JBUON 2019; 24(3): 943-954
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Circulating plasminogen activator inhibitor-1 activity: a biomarker for resectable non-small cell lung cancer?

George P. Sotiropoulos[1,2], Marianna Kotopouli[1], Irene Karampela[3], Gerasimos-Socrates Christodoulatos[1], Georgios Antonakos[4], Ioanna Marinou[5], Evaggelos Vogiatzakis[5], Antigoni Lekka[6], Athanasios G. Papavassiliou[1], Maria Dalamaga[1]

[1]Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, 11527 Athens, Greece; [2]Department of Thoracic Surgery, 'Sotiria' General Hospital, 11527 Athens, Greece; [3]Laboratory of Clinical Biochemistry, Attikon General University Hospital, Medical School, National and Kapodistrian University of Athens, Chaidari, Athens, Greece; [4]Second Department of Critical Care, Attikon General University Hospital, Medical School, National and Kapodistrian University of Athens, Chaidari, Athens, Greece; [5]Laboratory of Microbiology, 'Sotiria' General Hospital, Athens, Greece; [6]Department of Laboratory Hematology, NIMTS General Hospital, Athens, Greece.

Summary

Purpose: *Plasminogen activator inhibitor-1 (PAI-1) participates in thrombotic, fibrinolytic, inflammatory and metabolic cascades. Since previous studies have focused on tissue and blood level concentrations, our goal was to investigate for the first time the independent relationship between plasma PAI-1 activity in resectable non small cell lung cancer (NSCLC) taking into consideration its several interfaces and study its diagnostic and prognostic potential.*

Methods: *In an adequately powered case-control study, plasma PAI-1 activity, metabolic parameters, classic adipokines, hemostatic, inflammatory and tumor biomarkers were measured in 110 consecutive patients with resectable NSCLC and 110 healthy subjects matched on age, sex and date of blood draw.*

Results: *NSCLC patients exhibited significantly higher PAI-1 activity compared to controls (p<0.001). In NSCLC cases, PAI-1 activity correlated with somatometric variables, insulin, WBC, antithrombin III, protein C, plasminogen, IL-6 and tumor size (p<0.05). Plasma PAI-1 activity was independently associated with NSCLC beyond risk factors associated with NSCLC (OR:6.9, 95%CI:2.9-16.6, p<0.001). Plasminogen activity and body mass index emerged as independent predictors of PAI-1 activity in cases. Due to its high specificity, PAI-1 activity could represent a potentially useful parameter in ruling out NSCLC, alone or in combination with serum tumor markers associated with NSCLC.*

Conclusions: *PAI-1 activity, reflecting PAI-1 functionality, may represent a potentially useful biomarker in NSCLC associated with thrombotic, tumor-promoting and metabolic networks. More clinical studies are needed to explore whether PAI-1 activity may be a practical biomarker in the risk assessment of NSCLC, at the crossroads of hemostasis and metabolism.*

Key words: *adipocytokine, biomarker, coagulation, fibrinolysis, lung cancer, plasminogen activator inhibitor-1*

Introduction

Plasminogen Activator Inhibitor-1 (PAI-1), a glycoprotein of 45 kDa belonging to the serine protease inhibitor superfamily, displays a pivotal role as a regulator of physiologic hemostatic balance *in vivo*, being the main inhibitor of urokinase-type plasminogen activator (uPA) and plasminogen, and as an adipocytokine [1,2]. PAI-1 is mainly synthesized by blood thrombocytes, adipocytes, vascular

Corresponding author: Maria Dalamaga, MD, PhD, MS, MPH. Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, 75 Mikras Asias Str, Goudi, 11527 Athens, Greece.
Tel: +30 2107462624, Fax:+30 2106082467, Email: madalamaga@med.uoa.gr
Received: 18/09/2018; Accepted: 19/10/2018

smooth muscle cells, epithelial cells, some tissue-specific parenchymal cells (e.g., renal epithelial cell, hepatocytes, etc) and tumor cells [3]. An upregulation of PAI-1 levels and activity has been observed in a plethora of disease states such as thrombotic disorders, atherosclerosis, hypertension, coronary heart disease, lung and renal fibrosis, insulin resistance/metabolic syndrome/obesity, sepsis and cancer [3-5].

Over the last decades, the incidence of lung cancer worldwide has increased significantly, being the most common cause of cancer-related death [6]. Non-small-cell lung cancer (NSCLC) represents the major category (85%) of lung cancer cases [6,7]. Smoking, family history of cancer, lifestyle, dietary, environmental, professional and infectious factors constitute the main risk factors of NSCLC [8,9]. Unfortunately, despite tremendous therapeutic efforts in NSCLC, the 5-year survival rate for patients with NSCLC is low [6]. Therefore, a practical biomarker that facilitates the early diagnosis of NSCLC as well as the prompt recognition of invasion, metastasis and prognosis would be very useful in the management of NSCLC.

A complex interconnection of inflammation, coagulation, fibrinolysis, insulin resistance and immune homeostasis is implicated in the pathogenesis of NSCLC promoting significantly neoplastic growth. Even early-stage resectable NSCLC exhibits a subclinical prethrombotic state and/or activation of fibrinolysis and inflammation [10,11]. PAI-1 participates in thrombotic, fibrinolytic, inflammatory and metabolic cascades [3]. Although the exact pathogenetic role of PAI-1 in humans is at the center of intensive translational research, the majority of evidence supports an association of PAI-1 with hemostasis, inflammation, insulin resistance and cancer, participating in the multi-step pathogenesis of NSCLC.

To date, in NSCLC clinical studies, the role of PAI-1 has been explored only regarding 1) its expression in lung cancer tissue [12,13] in several retrospective studies and 2) its circulated antigen levels in very few retrospective studies, mainly in advanced disease, without considering risk factors of NSCLC and several interconnections of PAI-1 pathophysiology [14-16]. Since PAI-1 activity reflects functionality and inhibitory capacity of PAI-1 which are not determined by its circulating antigen levels [17], our aim was to investigate the independent relationship between plasma PAI-1 activity and resectable NSCLC risk as well as its interplay with hemostatic, inflammatory, metabolic, tumor and clinicopathologic biomarkers in a large case control-study. Other goals were: 1) to examine clinical and laboratory parameters related to PAI-1 activity variation in NSCLC as well as 2) to assess the prognostic and diagnostic potential of circulating PAI-1 activity in NSCLC.

Methods

Study patients and controls

The group of cases included 110 consecutive patients with previously untreated, resectable NSCLC. Patients were enrolled from the 3rd Department of Thoracic Surgery of Sotiria General Hospital, which is the main Greek hospital for respiratory diseases. NSCLC was subsequently histologically confirmed according to the revised WHO classification for lung cancer and staging relied on the revised staging for lung cancer [18].

The group of controls comprised 110 consecutive healthy subjects matched on age (±5 years), gender and date of diagnosis (±1 month) with cases. Controls presented at the Laboratory Department for an annual check-up examination between July, 2012 and June, 2014 inclusive. All study subjects were of Greek nationality and the same residency (Attiki region and Metropolitan area of Athens, Greece). Written informed consent was obtained from all study participants before enrollment in the study. The Scientific and Ethics Committee of Sotiria General Hospital approved the study protocol (#4161/15-2-12) which conformed to the ethical guidelines of the Helsinki Declaration of 1975, as revised in 2000.

Eligibility criteria

Study participants were exclude from the study if they presented: cardiovascular disease (CVD), prior malignancy, diabetes mellitus (DM), endocrine disorders, hypertension, autoimmune, renal and hepatic disorders, hematologic (including coagulation disorders), neurologic, muscular or psychiatric disorders, HIV infection, recent infection at the time of blood drawn, asthma and alcoholism; if they routinely received any drugs including drugs for hyperlipidemia; and if they were pregnant and in post-partum period for women.

Data collection

Information was retrieved from medical records and interviews were done by the same physician (GS) using a structured questionnaire comprising sociodemographic, diet and lifestyle items, professional exposure to substances related to lung tumors (mainly asbestos and silica) as well as physical exercise. Family history of cancer, particularly lung cancer, and DM was recorded for first-degree and second-degree family relatives. Weight, height, waist (WC) and hip circumference (HC), and blood pressure (BP) measurements were performed for all subjects by the same physician (GS). Two BP measurements with the same instrument were obtained 5 min apart after 10 min of rest for each subject. BP was determined by taking into account the average of 2 measurements. Body mass index (BMI) was determined based on the formula: body weight (kg)/height^2 (m^2). Waist-to-hip ratio (WHR) was calculated based on the ratio WC to HC. Body fat percentage was determined

taking into account the Deurenberg equation [19]. All interviews and measurements were performed at the same time in the morning and under similar conditions.

Laboratory determinations

All blood specimens were collected early in the morning (8.00-9.30 am) after 12 h of fasting to avoid any effects of the circadian rhythm on the measured biomarkers, and before surgery or any other therapeutic approach for the cases. Blood samples for PAI-1 activity and hemostatic variables were collected in citrated tubes on enrollment, were centrifuged for 10 min at 4000 rounds per min, frozen at -20°C, and analyzed after no more than 28 days following the instructions. PAI-1 activity and other hemostatic parameters (PT, aPTT, fibrinogen, antithrombin III/ATIII, Protein C/PrC,

Table 1A. Demographic characteristics and risk factors of patients suffering from resectable NSCLC (n=110) and controls (n=110)

Categorical variable	*Cases (n=110) n (%)*	*Controls (n=110) n (%)*	*p value*
Demographic characteristics			
Sex (male, %)	91 (82.7)	91 (82.7)	1.00
Educational level (years)			
<6	10 (9.1)	2 (1.8)	0.04
6	46 (41.8)	40 (36.4)	
9	7 (6.4)	11 (10)	
12	27 (24.5)	24 (21.8)	
>12	20 (18.2)	33 (30)	
Area of residence (urban, %)	87 (79.1)	73 (66.4)	0.03
Risk factors of NSCLC			
Hx of tobacco smoking			<0.001
Non-smoker	4 (3.6)	38 (34.5)	
Ex-smoker	26 (23.6)	43 (39.1)	
Current smoker	80 (72.7)	29 (23.6)	
Hx of passive smoking (yes, %)	59 (53.6)	26 (23.6)	<0.001
Occupational exposure to substances (yes, %)	28 (25.5)	9 (8.2)	<0.001
Hx of alcohol consumption			<0.001
Never	15 (13.6)	16 (14.5)	
Rarely (1-2 glasses per month)	14 (12.7)	3 (2.7)	
Weekly (1-2 glasses per week)	31 (28.2)	76 (69.1)	
Weekly (3-4 glasses per week)	9 (8.2)	5 (4.5)	
Daily	41 (37.3)	10 (9.1)	
Hx of Daily coffee consumption (yes, %)	105 (95.5)	91 (82.7)	<0.002
Physical exercise (≥ 2 hours/week)	53 (48.2)	72 (65.5)	<0.01
Family history of lung cancer (yes, %)	19 (17.3)	9 (8.2)	<0.04
Family history of cancer (yes, %)	44 (40)	25 (22.7)	<0.006
Family history of diabetes (yes, %)	29 (26.4)	17 (15.5)	<0.047
Continuous variables	*Cases (n=110) Mean (SD)*	*Controls (n=110) Mean (SD)*	*p value*
Demographic variables			
Age, years	65.11 (7.90)	65.04 (7.54)	0.85
Risk factors of NSCLC			
Hx of smoking, daily smoked cigarettes X years smoking	1423.24 (907.09)	483.27 (497.36)	<0.001
Red meat, weekly servings	2.47 (1.39)	1.79 (0.96)	<0.001
Fruits, daily servings	0.71 (0.68)	0.88 (0.35)	<0.001
Vegetables, daily servings	0.82 (0.60)	0.76 (0.33)	0.67
Plant food, daily servings	1.50 (1.10)	1.45 (0.51)	0.38

SD: standard deviation

D-dimers and plasminogen) were evaluated using commercially available reagents (Berichrom® PAI) on an automated hemostasis analyzer (BCS XP system, Siemens Healthineers, Erlangen, Germany). The detection limit as well as the inter-assay and intra-assay coefficients of variations for Berichrom® Siemens PAI-1 activity determination were 0.5 U/mL, <4% and between 3-6%, respectively.

Additional serum was separated from initial blood drawn and stored at –80°C until analysis of tumor and inflammatory biomarkers, classic adipocytokines and insulin. Mean storage interval was similar for case and control specimens. Classic adipokines (leptin and adiponectin), hematologic biomarkers, glucose, insulin, urea, creatinine, lipid biomarkers, inflammatory markers such as high-sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and tumor markers such as CEA, CA 125, CA 15-3 and CYFRA 21-1 were evaluated as previously described [20,21]. IL-6 was determined using an automated analyzer (Cobase411, Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Finally, HOMA-IR (Homeostasis model assessment score of insulin resistance) was calculated using the formula: [fasting serum insulin (μU/mL) x fasting serum glucose (mmol/L)] / 22.5.

Statistics

Categorical data were expressed as number and percentage while continuous data were expressed as mean±standard deviation (SD). The following statistical tests were performed: Pearson's x^2 or Fisher's exact test for categorical variables, Student's *t*-test for normally distributed continuous variables, and Mann-Whitney U test for non-normally distributed continuous variables. Normality hypothesis was tested using the Shapiro-Wilk test. Spearman's correlation was used to assess correlations for continuous and ordinal variables. Taking into account prior studies on classic adipokines and lung cancer risk [9,22], multiple binary logistic regression analysis was employed to explore whether PAI-1 activity, expressed also in quartiles, was independently associated with NSCLC risk (outcome variable), controlling for matching factors and important clinical and laboratory biomarkers retrieved in univariate analyses [23]. Subsequently, linear regression models were used to explore the independent contribution of an individual predictor to PAI-1 activity variability in NSCLC. Stepwise binary logistic regression included significant predictors found in the univariate analysis to come up with a final model predicting PAI-1 activity in NSCLC (stepwise criteria of entry in the model ≤0.05 and removal from the model ≥0.1 [23]). Receiver Operator Characteristic (ROC) curve and area under the curve (AUC) analyses were performed to evaluate the discriminative ability of PAI-1 activity and serum tumor markers in NSCLC. The statistical package IBM-SPSS® version 24 for Windows was used. A two-sided p value less than 0.05 was considered significant for all tests. We calculated that we required a total sample size of at least 220 patients to achieve 90% power at the 0.05 level of significance in order to detect a 1 KU/L difference in PAI-1 activity.

Results

Clinical characteristics of study participants

Tables 1A, 1B and 1C depict the distribution of sociodemographic and clinical characteristics as well as laboratory biomarkers of all study subjects. Prior established connections of NSCLC with smoking, passive smoking, lower education level and physical activity, urban residency, alcohol consumption, red meat and lower fruit consumption,

Table 1B. Clinical characteristics of patients suffering from resectable NSCLC (n=110) and controls (n=110)

Clinical variables	*Cases (n=110) n (%)*	*Controls (n=110) n (%)*	*p value*
Blood pressure parameters			
SBP, mmHg	127.00 (12.50)	126.07 (9.69)	0.69
DBP, mmHg	78.61 (9.42)	78.93 (6.08)	0.35
Mean arterial BP[a], mmHg	94.74 (9.57)	94.65 (5.88)	0.53
Somatometric variables			
Weight at diagnosis, kg	77.07 (14.34)	80.25 (14.76)	0.14
Height, m	1.71 (0.07)	1.70 (0.08)	0.30
BMI at diagnosis, kg/m^2	26.35 (4.12)	27.67 (4.52)	0.01
Waist circumference, cm	100.90 (13.71)	103.77 (14.40)	0.20
Hip circumference, cm	102.40 (8.45)	104.85 (10.95)	0.24
WHR	0.98 (0.08)	0.99 (0.14)	0.16
Fat, %	32.26 (6.20)	33.82 (7.56)	0.11
Fat mass, kg	25.28 (8.38)	27.71 (9.93)	0.05
Free fat mass, kg	51.80 (8.26)	52.53 (8.40)	0.80

[a]Mean Arterial BP= (2*DBP+ SBP)/3
BMI: Body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; SD: standard deviation; WHR: waist-hip ratio

professional exposure to carcinogenic substances, family history of cancer and diabetes are confirmed in this Table data [8]. Patients exhibited significantly elevated levels of platelet count, HOMA-IR, insulin, fibrinogen, fibrinolytic, inflammatory and tumor biomarkers. However, patients presented significantly lower fat mass, BMI, cholesterol, hemoglobin and activity of anti-coagulant biomarkers such as ATIII and PrC. Notably, NSCLC patients showed significantly elevated plasma PAI-1 activity ($p<0.001$; Figure 1) but not adiponectin ($p=0.08$) and leptin ($p=0.06$) concentrations in comparison to controls. Table 2 portrays special clinicopathological characteristics of NSCLC cases.

Table 1C. Laboratory characteristics of patients suffering from resectable NSCLC (n=110) and controls (n=110)

Laboratory variables	*Cases (n=110) n (%)*	*Controls (n=110) n (%)*	*p value*	*Reference range*
Metabolic variables				
Glucose, mmol/L	5.80 (1.48)	5.42 (0.98)	0.15	4.11- 5.88
Insulin, pmol/L	111.75 (35.77)	99.66 (47.36)	0.004	18.06-172.93
HOMA-IR score	4.25 (2.02)	3.59 (2.33)	<0.001	-
Total cholesterol, mmol/L	4.66 (0.86)	5.39 (0.88)	<0.001	3.63- 5.70
Triglycerides, mmol/L	1.30 (0.69)	1.45 (0.57)	0.07	<2.26
HDL-C, mmol/L	1.12 (0.25)	1.31 (0.29)	<0.001	♂: 0.91-1.42
				♀: 1.17-1.68
LDL-C, mmol/L	2.95 (0.73)	3.41 (0.79)	<0.001	<4.12
Hematologic variables				
Hemoglobin, g/L	132.90 (14.0)	147.5 (15.8)	<0.001	♂: 135-175
				♀: 120-155
WBC, $\times 10^9$/L	8.839 (2.157)	6.960 (1.759)	<0.001	4-11
Platelets, $\times 10^9$/L	284 (97)	243 (46)	<0.001	150-400
Hemostatic variables				
PT, s	12.10 (0.68)	11.98 (0.75)	0.07	9.8-12.1
INR	1.03 (0.07)	1.00 (0.12)	0.12	-
aPTT, s	36.14 (7.11)	34.44 (7.10)	0.08	26-36
Fibrinogen, μmol/L	10.34 (3.20)	9.11 (1.29)	0.001	5.29-10.29
ATIII, % of the norm	92.92 (15.61)	105.53 (9.13)	<0.001	79-112
PrC, % of the norm	100.88 (25.24)	110.79 (15.15)	<0.001	70-140
D-dimers, nmol/L	6.19 (13.03)	2.25 (1.26)	<0.001	0s.01
Plasminogen, % of the norm	114.30 (19.74)	105.83 (8.51)	<0.001	75-150
Inflammatory variables				
hsCRP, nmol/L	69.33 (79.05)	32.10 (15.05)	<0.001	<47.62
IL-6, ng/L	8.33 (8.97)	4.84 (1.86)	<0.001	<7
TNF-α, ng/L	9.84 (13.89)	5.91 (3.01)	0.007	<8
Adipokines				
Leptin, μg/L	15.48 (5.39)	14.18 (4.63)	0.06	-
Adiponectin, mg/L	7.19 (2.25)	6.83 (1.87)	0.08	-
PAI-1 activity, kU/L	6.38 (3.24)	3.91 (1.53)	<0.001	2-7
Tumor biomarkers				
CEA, μg/L	7.02 (7.55)	2.20 (1.56)	<0.001	NS <3.8
				S<5.5
CA 125, kU/L	33.11 (22.46)	24.04 (9.41)	0.003	<35
CA 15-3, kU/L	25.98 (19.20)	15.10 (9.44)	<0.001	<25
CYFRA 21-1, μg/L	8.20 (11.14)	1.84 (1.36)	<0.001	<3.3

aPTT: activated Partial Thromboplastin Time; ATIII: Antithrombin III; CEA: Carcinoembryonic antigen; CYFRA 21-1: Cytokeratin 19 Fragment; HDL-C: High-density lipoprotein cholesterol; HOMA: Homeostasis model assessment score of insulin resistance; hsCRP: high sensitive C-Reactive Protein; IL-6: interleukin-6; INR: International Normalized Ratio; LDL-C: Low-density lipoprotein Cholesterol; PAI-1: Plasminogen activator inhibitor-1; PLT: Platelets; PrC: Protein C; PT: Prothrombin Time; SD: standard deviation; TNF-α: Tumor Necrosis Factor-α; WBC: White Blood Cells.

Correlations of PAI-1 activity with metabolic, hemostatic, inflammatory, tumor, clinicopathological variables and adipokines

In NSCLC patients, PAI-1 activity showed positive correlations with somatometric variables, insulin, WBC, ATIII, PrC, plasminogen, IL-6 and tumor size as depicted in Table 3. In control subjects, PAI-1 activity was associated with somatometric, metabolic (insulin, HOMA-IR, triglycerides, total cholesterol and LDL-C), fibrinogen, D-dimers, plasminogen, inflammatory biomarkers (hsCRP and IL-6) and leptin. Furthermore, PAI-1 activity showed negative correlations with adiponectin and CYFRA-21-1.

Plasma PAI-1 activity is independently associated with NSCLC

As shown in Table 4, multivariable logistic regression analysis revealed that higher plasma activity of PAI-1, expressed also as quartiles, was significantly related with NSCLC, before and after adjustment for age, gender, date of blood draw, residence, level of education, smoking, passive smoking, family history of cancer and diabetes, coffee, alcohol and red meat consumption, professional exposure to substances, physical exercise, BMI, HOMA-IR, TNF-α, IL-6, hsCRP, adiponectin and leptin (p value for trend <0.001). Notably, individuals in the 4th quartile of PAI-1 activity showed significantly higher odds for NSCLC (OR=21.57, 95%C.I. 3.99-116.81, p<0.001), while subjects in the 1st quartile of PAI-1 activity presented lower odds for NSCLC (OR=0.05, 95%C.I. 0.01-0.22, p<0.001) after adjusting for matching factors, risk factors and significant variables from univariate logistic regression analyses.

Independent determinants of plasma PAI-1 activity in NSCLC patients

As shown in Table 5, the only significant predictors of PAI-1 activity in NSCLC cases derived from the final model of multiple stepwise linear regression were plasminogen (p<0.001) and BMI (p=0.001).

PAI-1 activity in clinicopathological characteristics of NSCLC

PAI-1 activity is depicted in NSCLC patients amid various categories of stage, tumor differentiation, histology, tumor size and lymph node involvement (Table 6). PAI-1 activity showed a borderline statistical association with tumor size (p=0.05).

PAI-1 activity could be a useful biomarker in excluding NSCLC

The ROC analysis of PAI-1 activity and serum tumor markers is depicted in Figure 2. For PAI-1 activity, the best cut-off point based on Youden's index was 6.60 kU/L. PAI-1 activity greater than 6.60 kU/L provided a sensitivity and a specificity of 66% and 81% respectively (AUC 0.77, 95%CI: 0.71-0.84)

Table 2. Clinicopathological characteristics in 110 cases with resectable NSCLC

Clinicopathological variables	*Cases (n=110) n (%)*
Stage	
IA	25 (22.7)
IB	15 (13.6)
IIA	30 (27.3)
IIB	23 (20.9)
IIIA	17 (15.5)
Tumor differentiation	
Well	6 (5.5)
Moderate	56 (50.9)
Poor	48 (43.6)
Histology	
Adenocarcinoma	58 (52.7)
Squamous	47 (42.7)
Adenosquamous	5 (4.5)
Tumor size, cm	
≤3	37 (33.6)
3-5	51 (46.4)
>5	22 (20)
Lymph node involvement	
0	63 (57.3)
≥1	47 (42.7)

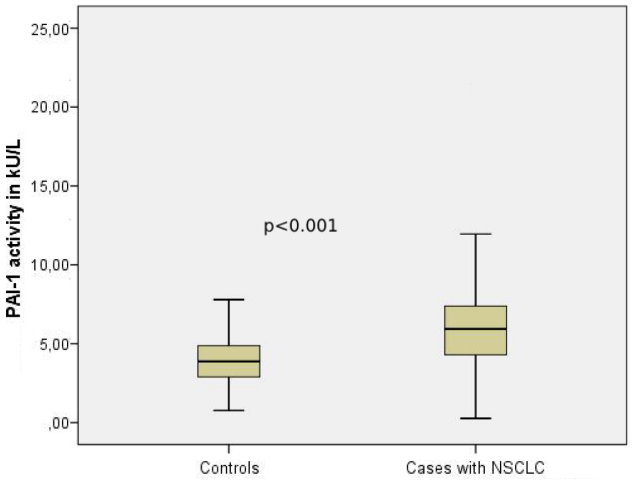

Figure 1. Circulating PAI-1 activity levels in NSCLC patients (n=110) and in controls (n=110). PAI-1 activity levels are significantly elevated in NSCLC cases (6.38 ± 3.24 kU/L) than in control subjects (3.91 ± 1.53 kU/L, p<0.001).

Table 3. Associations of study biomarkers with plasma PAI-1 activity

Variables	*Cases with NSCLC n=110*		*Controls n=110*	
	r	*p*	*r*	*p*
Blood pressure variables				
SBP	-0.02	0.82	-0.14	0.15
DBP	-0.06	0.52	-0.01	0.90
Mean ABP	-0.03	0.73	-0.11	0.28
Somatometric variables				
BMI	0.34	<0.001	0.53	<0.001
Waist circumference	0.35	<0.001	0.49	<0.001
WHR	0.26	0.01	0.10	0.31
Fat mass %	0.21	0.03	0.36	<0.001
Metabolic variables				
Glucose	0.10	0.31	0.15	0.11
Insulin	0.21	0.03	0.41	<0.001
HOMA-IR	0.18	0.06	0.41	<0.001
Triglycerides	-0.02	0.85	0.42	<0.001
Total cholesterol	0.07	0.49	0.37	<0.001
LDL-C	0.13	0.19	0.28	0.003
HDL-C	0.02	0.83	-0.09	0.35
Hematologic variables				
Hemoglobin	0.07	0.47	0.08	0.42
WBC	0.21	0.03	0.22	0.02
PLT	0.16	0.10	-0.12	0.21
Hemostatic variables				
PT	-0.15	0.23	-0.03	0.76
INR	-0.07	0.44	-0.06	0.55
aPTT	-0.07	0.47	0.08	0.40
Fibrinogen	0.07	0.46	0.18	0.05
ATIII	0.20	0.04	-0.02	0.81
PrC	0.28	0.003	-0.01	0.94
D-dimers	0.05	0.58	0.38	<0.001
Plasminogen	0.38	<0.001	0.54	<0.001
Inflammatory variables				
hsCRP	0.09	0.38	0.46	<0.001
IL-6	0.24	0.01	0.29	0.002
TNF-α	0.06	0.57	0.02	0.87
Adipokines				
Leptin	0.09	0.37	0.40	<0.001
Adiponectin	-0.07	0.48	-0.34	<0.001
Tumor biomarkers				
CEA	0.05	0.60	-0.10	0.29
CA125	0.01	0.95	-0.09	0.35
CA15-3	0.06	0.52	-0.14	0.14
CYFRA 21-1	-0.01	0.90	-0.21	0.03
Clinicopathological variables				
Stage	0.08	0.40	-	-
Tumor Differentiation	0.06	0.53	-	-
Tumor size	0.19	0.04	-	-
Number of infiltrated lymph nodes	-0.01	0.93	-	-

aPTT: activated partial thromboplastin time; ATIII: Antithrombin III; BP: blood pressure; BMI: body mass index; CEA: carcinoembryonic antigen; CYFRA 21-1: cytokeratin 19 fragment; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment score of insulin resistance; hsCRP: high sensitive C-reactive protein; IL-6: interleukin-6; LDL-C: low-density lipoprotein cholesterol; PAI-1: plasminogen activator inhibitor-1; PLT: platelets; PrC: protein C; PT: prothrombin time; SBP: systolic blood pressure; TNF-α: tumor necrosis factor-α; WBC: white blood cells; WHR: waist-hip ratio.

Table 4. Odd ratios (OR) and 95% confidence intervals (CI) for risk of resectable NSCLC in relation to plasma PAI-1 activity by quartiles and continuously

PAI-1 activity (kU/L)	*Cases (n)/Total (n), %*	*Crude OR (95% CI)*	*p value*	*p trend*	*Adjusted OR[a] (95% CI)*	*p value*	*p trend*
PAI-1 quartile (range)							
1st (0.5-3.40)	17/56	0.33	<0.001	<0.001	0.05	<0.001	<0.001
median: 2.70	(30.4)	(0.17-0.64)			(0.01-0.22)		
2nd (3.41-4.69	16/54	0.32	<0.001		0.34	0.09	
median: 3.90	(29.6)	(0.17-0.62)			(0.10-1.18)		
3rd (4.70-6.48)	30/55	1.28	0.44		4.41	0.03	
median: 5.19	(54.5)	(0.70-2.35)			(1.17-16.60)		
4th (6.49-21.2)	47/55	9.51	<0.001		21.57	<0.001	
median: 7.50	(85.5)	(4.22-21.44)			(3.99-116.81)		
For 1 SD of PAI-1 more	-	4.61	<0.001		6.93	<0.001	
		(2.83-7.52)			(2.90-16.59)		

SD: standard deviation. [a]OR adjusted for age, gender, date of blood draw, residence area, education level, smoking, passive smoking, coffee consumption, alcohol consumption, red meat consumption, professional exposure to carcinogenic substances, physical exercise, family history of cancer, family history of diabetes, BMI, HOMA-IR, IL-6, TNF-α, hsCRP, adiponectin and leptin.

Table 5. Final model of multiple linear regression analysis (stepwise method) portraying independent predictors of PAI-1 activity (as dependent variable) in 110 cases suffering from NSCLC; regression coefficients (b), standard error of b (SE_b) and t-statistic with corresponding p value

Independent variables	*b*	*SE_b*	*t-statistic*	*p value*
Plasminogen	0.05	0.01	3.75	<0.001
BMI	0.24	0.70	3.45	<0.001

Table 6. Circulating PAI-1 activity (kU/L) and its association with clinicopathological variables in 110 cases with resectable NSCLC

Clinicopathologic features	*PAI-1 activity (mean ± SD)*	*p value*
Stage		0.45
IA	5.79 ± 3.56	
IB	6.52 ± 1.23	
IIA	5.97 ± 1.98	
IIB	6.73 ± 4.32	
IIIA	7.38 ± 4.04	
Tumor differentiation		0.70
Well	5.08 ± 2.89	
Moderate	6.37 ± 3.16	
Poor	6.56 ± 3.40	
Histology		0.72
Adenocarcinoma	6.45 ± 2.90	
Squamous	6.25 ± 3.76	
Adenosquamous	6.67 ± 2.02	
Tumor size, cm		0.05
≤3	5.58 ± 3.17	
3-5	6.71 ± 3.07	
>5	6.95 ± 3.65	
Lymph node involvement		0.66
0	6.26 ± 2.87	
≥1	6.55 ± 3.72	

for the diagnosis of NSCLC. The negative predictive value was 71%. Only serum CEA outperformed PAI-1 in distinguishing NSCLC (CEA AUC 0.84, 95%CI: 0.79-0.90). PAI-1 activity was above the optimum cut-off point in 43 cases with NSCLC (39.1%) versus 8 (7.3%) controls (x^2=31.27, p<0.001). Despite its moderate discriminative ability (based on the AUC), PAI-1 activity could represent a potentially useful parameter in ruling out NSCLC due to its high specificity. Notably, the combination of plasma PAI-1 activity with all tumor markers yielded a better discriminative ability in terms of sensitivity (85%) and specificity (92%) compared to all biomarkers individually.

Discussion

To the best of our knowledge, this is the first study demonstrating the independent association of higher preoperative plasma PAI-I activity levels with resectable NSCLC, taking into account significant risk factors of NSCLC. Although its moderate discriminative ability, plasma PAI-1 activity could represent a potential helpful hemostatic biomarker excluding early NSCLC in combination with other serum tumor markers for lung cancer. So far, *in vivo* clinical studies have explored the blood con-

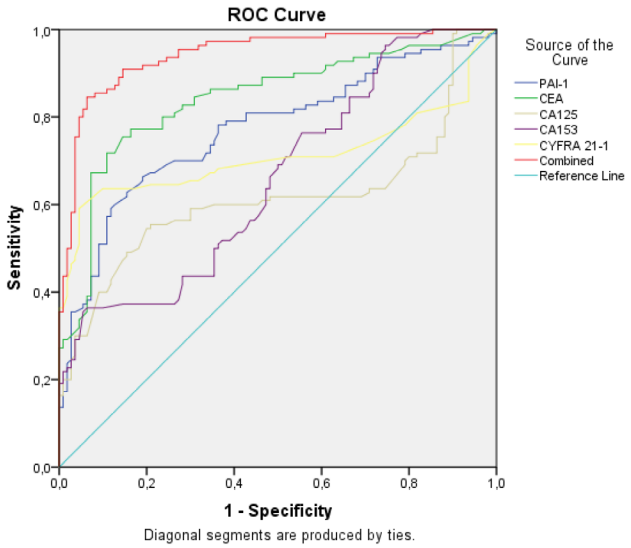

Figure 2. Receiver operating characteristic (ROC) analysis of PAI-1 activity, CEA, CA 15-3, CA 125, CYFRA 21-1 and combination of all biomarkers (patients with NSCLC versus controls): PAI-1 AUC (area under the curve), 0.77 (95%CI: 0.71-0.84); CEA AUC, 0.84 (95%CI: 0.79-0.90); CA 15-3 AUC, 0.66 (95%CI: 0.59-0.73), CA 125 AUC, 0.61 (95%CI: 0.54-0.69); CYFRA 21-1 AUC, 0.71 (95%CI: 0.64-0.79); combination of all tumor biomarkers including PAI-1 AUC, 0.94 (95%CI: 0.91-0.97).

centration of PAI-1 antigen as well as the tissue expression of PAI-1 in NSCLC, showing positive associations in general [12,14,16,25-31]. These studies included a heterogeneous case population regarding staging, focusing more on advanced cases with NSCLC, with a lower number of patients with resectable NSCLC. Furthermore, these studies did not take into account significant risk factors of NSCLC in multivariate models as well as the potential interfaces of PAI-1 with hemostatic, metabolic, inflammatory and tumor biomarkers in correlation analyses. Whilst PAI-1 antigen concentration levels are not able to discern the PAI-1 protein form (inert, active, latent, substrate noninhibitory) [13], PAI-1 activity, which reflects also antigen concentration, represents the functional inhibitory capacity of PAI-1 molecule [17]. Thus, it is more appropriate to study PAI-1 activity rather than PAI-1 antigen concentration in biological systems and *in vivo* studies.

The results of our study are in agreement with: 1) observational prospective epidemiological studies showing increased risk of cancer, particularly breast and colorectal cancers, in individuals in the upper quartile of PAI-1 expression [32]; 2) retrospective studies demonstrating higher PAI-1 concentration levels in cases with NSCLC than controls [14,30,31]; 3) clinical studies presenting higher expression levels of PAI-1 in lung cancer tissues than normal tissues [12,16,25-29,31,33]; and 4) experimental studies showing higher expression levels of PAI-1 in many tumor cells, including lung carcinoma [3,34-36].

The role of PAI-1 is not clear in carcinogenesis and tumor progression. PAI-1 could be implicated in lung cancer pathophysiology via the following mechanisms:

1) As an anti-apoptotic and proliferative factor. Indeed, PAI-1 may alter cell signaling, promoting proliferative signaling in many cell types [34]. In particular, intracellular PAI-1 may stimulate cell survival through inhibition of caspase-3, which diverts intracellular pro-apoptotic signaling to a proliferative one [37]. PAI-1 is found in elevated concentrations in blood, particularly in cancer, after secretion from a variety of cells including tumor cells which release it in the vicinity of cells [29].

Emerging evidence has related PAI-1 with tumor development, invasion, angiogenesis and progression. PAI-1 has been found to activate cell migration and capacity as well as the expression of Epithelial to Mesenchymal Transition (EMT) markers *in vivo* and *in vitro* [26]. Interestingly, we have found that PAI-1 correlated with tumor size as evidenced in many studies [2]. However, PAI-1 activity did not correlate with other clinicopathological features such as staging and lymph node involvement. The role of PAI-1 in tumor angiogenesis is not clear since many animal and experimental studies have shown conflicting results with both i) inhibition [13,38], attributed to the suppression of u-PA, which propels matrix degradation and cancer invasion, ii) stimulation [2,39], due to the inhibition of excessive proteolysis which prevents from successful tumor vessels assembly, and iii) lack of any effect [40,41]. It seems that this phenomenon depends on optimal range of PAI-1 levels and activity as supraphysiological doses of PAI-1 favor tumor size development and angiogenesis, whereas higher doses exhibit inhibitory action [13,41]. More experimental studies are needed to elucidate the oncogenic properties of PAI-1 and its role in tumor invasion, angiogenesis and metastasis.

2) As a biomarker reflecting disturbed coagulation and fibrinolysis, which are frequently seen in lung cancer even at earlier stages [42-44]. We have found that, in NSCLC patients, PAI-1 was positively associated with ATIII, protein C and plasminogen, which was the most important determinant of plasma PAI-1 activity. PAI-1 are an essential inhibitor of plasminogen activator in plasma, presenting high affinity for both uPA, tissue PA and plasminogen [2].

3) As a metabolic mediator reflecting the insulin-resistant and inflammatory state observed in

NSCLC [45]. Insulin resistance and elevated PAI-1 may be intertwined in promoting lung carcinogenesis. Adipocytes may synthesize PAI-1 in response to insulin [46] while insulin may release PAI-1 from a variety of cell types [47]. Current evidence supports a relationship between PAI-1 (characterized as an adipocytokine), obesity, insulin resistance and cancer [13]. Visceral fat and BMI are associated with PAI-1 levels and activity [3]. In cases, BMI emerged as an important determinant of PAI-1 activity in our study. Notably, we have shown positive associations between PAI-1 and BMI, insulin, fat mass, WC, HOMA-IR (of borderline significance) and IL-6 in cases, and with somatometric, metabolic and inflammatory biomarkers in controls, in agreement with many studies [13]. As adiposity increases, so does the secretion of PAI-1 and its activity resulting in an impairment of the hemostatic system [48].

Important strengths of our study are 1) the determination of plasma PAI-1 activity which reflects not only its concentration but also its functional capacity; 2) the determination in the morning which better reflects the circadian rhythm of PAI-1 activity; 3) the appropriately powered study, which was sufficient to document novel significant associations with PAI-1 and replicate previous established risk factors of NSCLC; 4) the use of detailed information on lifestyle, somatometric and biological variables, allowing to control for their potential confounding effect in multivariate models; 5) the blind plasma determinations by the laboratory staff, eliminating bias from laboratory sources; 6) the selection of controls from the same study population, who were adequately matched to cases, avoiding selection bias. Although the abovementioned strengths, this study presents noteworthy limitations. Its case-control design cannot prove causality, however it may raise strong hypotheses to be explored in future prospective studies. Also, residual confounding by other undetermined variables remains a possibility.

In conclusion, circulating PAI-1 activity was independently associated with resectable NSCLC and correlated with hemostatic, metabolic, inflammatory biomarkers and tumor size. Further mechanistic and prospective studies are required to elucidate whether this relationship is causative or an epiphenomenon. If the former is true, additional investigations on PAI-1 inhibitors suppressing tumor invasion and metastatic potential are required [13]; More longitudinal studies are needed to explore whether plasma PAI-1 activity may be a practical biomarker in the risk assessment of NSCLC, at the crossroads of thrombosis, fibrinolysis and metabolism.

Acknowledgements

The study is dedicated to the memory of Dr Marios Konstantinou†, Director of the Department of Thoracic Surgery, 'Sotiria' General Hospital of Athens, who provided his support throughout this work.

Conflict of interests

The authors declare no conflict of interests.

References

1. Dupont DM, Madsen JB, Kristensen T et al. Biochemical properties of plasminogen activator inhibitor-1. Front Biosci (Landmark Ed) 2009;14:1337-61.
2. Chorostowska-Wynimko J, Skrzypczak-Jankun E, Jankun J. Plasminogen activator inhibitor type-1: its structure, biological activity and role in tumorigenesis (Review). Int J Mol Med 2004;13:759-66.
3. Yamamoto K, Takeshita K, Saito H. Plasminogen activator inhibitor-1 in aging. Semin Thromb Hemost 2014;40:652-9.
4. Aune G, Stunes AK, Lian AM et al. Circulating interleukin-8 and plasminogen activator inhibitor-1 are increased in women with ovarian carcinoma. Results Immunol 2012;2:190-5.
5. Newland JR, Haire WD. Elevated plasminogen activator inhibitor levels found in patients with malignant conditions. Am J Clin Pathol 1991;96:602-4.
6. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA: Cancer J Clin 2018;68:7-30.
7. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008;359:1367-80.
8. Akhtar N, Bansal JG. Risk factors of Lung Cancer in nonsmoker. Curr Probl Cancer 2017;41:328-39.
9. Terzidis A, Sergentanis TN, Antonopoulos G et al. Elevated serum leptin levels: a risk factor for non-small-cell lung cancer? Oncology 2009;76:19-25.
10. Gabazza EC, Taguchi O, Yamakami T, Machishi M, Ibata H, Suzuki S. Evaluating prethrombotic state in lung cancer using molecular markers. Chest 1993;103:196-200.

11. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436-44.
12. Lu JJ, Guo H, Gao B et al. Prognostic value of urokinase plasminogen activator system in non-small cell lung cancer: A systematic review and meta-analysis. Mol Clin Oncol 2018;8:127-32.
13. Murphy EA. Novel Adipocytokines: Monocyte Chemotactic Protein-1, Plasminogen Activator Inhibitor-1, Chemerin. In: Reizes O, Berger NA (Eds): Adipocytokines, Energy Balance, and Cancer. Cham: Springer International Publishing, 2017, pp 161-86.
14. Zekanowska E, Cieslinski K, Rosc D. [Plasminogen activator inhibitor type 1 (PAI-1) in blood and tissue extracts of patients with non-small cell lung cancer]. Pneumonol Alergol Pol 2004;72:409-14.
15. Izbicka E, Streeper RT, Michalek JE, Louden CL, Diaz A, 3rd, Campos DR. Plasma biomarkers distinguish non-small cell lung cancer from asthma and differ in men and women. Cancer Genomics Proteomics 2012;9:27-35.
16. Werle B, Kotzsch M, Lah TT et al. Cathepsin B, plasminogen activator-inhibitor (PAI-1) and plasminogen activator-receptor (uPAR) are prognostic factors for patients with non-small cell lung cancer. Anticancer Res 2004;24:4147-61.
17. Pavlov M, Nikolic-Heitzler V, Babic Z et al. Plasminogen activator inhibitor-1 activity and long-term outcome in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention: a prospective cohort study. Croat Med J 2018;59:108-17.
18. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The Eighth Edition Lung Cancer Stage Classification. Chest 2017;151:193-203.
19. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Br J Nutr 1991;65:105-14.
20. Sotiropoulos G, Dalamaga M, Antonakos G et al. Chemerin as a biomarker at the intersection of inflammation, chemotaxis, coagulation, fibrinolysis and metabolism in resectable non-small cell lung cancer. Lung Cancer 2018;125;291-9.
21. Bluher M, Brennan AM, Kelesidis T et al. Total and high-molecular weight adiponectin in relation to metabolic variables at baseline and in response to an exercise treatment program: Comparative evaluation of these assays. Diabetes Care 2007;30:280-5.
22. Petridou ET, Mitsiades N, Gialamas S et al. Circulating adiponectin levels and expression of adiponectin receptors in relation to lung cancer: two case-control studies. Oncology 2007;73:261-9.
23. Cook F. Advanced Methods in Clinical Epidemiology. Harvard School of Public Health. Boston, MA, USA 2000.
24. de Groot P, Munden RF. Lung cancer epidemiology, risk factors, and prevention. Radiol Clin North Am 2012;50:863-76.
25. Salden M, Splinter TA, Peters HA et al. The urokinase-type plasminogen activator system in resected non-small-cell lung cancer. Rotterdam Oncology Thoracic Study Group. Ann Oncol 2000;11:327-32.
26. Kang J, Kim W, Kwon T, Youn H, Kim JS, Youn B. Plasminogen activator inhibitor-1 enhances radioresistance and aggressiveness of non-small cell lung cancer cells. Oncotarget 2016;7:23961-74.
27. Pedersen H, Brunner N, Francis D et al. Prognostic impact of urokinase, urokinase receptor, and type 1 plasminogen activator inhibitor in squamous and large cell lung cancer tissue. Cancer Res 1994;54:4671-5.
28. Nagayama M, Sato A, Hayakawa H, Urano T, Takada Y, Takada A. Plasminogen activators and their inhibitors in non-small cell lung cancer. Low content of type 2 plasminogen activator inhibitor associated with tumor dissemination. Cancer 1994;73:1398-405.
29. Robert C, Bolon I, Gazzeri S, Veyrenc S, Brambilla C, Brambilla E. Expression of plasminogen activator inhibitors 1 and 2 in lung cancer and their role in tumor progression. Clin Cancer Res 1999;5:2094-102.
30. Davies NA, Harrison NK, Sabra A et al. Application of ROTEM to assess hypercoagulability in patients with lung cancer. Thromb Res 2015;135:1075-80.
31. McMahon BJ, Kwaan HC. Components of the Plasminogen-Plasmin System as Biologic Markers for Cancer. Adv Exp Med Biol 2015;867:145-56.
32. Iacoviello L, Agnoli C, De Curtis A et al. Type 1 plasminogen activator inhibitor as a common risk factor for cancer and ischaemic vascular disease: the EPICOR study. BMJ Open 2013;3:e003725.
33. Pedersen H, Grondahl-Hansen J, Francis D et al. Urokinase and plasminogen activator inhibitor type 1 in pulmonary adenocarcinoma. Cancer Res 1994;54:120-3.
34. Gramling MW, Church FC. Plasminogen activator inhibitor-1 is an aggregate response factor with pleiotropic effects on cell signaling in vascular disease and the tumor microenvironment. Thromb Res 2010;125:377-81.
35. Kristensen P, Pyke C, Lund LR, Andreasen PA, Dano K. Plasminogen activator inhibitor-type 1 in Lewis lung carcinoma. Histochemistry 1990;93:559-66.
36. Zhu C, Shen H, Zhu L, Zhao F, Shu Y. Plasminogen Activator Inhibitor 1 Promotes Immunosuppression in Human Non-Small Cell Lung Cancers by Enhancing TGF-Beta1 Expression in Macrophage. Cell Physiol Biochem 2017;44:2201-11.
37. Schneider DJ, Chen Y, Sobel BE. The effect of plasminogen activator inhibitor type 1 on apoptosis. Thromb Haemost 2008;100:1037-40.
38. Chen H, Peng H, Liu W et al. Silencing of plasminogen activator inhibitor-1 suppresses colorectal cancer progression and liver metastasis. Surgery 2015;158:1704-13.
39. Bajou K, Noel A, Gerard RD et al. Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. Nat Med 1998;4:923-8.
40. McMahon GA, Petitclerc E, Stefansson S et al. Plasminogen activator inhibitor-1 regulates tumor growth and angiogenesis. J Biol Chem 2001;276:33964-8.

41. Stefansson S, McMahon GA, Petitclerc E, Lawrence DA. Plasminogen activator inhibitor-1 in tumor growth, angiogenesis and vascular remodeling. Curr Pharm Des 2003;9:1545-64.
42. Marinho FC, Takagaki TY. Hypercoagulability and lung cancer. J Bras Pneumol 2008;34:312-22.
43. Seitz R, Rappe N, Kraus M et al. Activation of coagulation and fibrinolysis in patients with lung cancer: relation to tumour stage and prognosis. Blood Coagul Fibrinolysis 1993;4:249-54.
44. Niki M, Yokoi T, Kurata T, Nomura S. New prognostic biomarkers and therapeutic effect of bevacizumab for patients with non-small-cell lung cancer. Lung Cancer (Auckl) 2017;8:91-9.
45. Petridou ET, Sergentanis TN, Antonopoulos CN et al. Insulin resistance: an independent risk factor for lung cancer? Metabolism 2011;60:1100-6.
46. Lundgren CH, Brown SL, Nordt TK, Sobel BE, Fujii S. Elaboration of type-1 plasminogen activator inhibitor from adipocytes. A potential pathogenetic link between obesity and cardiovascular disease. Circulation 1996;93:106-10.
47. Loskutoff DJ, Samad F. The adipocyte and hemostatic balance in obesity: studies of PAI-1. Arterioscler Thromb Vasc Biol 1998;18:1-6.
48. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. Int J Obes Relat Metab Disord 2004;28:1357-64.