytic function.[12] In humans, TAFI concentrations exceeding 129 nmol/L correlate with a doubled risk of deep vein thrombosis.[16]

So far, an increased circulating level of plasminogen activator inhibitor-1 has been ascribed as the main causative factor of hypofibrinolysis in patients with malignancy, including those with

lung cancer. The role of TAFI in coagulation abnormalities associated with lung cancer has been reported by Hataji et al.[7] Their study assessed the circulating level of TAFI in patients with lung cancer and its expression in several lung cancer cell lines. They found that plasma concentrations of TAFI were significantly increased in patients with lung cancer compared with healthy subjects. The concentration of TAFI was particularly higher in patients with small-cell carcinoma compared with those with adenocarcinoma or squamous cell carcinoma and also in patients with cancer who responded to chemotherapy compared with nonresponders. In vitro studies showed more expression of TAFI in small-cell carcinoma than in adenocarcinoma cell lines and more expression in lung cancer cell lines sensitive to anticancer agents than in resistant cell lines. The Hataji et al study suggests that TAFI, in part secreted from lung cancer cells, might play a role in the pathogenesis of thrombotic disorders in patients with lung cancer. Moreover, it suggests that TAFI can be used as a tumor marker to assess the response of tumor cells to chemotherapy.

In the present study, we found increased circulating levels of TAFI activity in patients with NSCLC, and TAFI activity is mentioned for the first time. In our study, the TAFI activity and levels of F 1 + 2 and TFPI were substantially higher in the patient group compared with the control group, and the difference was statistically significant ($P > .05$). There was, however, no statistically significant difference for the relation of the TAFI activity and TFPI levels with age, sex, BMI, histopathologic diagnosis, and stage of disease. Only the levels of F 1 + 2 showed a significant difference in the patients with stage III and IV disease ($P = .026$).

A study conducted with 249 healthy subjects by Chetaille et al indicated a broad range of variation in the levels of TAFI antigen (41%-259%).[17] It has been shown that there is a positive correlation between the TAFI antigen and the age in female patients but not in male patients. Generally, the levels of TAFI antigen have been found at constant levels in a wide range among the patients and show no significant variations by the hourly, daily, or monthly periods. However, variations have been shown among ethnicities. The present study showed no difference for the relationship between patient age, sex, and BMI and the levels of TAFI antigen.

Conclusion

In conclusion, high TAFI levels in the patients with lung cancer in our study could help to explain the pathogenesis of thromboembolic events, which are frequently seen in patients with cancer. Therefore, we suggest that, in patients with NSCLC, increased thrombin generation, decreased fibrinolytic activity, and coagulation inhibition might contribute to the multifactorial etiology of the hypercoaguable state.

References

1. Rickles FR, Edwards RL. Activation of blood coagulation in cancer: Trousseau's syndrome revisited. *Blood* 1983; 62:14-31.
2. Zacharski LR, Ornstein DL. Venous thromboembolism and cancer. *N Engl J Med* 2000; 343:1338.
3. Tallman MS, Hakimian D, Kwaan HC, et al. New insights into the pathogenesis of coagulation dysfunction in acute promyelocytic leukemia. *Leuk Lymphoma* 1993; 11:27-36.
4. Henderson ES, Lister TA, Greaves MF. (eds). *The Management of Bleeding and Thrombosis in Leukemia.* Philadelphia, PA: W. B. Saunders Company; 1996:291-497.
5. Wang W, Hendriks DF, Scharpé SS. Carboxypeptidase U, a plasma carboxypeptidase with high affinity for plasminogen. *J Biol Chem* 1994; 269:15937-44.
6. Bajzar L. Thrombin activatable fibrinolysis inhibitor and an antifibrinolytic pathway. *Arterioscler Thromb Vasc Biol* 2000; 20:2511-8.
7. Hataji O, Taguchi O, Gabazza EC, et al. Increased circulating levels of thrombin-activatable fibrinolysis inhibitor in lung cancer patients. *Am J Hematol* 2004; 76:214-9.
8. Parisi V, Capasso F, Pudore L, et al. New thrombophilia markers in digestive tract neoplasia [in Italian]. *Tumori* 2003; 89(4 suppl):129-32.
9. Meijers JC, Oudijk EJ, Mosnier LO, et al. Reduced activity of TAFI (thrombin-activatable fibrinolysis inhibitor) in acute promyelocytic leukaemia. *Br J Haematol* 2000; 108:518-23.
10. Demirkan B, Ozcan MA, Glu AA, et al. The effect of anthracycline-based (epirubicin) adjuvant chemotherapy on plasma TAFI and PAI-1 levels in operable breast cancer. *Clin Appl Thromb Hemost* 2006; 12:9-14.
11. Luxembourg B, Bauersachs R. Malignancy and thrombosis: a double-sided clinical relationship [in German]. *Vasa* 2005; 34:225-34.
12. Pavey SJ, Hawson GA, Marsh NA. Impact of the fibrinolytic enzyme system on prognosis and survival associated with non-small cell lung carcinoma. *Blood Coagul Fibrinolysis* 2001; 12:51-8.
13. Gale AJ, Gordon SG. Update on tumor cell procoagulant factors. *Acta Haematol* 2001; 106:25-32.
14. Kakkar AK, DeRuvo N, Chinswangwatanakul V, et al. Extrinsic-pathway activation in cancer with high factor VIIa and tissue factor. *Lancet* 1995; 346:1004-5.
15. Rickles FR, Falanga A. Molecular basis for the relationship between thrombosis and cancer. *Thromb Res* 2001; 102:V215-24.
16. van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. *Blood* 2000; 95:2855-9.
17. Chetaille P, Alessi MC, Kouassi D, et al. Plasma TAFI antigen variations in healthy subjects. *Thromb Haemost* 2000; 83:902-5.