



ELSEVIER

**LUNG
CANCER**


www.elsevier.com/locate/lungcan

Correlation between tumor necrosis factor-alpha and D-dimer levels in non-small cell lung cancer patients

F. Guadagni^a, P. Ferroni^{b,*}, S. Basili^c, F. Facciolo^d, S. Carlini^d,
M. Crecco^e, F. Martini^b, A. Spila^a, R. D'Alessandro^a, S. Aloe^a,
V. Cerasoli^d, G. Del Monte^{a,f}, S. Mariotti^f, T.C. Mineo^g, M. Roselli^f

^a Laboratory of Clinical Pathology, Regina Elena Cancer Institute, Via E. Chianesi 53, Rome 00144, Italy

^b Department of Experimental Medicine and Pathology, University La Sapienza, Viale Regina Elena 324, Rome 00161, Italy

^c Department of Medical Therapy, University La Sapienza, Viale del Policlinico, Rome 00185, Italy

^d Division of Thoracic Surgery, Regina Elena Cancer Institute, Via E. Chianesi 53, Rome 00144, Italy

^e Division of Radiology, Regina Elena Cancer Institute, Via E. Chianesi 53, Rome 00144, Italy

^f Medical Oncology, Department of Surgery, University "Tor Vergata", Via di Tor Vergata 135, Rome 00133, Italy

^g Thoracic Surgery, Department of Surgery, University "Tor Vergata", Via di Tor Vergata 135, Rome 00133, Italy

Received 24 July 2003; received in revised form 17 November 2003; accepted 18 November 2003

KEYWORDS

Non-small-cell lung cancer;
Fibrinolysis;
Coagulation;
Tumor necrosis factor-alpha;
D-Dimer;
Thrombin-antithrombin complexes

Summary The present study was designed to investigate whether a correlation exists between IL-6, TNF- α and coagulation (Thrombin-antithrombin, TATc) or fibrinolysis (D-dimer) activation in non-small cell lung cancer (NSCLC) patients. One hundred thirty patients with NSCLC ($n = 65$, 53 males, mean age 65 ± 8 , adenocarcinoma $n = 32$, squamous cancer $n = 33$) or chronic obstructive pulmonary disease (COPD) ($n = 65$, 51 males, mean age 67 ± 9) were studied. As control group 65 healthy donors (51 males, mean age 61 ± 14) were also evaluated. The results obtained showed that median D-dimer levels were higher in NSCLC patients ($3.0 \mu\text{g/ml}$) compared either to COPD patients ($1.1 \mu\text{g/ml}$, $P < 0.05$) or controls ($0.3 \mu\text{g/ml}$, $P < 0.0001$). Positive TNF- α levels ($>10 \text{ pg/ml}$) were found in 26% of NSCLC compared to 3% of COPD ($P < 0.002$) and 5% of controls ($P < 0.0005$). On the other hand, positive ($>8.5 \text{ pg/ml}$) IL-6 levels were found in 53% of NSCLC and 21% of COPD patients, compared to 5% of control subjects ($P < 0.001$). Median TATc levels were elevated in either NSCLC ($6.9 \mu\text{g/l}$) or COPD ($5.7 \mu\text{g/l}$) patients compared to controls ($1.8 \mu\text{g/l}$, $P < 0.0001$). Elevated D-dimer levels were significantly associated to positive TNF- α levels in patients without distant metastasis ($F = 4.3$, $P < 0.05$). Moreover, TNF- α levels ($P < 0.01$) were independently related to the presence of positive D-dimer levels in patients with non-metastatic NSCLC. These results suggest that increased levels of TNF- α might be responsible for an activation of fibrinolysis in patients with NSCLC.

© 2003 Elsevier Ireland Ltd. All rights reserved.

*Corresponding author. Tel.: +39-06-4452955; fax: +39-06-4454820.

E-mail address: patrizia.ferroni@uniroma1.it (P. Ferroni).

1. Introduction

Haemostatic abnormalities can be found in more than 90% of cancer patients, regardless of the presence or absence of clinical evidence for thromboembolic disease, and there is reason to believe that these abnormalities contribute substantially to solid tumor organization, demarcation of tumor from normal host tissues, regulation of inflammatory cell accumulation, tumor angiogenesis and tumor stroma generation [1].

Although a number of studies have found an association between fibrinolysis and non-small cell lung cancer (NSCLC), this relationship is by no means completely understood. Indeed, significant elevation in the blood concentrations of the split product from cross-linked fibrin, D-dimer, was found in lung cancer patients, with either extensive or limited disease [2–6], suggesting that a sub-clinical activation of blood coagulation and fibrinolysis can occur in NSCLC from the early clinical stages of disease. Furthermore, the increase of D-dimer levels has been related to unresponsiveness to treatment [7] and unfavorable prognosis [7–10] in patients with NSCLC.

Despite these studies, the origin of the activation of fibrinolysis in NSCLC is still unclear. The elevated levels of D-dimer may, in fact, indicate a host compensatory response to the increased formation of cross-linked fibrin [5,11], as indicated by the finding of a concomitant activation of the coagulation pathway in NSCLC [2–6]. Indeed, in a recent study we have shown that D-dimer levels are increased in NSCLC concomitantly to thrombin/anti-thrombin III complexes (TATc), an activation marker of coagulation [5]. However, we did not find any correlation between D-dimer and TATc, suggesting that mechanism(s) other than the compensatory response to fibrin production might be operating in NSCLC, such as an accelerated activity of the tumor-derived plasmin-mediated activation of fibrinolysis. Yet, in a comprehensive study of systemic fibrinolysis in patients with NSCLC, Pavey et al. [6] demonstrated that significant activation of fibrinolysis occurs in NSCLC patients, although the origin of this activation could not be related to consistent changes in plasminogen activator and inhibitor levels. Thus, the question that remains to be answered is whether the increased fibrinolysis found in NSCLC is due to the presence of cancer and/or cancer-related products or represents a normal physiological process to balance the activation of coagulation.

It is well known that tumor cells and/or tumor-associated leukocytes may produce inflammatory cytokines, such as IL-6 and TNF- α [12], and it has

been recently demonstrated by in-situ hybridization that elevated TNF- α mRNA levels can be found in both non-small cell lung cancer cells and tumor infiltrating lymphocytes (TIL) [13]. Moreover, circulating levels of these cytokines have been associated with the disease status of lung cancer patients [14–16].

The release of inflammatory cytokines is involved in activation of the fibrinolytic/coagulative system. IL-6, for example, is thought to act over the extrinsic pathway of coagulation through tissue factor expression [17], whereas TNF- α is thought to play a direct role in the activation pathway of the fibrinolytic system [18,19]. Thus, the present study was aimed at analyzing the possible association between TNF- α , IL-6 and D-dimer or TATc levels in patients with different stages of NSCLC, to better characterize the possible link between these inflammatory cytokines and fibrinolysis activation in NSCLC.

2. Patients and methods

Sixty-five patients with NSCLC, treated at our Institutions, entered the study. Patients (53 males, 12 females; mean age 65 ± 8 , ranging from 48 to 82 years; 22 current and 22 ex-smokers) were histologically diagnosed with lung adenocarcinoma ($n = 32$) or squamous cancer ($n = 33$). NSCLC was pathologically staged according to the tumor-nodes-metastasis classification (UICC—Union International Contre le Cancer TNM classification of malignant tumors). Nineteen (29.2%) patients were classified as stage I, 6 (9.2%) as stage II, 23 (35.4%) as stage III, and 17 (26.2%) as stage IV lung cancer. Sixty-five patients with chronic obstructive pulmonary disease (COPD) (51 males, 14 females; mean age 67 ± 9 , ranging from 46 to 85 years; 11 current and 33 ex-smokers) and 65 healthy subjects (51 males, 14 females; mean age 61 ± 14 , ranging from 47 to 83 years; 12 current and 25 ex-smokers) were evaluated as control groups. The latter were chosen among healthy subjects with a family history of cardiovascular disease participating in a prevention program for atherothrombotic disorders at the University of Rome La Sapienza. Selection of controls was performed by an independent investigator, blind to laboratory data, in order to have a population similar for age, sex and smoking habit to the NSCLC and COPD groups. Diabetes mellitus (fasting blood glucose level >115 mg/dl or treatment with a hypoglycemic agent), acute inflammatory disease, history of alcohol or drug abuse, peripheral-, cardio- and cerebro-vascular atherosclerotic diseases (by clinical history,

physical examination, and instrumental diagnosis) were considered as exclusion criteria. No subject was on non-steroidal anti-inflammatory drugs, anticoagulant or antiplatelet agents in the 2 weeks preceding the study. The study was performed under the appropriate institutional ethics approvals and in accordance with the principles embodied in the Declaration of Helsinki. Written informed consent was obtained from each participating subject.

2.1. Sample collection and immunoassays

Plasma samples from resectable lung cancer patients were drawn within 1 week before surgery, while samples from patients with metastatic disease were obtained at the time of clinical diagnosis, and prior to any treatment. Blood samples from COPD patients were drawn at the time of clinical and/or instrumental diagnosis and prior to any treatment. After a rest period of at least 20 min, blood samples were withdrawn from each consenting subject, without stasis, from the antecubital vein using a 20 G needle, and anticoagulated in Na citrate 3.8% (1:9, v:v). Samples were immediately centrifuged at $1500 \times g$ for 10 min to obtain plasma, aliquoted, coded and stored at -40°C until the assays were performed.

Plasma TNF- α , IL-6 (both by R&D Systems, Minneapolis, MN, USA) and TATc (Enzygnost TAT, Dade-Behring, Marburg, Germany) levels were measured by commercially available enzyme immunoassays according to the manufacturers' instructions. D-Dimer concentration was determined on a STA analyzer (Roche Diagnostics, Mannheim, Germany) using the STA LIATEST D-DI (Roche Diagnostics). Measurements were done blinded. All samples were assayed in duplicate and those showing values above the standard curve were re-tested with appropriate dilutions. The cutoff limits for TNF- α and IL-6 plasma levels were calculated as the mean \pm 25.D. of the values observed in the control population, and were set at 10 and 8.5 pg/ml, respectively. As previously reported, the cutoff limits for TATc and D-dimer levels were set at 3.7 $\mu\text{g/l}$ and 0.72 $\mu\text{g/ml}$, respectively [5].

2.2. Statistical analysis

Statistical analysis was performed by χ^2 statistics, Pearson's correlation coefficient, ANOVA and/or unpaired *t*-test. When necessary, log transformation was used to normalize the data, or appropriate non-parametric tests were employed (Spearman correlation analysis, Kruskal–Wallis and/or Mann–Whitney *U*-tests). Data are presented as median and interquartile range (IRQ; 25th percentile

to 75th percentile) or as percentages of positive patients. Only *P*-values lower than 0.05 were regarded as statistically significant. All calculations were made using a computer software package (Statistica, StatSoft Inc., Tulsa, OK, USA).

3. Results

IL-6, TNF- α , D-dimer and TATc levels were determined in plasma samples obtained from 195 subjects, including 65 NSCLC patients with histologically diagnosed lung adenocarcinoma ($n = 32$) or squamous cancer ($n = 33$), 65 patients with COPD and 65 control subjects. Median levels of all four variables did not significantly differ between current smokers, ex-smokers and never smokers in the three groups under evaluation (data not shown). As summarized in Table 1, median TNF- α levels were higher in NSCLC compared to either COPD patients ($P < 0.05$) or controls ($P < 0.0001$). Indeed, positive (above the cutoff value) TNF- α levels were found in a higher percentage of patients with NSCLC (26%) compared to either COPD patients (3%, $P < 0.001$) or healthy subjects (5%, $P < 0.001$). Similarly, median IL-6 levels were higher in NSCLC compared to either COPD patients ($P < 0.01$) or controls ($P < 0.0001$). Indeed, positive (above the cutoff value) IL-6 levels were found in a higher percentage of patients with NSCLC (53%) compared to either COPD patients (21%, $P < 0.001$) or healthy subjects (5%, $P < 0.001$). Furthermore, COPD patients had IL-6 levels higher than controls ($P < 0.0001$).

On the other hand, both patients with COPD and NSCLC had higher median TATc levels compared with healthy subjects ($P < 0.0001$), but no difference was observed between the two former groups (Table 1). Median D-dimer plasma levels were also significantly elevated in COPD patients compared to healthy subjects ($P < 0.0001$), but were significantly lower than in patients with NSCLC ($P < 0.05$). Positive D-dimer levels were found in 86% of patients with NSCLC compared to 52% of COPD patients and 9% of healthy subjects ($P < 0.0001$) (Table 1).

Positive levels of either TNF- α or TATc, but not IL-6 or D-dimer, were found in a higher percentage of patients with distant metastasis compared to those without. However, the measured levels of both analytical variables did not significantly differ in the two subgroups of patients (Fig. 1). Of interest, COPD patients had TATc levels significantly higher than those observed in non-metastatic patients ($P < 0.05$) and quite similar to those found in Stage IV NSCLC (Fig. 1). No significant association was found between all laboratory variables and

Table 1 Plasma TNF- α , TATc and D-dimer levels in patients with non-small cell lung cancer (NSCLC) or chronic obstructive pulmonary disease (COPD) and control subjects

	TNF- α (pg/ml)	IL-6 (pg/ml)	TATc (μ g/l)	D-Dimer (μ g/ml)
NSCLC patients				
N. positive (%)	17 (26)	34 (53)	46 (71)	56 (86)
Median (IQR)	4.5 (3.0–11.9)	8.8 (3.7–21.6)	6.9 (3.4–7.4)	3.0 (1.5–5.8)
χ^2 -test	$P < 0.001$	$P < 0.001$	NS	$P < 0.0001$
Mann–Whitney	$P < 0.05$	$P < 0.01$	NS	$P < 0.05$
COPD patients				
N. positive (%)	2 (3)	8 (21)	46 (71)	34 (52)
Median (IQR)	4.0 (2.2–6.4)	4.7 (2.7–6.8)	5.7 (3.7–7.7)	1.1 (0.4–6.7)
χ^2 -test	NS	$P < 0.05$	$P < 0.0001$	$P < 0.0001$
Mann–Whitney	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Control subjects				
N. positive (%)	3 (5)	3 (5)	17 (26)	6 (9)
Median (IQR)	0.4 (0.2–1.2)	1.5 (0.5–3.8)	1.8 (1.1–4.1)	0.3 (0.2–0.4)
χ^2 -test ^a	$P < 0.001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Mann–Whitney ^a	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Kruskal–Wallis	$H = 62.3$	$H = 48.5$	$H = 46.7$	$H = 81.9$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

^a NSCLC vs. control subjects.

tumor size, lymph node involvement or histological diagnosis (Table 2). Thus, the interactions among TNF- α , D-dimer levels and the presence of distant metastases was analyzed by two-way

Anova (Fig. 2). Of interest, elevated D-dimer levels were significantly associated to positive TNF- α levels only in patients without distant metastasis ($F = 4.3$, $P < 0.05$).

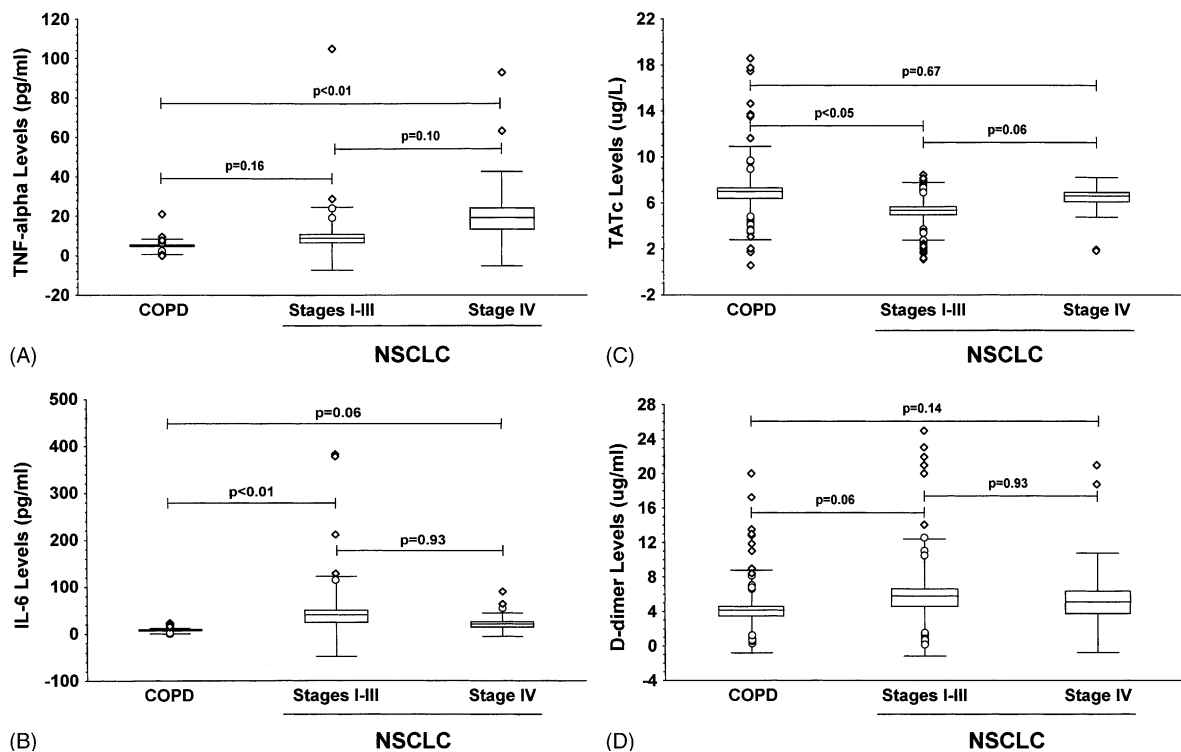


Fig. 1 Box-plot analysis of plasma TNF- α (Panel A), IL-6 (Panel B), Thrombin–antithrombin complex (TATc) (Panel C) and D-dimer (Panel D) levels in 65 patients with chronic obstructive pulmonary disease (COPD) and 65 non-small cell lung cancer (NSCLC). Solid lines indicate mean values, open circles indicate outlier values, open diamonds indicate extreme values, bars indicate standard deviations, columns indicate standard errors of the mean.

Table 2 Association of positive levels of TNF- α , TATc and D-dimer with different pathological variables in 65 non-small cell lung cancer patients

Variable	Plasma levels above the cutoff value				
	N (%)	TNF- α (>10 pg/ml)	IL-6 (>8.5 pg/ml)	TATc (>3.7 μ g/l)	D-Dimer (>0.72 μ g/ml)
Tumor size					
T1	15	2 (13)	5 (33)	11 (73)	11 (73)
T2	21	5 (24)	10 (48)	11 (52)	20 (95)
T3	25	10 (40)	17 (68)	20 (80)	22 (88)
T4	4	0 (0)	2 (50)	4 (100)	3 (75)
Lymph node involvement					
N0	31	7 (23)	18 (58)	20 (65)	26 (84)
N1	9	2 (22)	1 (11)	5 (56)	8 (89)
N2	25	8 (32)	15 (69)	21 (84)	22 (88)
Distant metastasis					
No	45	8 (18)	22 (49)	28 (62)	38 (84)
Yes	20	9 (45)*	12 (69)	18 (90)*	18 (90)
Diagnosis of primary tumor					
Adenocarcinoma	32	9 (28)	14 (44)	25 (78)	28 (88)
Squamous	33	8 (24)	20 (61)	21 (64)	28 (85)

* $P < 0.05$.

Correlation analysis among TNF- α , IL-6, D-dimer and TATc levels in the three groups of patients demonstrated that D-dimer directly correlated with TATc either in COPD patients ($P < 0.001$) or control subjects ($P < 0.05$), whereas the former significantly correlated with TNF- α ($P < 0.01$) or IL-6 ($P < 0.05$) in NSCLC patients ($P < 0.01$). However, subgroup analysis of patients with or without distant metastasis, showed that D-dimer and TNF- α correlated only in the subgroup of patients with-

out metastatic disease ($P < 0.001$) (Table 3). Thus, to further analyze the nature of this relationship a multiple regression analysis including age, sex, smoking status, histological diagnosis, stage, TNF- α , IL-6 and TATc levels was carried out. The final model obtained by stepwise regression analysis revealed that only TNF- α levels (regression coefficient = 0.36, $P < 0.01$) were independently related to the presence of elevated D-dimer levels in patients with non-metastatic NSCLC. On the

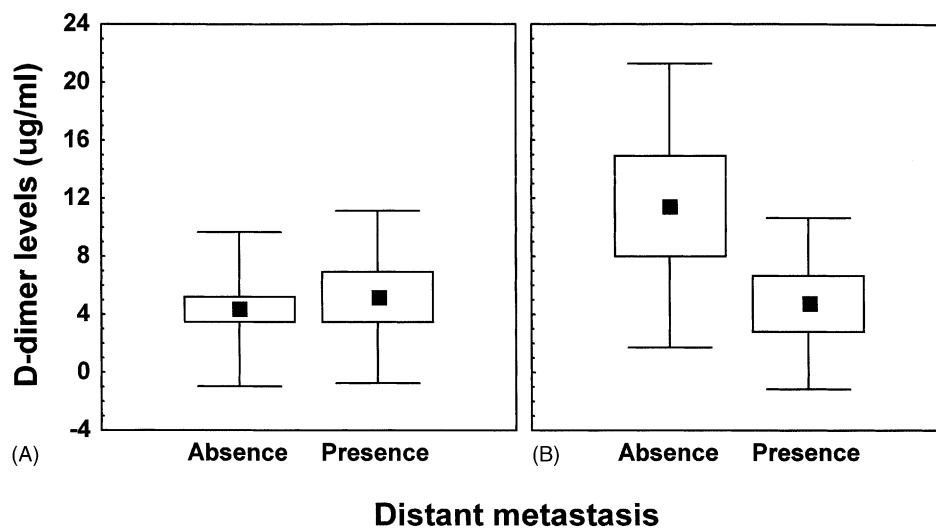


Fig. 2 Two-way Anova analysis of D-dimer levels in patients with or without distant metastasis of NSCLC and negative (panel A) or positive (panel B) TNF- α levels. Closed squares indicate mean values, bars indicate standard deviations, columns indicate standard errors of the mean.

Table 3 Summary of Spearman rank correlation coefficients among the variables analyzed in NSCLC, COPD patients and control subjects

	NSCLC patients									COPD patients		
	All patients (n = 65)			Stages I through III (n = 45)			Control subjects (n = 65)			(n = 65)		
	TNF- α	IL-6	TATc	TNF- α	IL-6	TATc	TNF- α	IL-6	TATc	TNF- α	IL-6	TATc
D-Dimer	0.345*	0.261 [‡]	0.027	0.522 [†]	0.136	0.035	0.122	0.161	0.246 [‡]	0.112	0.166	0.444 [†]
TATc	-0.046	-0.043		-0.085	-0.105		0.163	-0.033		0.233	0.166	
IL-6	0.354*			0.352 [‡]			0.325*			0.179		

* $P < 0.01$.† $P < 0.001$.‡ $P < 0.05$.

other hand, regression analysis of COPD patients including age, sex, smoking status, TNF- α , IL-6 and TATc levels showed that only TATc levels (regression coefficient = 0.52, $P < 0.001$) were independently related to the presence of elevated D-dimer levels.

4. Discussion

Several studies have demonstrated that pulmonary malignancy is frequently accompanied by alterations of fibrinolysis. The process of fibrinolysis involves an enzymatic cascade, which helps to break down cross-linked fibrin molecules. Fibrinolysis may be activated primarily—and thus independently of activation of the coagulation cascade—or secondarily in response to fibrin formation. However, the complex biochemical mechanisms of the activation of fibrinolysis in cancer patients are not completely understood.

In the last few decades a series of in vivo and in vitro studies has provided more insight into the pathogenetic mechanisms and the role of cytokines in the activation and regulation of the coagulation/fibrinolytic cascade, although the interactions are complicated, and the effects are time-dependent and transient [20]. To date, it is widely accepted that, on a cellular level, TNF- α may directly activate the fibrinolytic system. Injection of TNF- α into healthy volunteers, in fact, results in a transient activation of fibrinolysis [21,22]. Furthermore, treatment with anti-TNF- α in the human and chimpanzee sepsis models inhibits the fibrinolytic system [23,24], whereas coagulation is not affected, showing that TNF plays an important role in regulation of the fibrinolytic response. Although this scenario has been derived largely from studies with purified endotoxin or gram-negative bacteria, we hypothesized similar alterations to occur in the activation

of fibrinolytic systems in NSCLC, because the key intermediate cytokine TNF- α has been correlated to the disease status of lung cancer patients [14–16].

In the present study, plasma IL-6 and TNF- α levels were significantly elevated in NSCLC compared to either COPD patients or healthy controls. One limitation of the methodology used is that it does not allow distinguishing what is the main source of circulating cytokines in patients with NSCLC. These may be produced directly by the tumor, or may be the result of the host inflammatory response to cancer cells and/or their released products, although both phenomena can occur. Indeed, Li et al. recently showed by in-situ hybridization that, despite an immunosuppressive state, TNF- α mRNA levels were elevated in both tumor cells and TIL of NSCLC patients [13]. Whatever the source might be our finding that TNF- α was the only variable independently associated to D-dimer in patients with non-metastatic NSCLC suggests an involvement of this cytokine in the primary activation of fibrinolysis in this malignancy.

The finding of increased plasma levels of TATc (an activation marker of coagulation) and of D-dimer (a marker of fibrinolysis) in a substantial percentage of NSCLC patients is in agreement with the findings by other authors [2–4,6], although we could not confirm the correlation of D-dimer levels with tumor stage previously observed [6]. Furthermore, the lack of a significant correlation between TATc and D-dimer levels suggests that these two analytical variables represent different pathological mechanisms of activated coagulation and fibrinolysis in patients with NSCLC. Elevated TATc levels were also found in COPD, which is not surprising, because of the occurrence of a pro-thrombotic state in this clinical setting [25–28]. The percentage of patients with positive TATc levels did not significantly differ between COPD and NSCLC

patients, and median TATc levels of COPD patients were similar to those measured in plasma samples obtained from metastatic NSCLC patients. On the other hand, median D-dimer levels of COPD patients were significantly lower than those of NSCLC. Furthermore, TATc levels were independently related to D-dimer levels in COPD patients, suggesting that the activation of the fibrinolytic pathway observed in COPD might be secondary to thrombin generation and fibrin deposition.

The significant association found in NSCLC between TNF- α and D-dimer levels, but not with TATc, suggests that the host inflammatory response to cancer cells, and/or their released products, as well as tumor-derived cytokines, could be responsible for a primary activation of the fibrinolytic pathway, unrelated to the coagulation activation, at least in the early stages of disease. Indeed, the association between TNF- α and D-dimer levels was confined to non-metastatic patients, despite the occurrence of elevated levels of TNF- α in Stage IV NSCLC. However, we should consider that approximately 90% of metastatic patients also had elevated TATc levels, which is in agreement with the opinion currently accepted that the incidence, nature, and extent of coagulation abnormalities is related to the tumor burden [1]. Activation of the coagulation system and consequent thrombin generation could be due to increased production of tissue factor (TF) by lung cancer cells [29]. TF is the key factor in the activation of the extrinsic pathway and a regulator of tumor angiogenesis [30]. Furthermore, TF expression has been correlated with the clinical stage of other types of human cancer, with the highest percentages found in metastatic disease [31]. Thus, we can hypothesize that in metastatic lung cancer a secondary activation of fibrinolysis might also occur due to the increased thrombin generation and consequent fibrin production.

In conclusion, the present study confirms previously published observation that NSCLC causes a profound alteration in fibrinolysis and suggests that TNF- α could be, at least in part, responsible for the increased D-dimer levels found in this malignant disease. There have been no other reports of such an association between TNF- α and D-dimer levels in NSCLC, but our results suggest that the increased D-dimer levels might be accounted for a primary activation of fibrinolysis by TNF- α in the early stages of NSCLC. Additional studies in a larger number of patients are required to better define the role of tumor-derived cytokines in NSCLC. Better knowledge of the biologic effects of TNF- α in human cancer will help to improve our understanding of the pathophysiological significance of tumor-induced coagulopathies.

Acknowledgements

The authors wish to thank Simone Giommi, Alessia Cialfi and Alessandro Catalini for their excellent technical assistance. This work has been partially supported by Grant P.F. Ministero della Salute 1999.

References

- [1] Rickles FR, Levine MN, Dvorak HF, Abnormalities of hemostasis in malignancy. In: Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN, editors. Hemostasis and thrombosis. Basic principles and clinical practice, 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1131–52.
- [2] van Wersch JW, Tjwa MK. Coagulation/fibrinolysis balance and lung cancer. *Haemostasis* 1991;21:117–23.
- [3] Gabazza EC, Taguchi O, Yamakami T, Machishi M, Iбата H, Suzuki S. Evaluating prethrombotic state in lung cancer using molecular markers. *Chest* 1993;103:196–200.
- [4] Matsuyama W, Hashiguchi T, Mizoguchi A, Iwami F, Kawabata M, Arimura K, et al. Serum levels of vascular endothelial growth factor dependent on the stage progression of lung cancer. *Chest* 2000;118:948–51.
- [5] Roselli M, Mineo TC, Basili S, Mariotti S, Martini F, Bellotti A, et al. Vascular endothelial growth factor (VEGF-A) plasma levels in non-small cell lung cancer: relationship with coagulation and platelet activation markers. *Thromb Haemost* 2003;89:177–84.
- [6] Pavey SJ, Hawson GA, Marsh NA. Alterations to the fibrinolytic enzyme system in patients with non-small cell lung carcinoma. *Blood Coagul Fibrinolysis* 1999;10:261–7.
- [7] Seitz R, Rappe N, Kraus M, Immel A, Wolf M, Maasberg 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.
- [8] Taguchi O, Gabazza EC, Yasui H, Kobayashi T, Yoshida M, Kobayashi H. Prognostic significance of plasma D-dimer levels in patients with lung cancer. *Thorax* 1997;52:563–5.
- [9] Ferrigno D, Buccheri G, Ricca I. Prognostic significance of blood coagulation tests in lung cancer. *Eur Respir J* 2001;17:667–73.
- [10] 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.
- [11] Gabazza EC, Taguchi O, Yamakami T, Machishi M, Iбата H, Tsutsui K, et al. Coagulation-fibrinolysis system and markers of collagen metabolism in lung cancer. *Cancer* 1992;70:2631–6.
- [12] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–45.
- [13] Li R, Rüttinger D, Li R, Si LS, Wang YL. Analysis of the immunological microenvironment at the tumor site in patients with non-small cell lung cancer. *Langenbecks Arch Surg* 2003;388:406–12.
- [14] Ardizzoia A, Lissoni P, Brivio F, Tisi E, Perego MS, Grassi MG, et al. Tumor necrosis factor in solid tumors: increased blood levels in the metastatic disease. *J Biol Regul Homeost Agents* 1992;6:103–7.
- [15] Matanic D, Beg-Zec Z, Stojanovic D, Matakoric N, Flego V, Milevoj-Ribic F. Cytokines in patients with lung cancer. *Scand J Immunol* 2003;57:173–8.

- [16] Aleman MR, Santolaria F, Batista N, de La Vega M, Gonzalez-Reimers E, Milena A, et al. Leptin role in advanced lung cancer. A mediator of the acute phase response or a marker of the status of nutrition? *Cytokine* 2002;19:21–6.
- [17] Stouthard JM, Levi M, Hack CE, Veenhof CH, Romijn HA, Sauerwein HP, et al. Interleukin-6 stimulates coagulation, not fibrinolysis, in humans. *Thromb Haemost* 1996;76:738–42.
- [18] Zwaveling JH, Maring JK, Mulder AB, Bom VJ, van Ginkel RJ, Schraffordt Koops H, et al. Effects of hyperthermic isolated limb perfusion with recombinant tumor necrosis factor alpha and melphalan on the human fibrinolytic system. *Cancer Res* 1996;56:3948–53.
- [19] Suharti C, van Gorp EC, Setiati TE, Dolmans WM, Djoko-moeljanto RJ, Hack CE, et al. The role of cytokines in activation of coagulation and fibrinolysis in dengue shock syndrome. *Thromb Haemost* 2002;87:42–6.
- [20] ten Cate JW, van der Poll T, Levi M, ten Cate H, van Deventer SJH. Cytokines: triggers of clinical thrombotic disease. *Thromb Haemost* 1997;78:415–9.
- [21] Van Deventer SJH, Buller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxaemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. *Blood* 1990;76:2520–6.
- [22] van der Poll T, Levi M, Buller HR, van Deventer SJ, de Boer JP, Hack CE, et al. Fibrinolytic response to tumor necrosis factor in healthy subjects. *J Exp Med* 1991;174:729–32.
- [23] van der Poll T, Levi M, van Deventer SJH, ten Cate H, Haagmans BL, Biemond BJ, et al. Differential effects of anti-tumor necrosis factor monoclonal antibodies on systemic inflammatory responses in experimental endotoxemia in chimpanzees. *Blood* 1994;83:446–51.
- [24] van der Poll T, Coyle SM, Levi M, Jansen PM, Dentener M, Barbosa K, et al. Effect of recombinant dimeric tumor necrosis factor receptor on inflammatory responses to intravenous endotoxin in normal humans. *Blood* 1997;89:3727–34.
- [25] Nakstad B, Lyberg T, Skjonsberg OH, Boye NP. Local activation of the coagulation and fibrinolysis systems in lung disease. *Thromb Res* 1990;57:827–38.
- [26] Alessandri C, Basili S, Violi F, Ferroni P, Gazzaniga PP, Cordova C. C.O.B.H. Group. Hypercoagulability state in patients with chronic obstructive pulmonary disease. *Thromb Haemost* 1994;72:343–6.
- [27] Davi G, Basili S, Vieri M, Cipollone F, Santarone S, Alessandri C, et al. C.O.B.H. Group. Enhanced thromboxane biosynthesis in patients with chronic obstructive pulmonary disease. *Am J Resp Crit Care Med* 1997;156:1794–9.
- [28] Ferroni P, Basili S, Martini F, Vieri M, Labbadia G, Cordova C, et al. Soluble P-selectin as a marker of platelet hyperactivity in patients with chronic obstructive pulmonary disease. *J Invest Med* 2000;48:21–7.
- [29] Keller T, Salge U, Konig H, Dodt J, Heiden M, Seitz R. Tissue factor is the only activator of coagulation in cultured human lung cancer cells. *Lung Cancer* 2001;31:171–9.
- [30] Rickles FR, Shoji M, Abe K. The role of the hemostatic system in tumor growth, metastasis, and angiogenesis: tissue factor is a bifunctional molecule capable of inducing both fibrin deposition and angiogenesis in cancer. *Int J Hematol* 2001;73:145–50.
- [31] Nakasaki T, Wada H, Shigemori C, Miki C, Gabazza EC, Nobori T, et al. Expression of tissue factor and vascular endothelial growth factor is associated with angiogenesis in colorectal cancer. *Am J Hematol* 2002;69:247–54.

Available online at www.sciencedirect.com

