ANTICANCER RESEARCH *25*: 403-408 (2005)

Plasma von Willebrand Factor Antigen Levels in Non-small Cell Lung Cancer Patients

FRANCESCA MARTINI[1], PATRIZIA FERRONI[1], FIORELLA GUADAGNI[2], STEFANIA BASILI[3], ANTONELLA SPILA[2], ROBERTA D'ALESSANDRO[2], DAVIDE MINEO[5], ANASTASIA LAUDISI[4], ILARIA PORTARENA[4], SABRINA MARIOTTI[4], VINCENZO AMBROGI[5], TOMMASO C. MINEO[5] and MARIO ROSELLI[4]

[1]*Department of Experimental Medicine and Pathology, University La Sapienza, Viale Regina Elena 324, 00161, Rome;*
[2]*Laboratory of Clinical Pathology, Regina Elena Cancer Institute, Via E. Chianesi 53, 00144, Rome;*
[3]*Department of Medical Therapy, University La Sapienza, Viale del Policlinico, 00185, Rome;*
[4]*Medical Oncology and* [5]*Thoracic Surgery, Department of Surgery, University "Tor Vergata", Policlinico Tor Vergata, Viale Oxford 81, 00133, Rome, Italy*

Abstract. *Background: To analyze the behavior of circulating von Willebrand factor antigen (vWf:Ag) in patients with non-small cell lung cancer (NSCLC). Patients and Methods: Pre-surgical vWf:Ag levels were measured in 64 patients with histologically diagnosed NSCLC compared to 64 patients with benign pulmonary diseases, as well as 64 age- and sex-matched controls. Results: Patients with NSCLC had mean vWf:Ag concentrations lower than either controls or benign patients ($p=0.001$). CEA was the only variable predictive of low vWf:Ag levels ($p<0.01$). Five of the 64 NSCLC patients had abnormally low vWf:Ag concentrations (<36 IU/dL). When these patients were excluded from the analysis, the vWf:Ag levels of NSCLC patients did not differ from those of controls ($p=0.19$). Conclusion: The vWf antigen levels of NSCLC patients are not substantially altered. A small subset of these patients will have a depletion of circulating vWf:Ag, probably because of a paraneoplastic process associated with an advanced stage of disease.*

von Willebrand factor (vWf) is a multimeric plasma glycoprotein synthesized in megakaryocytes/platelets and in endothelial cells, with the latter being the major source of plasma vWf (1). In endothelial cells, newly synthesized vWf is either secreted constitutively or stored in Weibel-Palade bodies that release their contents on endothelial cell activation (1). vWf plays an essential role in haemostasis, mediating adhesion of platelets to sub-endothelial surfaces at sites of vascular injury and acting as a carrier protein for factor VIII (1). Changes to physiological systems such as haemostasis and vascular function are commonly found in human cancer, and, as a consequence, often confer an increased risk of arterial and venous thrombosis (1). Evidence of these changes include abnormalities in the levels of plasma molecules involved in coagulation and fibrinolysis (2) and markers of endothelial cell integrity (3).

The plasma vWf level is currently regarded as a marker of endothelial cell activation. Furthermore, recent data showed that vWf mRNA and protein levels are controlled by angiogenic factors, thus suggesting its potential utility as a marker of angiogenesis (4). However, the use of this marker has been questioned because of its release by all endothelial cells, making vWf a pan-endothelial marker that would not accurately report angiogenesis (5).

Nevertheless, increased plasma levels of vWf antigen (vWf:Ag) have been found in solid tumors such as ovarian, colorectal and breast cancer (6-10) and it has been suggested that the levels of vWf:Ag in plasma of cancer patients increase with clinical staging and may be of prognostic significance (7, 10), although other authors did not confirm such an association (9, 11). Presently, no data are available on the variability of plasma vWf:Ag levels in patients with non-small cell lung cancer (NSCLC). NSCLC is characterized by a profound activation of the haemostatic system (3) and by the occurrence of elevated soluble (s) E-selectin (another marker of endothelial cell dysfunction) levels (2). The present study was aimed at analyzing plasma vWf:Ag levels in patients with NSCLC compared to patients with benign pulmonary diseases, as well as healthy subjects. The association with clinicopathological variables and sE-selectin levels was also analyzed.

Correspondence to: Mario Roselli, M.D., Ph.D., Medical Oncology, Dept. of Surgery, University of Rome "Tor Vergata", Policlinico Tor Vergata, Viale Oxford 81, 00133, Rome, Italy. Tel: +39 (06) 20908190, Fax: +39 (06) 20902894, e-mail: mario.roselli@ptvonline.it

Key Words: Lung cancer, von Willebrand antigen, carcinoembryonic antigen.

Patients and Methods

Sixty-four patients with NSCLC, treated at our Institutions, entered the study. Patients [52 males, 12 females; mean age 65±8, ranging from 48 to 82 years] were histologically diagnosed with lung adenocarcinoma (n=32) or squamous cancer (n=32). NSCLC was pathologically staged according to the tumor-nodes-metastasis classification (UICC - Union International Contre le Cancer TNM classification of malignant tumors). Twenty-two (34.4%) patients were classified as stage I, 7 (10.9%) as stage II, 16 (25.0%) as stage III and 19 (29.7%) as stage IV lung cancer. Sixty-four patients with benign pulmonary diseases [52 males, 12 females; mean age 66±10, ranging from 34 to 85 years] and 64 healthy subjects [52 males, 12 females; mean age 64±9, ranging from 47 to 82 years] 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, Italy. Selection of controls was performed by an independent investigator, blind to laboratory data, in order to achieve a population matched for age and sex to the patient groups. Patients with benign lung diseases were diagnosed with hamartomas (n=5), chronic obstructive pulmonary disease (COPD) (n=54) and other miscellaneous diseases (1 cyst, 1 fibrosing alveolitis, 2 chronic pneumonia and 1 indurative pleurisy). Clinical conditions were stable in all COPD patients in the two months before the examination, and no signs of malnutrition or muscle wasting were present.

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 hormone replacement therapy, nonsteroidal anti-inflammatory drugs, anticoagulant or antiplatelet agents in the two 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.

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 patients with benign lung disease were drawn at the time of clinical and/or instrumental diagnosis and prior to any treatment. After a rest period of at least 20 minutes, 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). The samples were immediately centrifuged at 1,500g for 10 minutes to obtain plasma, aliquoted, coded and stored at - 40°C until the assays were performed. Plasma vWf:Ag (Imubind vWF ELISA, American Diagnostica) and sE-selectin (R&D Systems, Minneapolis, MN, USA) levels were measured by enzyme-immunoassays according to the manufacturers' instructions. The minimum detectable doses were 0.6 IU/dL and 0.1 ng/ml for vWf:Ag and sE-selectin, respectively. Tumor marker assessment was performed by carcinoembryonic antigen (CEA) determination using the conventional cut-off of 5 ng/ml. Measurements were done blinded.

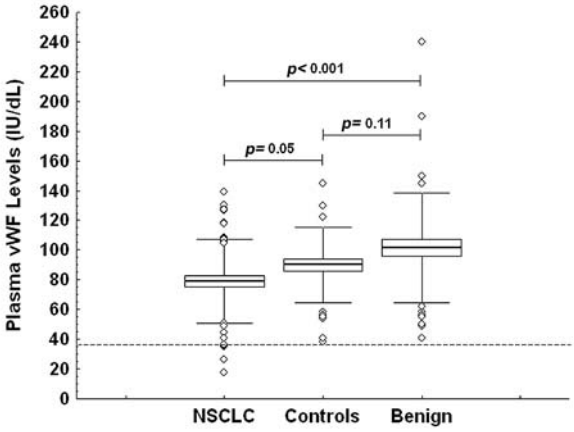

Figure 1. *Box-plot analysis of plasma vWf:Ag levels in 64 patients with non-small cell lung cancer (NSCLC), 64 patients with benign pulmonary diseases and 64 healthy subjects (HS). Solid lines indicate mean values; open diamonds indicate extreme values; bars indicate standard deviations; columns indicate standard errors of the mean. Dotted line represents the 10th percentile of vWf:Ag in the overall population.*

All samples were assayed in duplicate and those showing values above the standard curve were re-tested with appropriate dilutions.

Statistical analysis. Statistical analysis was performed by 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's correlation coefficient, Kruskall-Wallis method and/or Mann-Whitney *U*-test). The relationship among variables was assessed by multiple regression analyses. Data are presented as mean value±standard deviation (SD), or median and interquartile range (IRQ; 25th percentile to 75th percentile). Only *p* values lower than 0.05 were regarded as statistically significant. All calculations were made using a computer software package (Statistica 5.5, StatSoft Inc., Tulsa, OK, USA).

Results

Pre-surgical plasma vWf:Ag and sE-selectin levels of 192 subjects, including 64 control subjects, 64 patients with benign lung diseases and 64 patients with histologically diagnosed lung cancer, were analyzed. As previously reported, plasma sE-selectin levels were significantly higher in patients with NSCLC [median (IQR)= 41 (30 – 57) ng/ml] compared with either benign patients [36 (21 – 46) ng/ml] or control subjects [36 (26 – 46) ng/ml](analysis of variance by Anova test: F=3.5, p=0.03). Conversely, patients with NSCLC had mean vWf:Ag concentrations (Mean±SD: 79±28 IU/dL) significantly lower than either healthy subjects (90±25 IU/dL) or benign patients [102±37 IU/dL (F=7.2, p=0.001] (Figure 1). The plasma VWF:Ag and sE-selectin levels of healthy controls and patients with benign diseases did not show any difference.

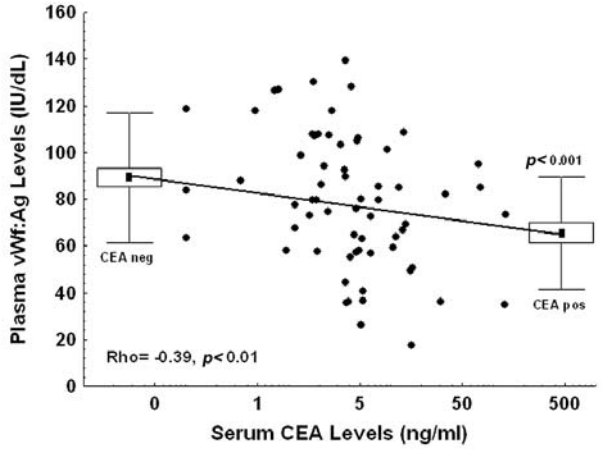

Figure 2. *Box-plot analysis of plasma vWf:Ag levels in patients with non-small cell lung cancer (NSCLC) and positive (≥5 ng/ml) or negative (<5 ng/ml) CEA levels. Closed squares indicate mean values; open circles indicate outlier values; bars indicate standard deviations; columns indicate standard errors of the mean. Correlation analysis of plasma vWf:Ag and CEA levels is displayed in the inset.*

No association was found between either sE-selectin or vWf:Ag concentrations and the stage of disease, grading or histological diagnosis. Significantly lower vWf:Ag levels were found in patients with positive CEA levels (65±24 IU/dL) compared to patients with negative levels (88±28 IU/dL, $p<0.001$). Plasma vWf:Ag levels, in fact, inversely correlated with CEA levels (Rho=-0.39, $p<0.01$) (Figure 2), but not with sE-selectin (Rho=0.001, $p<0.99$). To further analyze the association between the vWf:Ag levels and clinicopathological variables, a multiple regression analysis including age, sex, histological diagnosis, grading, tumor size, lymph node involvement, distant metastasis and CEA and sE-selectin levels was carried out. Multivariate analysis revealed that CEA was the only variable independently related to low levels of vWf:Ag (Beta coefficient= -0.34, $p< 0.01$) (Table I).

Finally, the plasma vWf:Ag levels were categorized according to a cut-off value of 36 IU/dL (*i.e.*, the 10th percentile of concentrations measured in the overall population). No patient with benign lung disease or healthy control had vWf:Ag levels below this cut-off. Conversely, 5 NSCLC patients had vWf:Ag concentrations below 36 IU/dL (Table II). When these patients were excluded from the analysis, the vWf:Ag levels of NSCLC patients (83±25 IU/dL) did not differ from those of healthy controls ($p=0.19$), but were still significantly lower than the vWf:Ag levels measured in patients with benign disease ($p<0.005$). Furthermore, the predictive value of CEA for low vWf:Ag levels was lost at multivariate analysis.

Discussion

In the present study, we report that patients with NSCLC may have vWf:Ag levels lower than those observed in healthy subjects. However, this difference was unrepresentative, and mostly due to the occurrence of abnormally low vWf:Ag levels in 5 patients with advanced disease. Their exclusion from the analysis, in fact, showed that the vWf:Ag levels of the NSCLC patients did not substantially differ from those of healthy controls. These results are in agreement with a recent study by Mills *et al.* (11) who showed that vWf antigen levels did not differ between women with non metastatic breast cancer and healthy controls. At present, no data are available on the behavior of vWf:Ag in NSCLC. Nevertheless, the finding of a similar distribution of vWf between cancer patients and control subjects is in conflict with the results obtained by other authors, who found increased plasma levels of vWf:Ag in solid tumors (6-10). In this respect, we must consider that, in the present study, the control population consisted of healthy subjects matched for age and sex. This choice was dictated by the need to avoid any bias due to confounding variables since patients with advanced age may have major risk factors for atherothrombotic disorders, which are often accompanied by increased levels of markers of endothelial dysfunction (*i.e.*, vWf:Ag and sE-selectin). An older age of controls or higher prevalence of metastatic disease might, thus, explain the discrepancies observed between our and other studies.

However, one limitation of the study should be emphasized, which was that no record of blood group distribution was available at the time of data analysis. This might be of importance since previous studies demonstrated that vWf:Ag levels vary with blood group, group 0 having lower levels than non-0 groups, particularly group A (1, 12). Hence, the present data do not allow us to exclude that an imbalance of blood group distribution between the healthy controls and patients with NSCLC might be responsible for the lack of differences between the two groups.

Nonetheless, the finding of abnormally low vWf:Ag levels in 5 of the 65 NSCLC patients is of particular interest. These patients had vWf:Ag levels lower than 36 IU/dL, which is below the mean minus 2SD (38 IU/dL) given for blood group 0 individuals in a recently published study (13). A depletion of vWf:Ag has occasionally been reported in patients with various underlying diseases, including lymphoproliferative disorders and solid tumors (reviewed in 14). This may be due to an absorption of vWf onto tumor cells, which is hypothesized to occur by an aberrant expression of glycoprotein Ib (GpIb), a major platelet vWf receptor whose expression was originally thought to be restricted to cells of the megakaryocytic lineage. Several authors have demonstrated that this is not the case, and have

Table I. *Multiple regression analysis.*

	Univariate Analysis			Multivariate Analysis		
Predictor variable	β-coefficient (SE)	95%CL	*P* value	β-coefficient (SE)	95%CL	*P* value
Sex	-0.075 (0.131)	-0.34 – 0.19	0.570			
Male *vs.* female						
Age	-0.024 (0.133)	-0.29 – 0.24	0.859			
Diagnosis Adenocarcinoma *vs.* squamous	-0.113 (0.140)	-0.39 – 0.17	0.420			
Grading	0.064 (0.136)	-0.21 – 0.34	0.639			
Tumor size T1/2 *vs.* T3/4	-0.010 (0.160)	-0.33 – 0.31	0.948			
Nodal status N0 *vs.* N+	-0.137 (0.171)	-0.48 – 0.21	0.426			
Distant metastasis M0 *vs.* M1	-0.045 (0.186)	-0.42 – 0.33	0.811			
CEA levels	**-0.310 (0.137)**	**-0.59 – -0.03**	**0.028**	**-0.34 (0.120)**	**-0.58 – -0.10**	**0.007**
sE-selectin levels	-0.043 (0.135)	-0.31 – 0.23	0.752			

SE: Standard Error; CL: Confidence Limit

Table II. *Clinic and laboratory variables of the 5 patients with abnormally low levels of vWf:Ag.*

Pt No.	Sex	Age	Diagnosis	Stage	TNM	vWf:Ag IU/dL	CEA ng/ml	sE-selectin ng/ml
1	Female	58	Adenocarcinoma	IIIA	T3N1M0	34.9	129.2	116.2
2	Male	68	Adenocarcinoma	IV	T3N2M1	35.9	30.5	38.5
3	Male	66	Squamous	IV	T3N2M1	17.4	16.0	42.4
4	Female	55	Squamous	IV	T3N2M1	26.1	5.1	34.0
5	Male	74	Squamous	IIIB	T3N2M0	35.7	3.6	73.7

shown that a functional GPIb-like complex resides on the surface of human carcinoma cells, and can mediate morphological and behavioral changes in response to vWf (15). In particular, a tumor cell GPIb-like complex could facilitate metastasis by allowing circulating cancer cells to bind plasma vWf and enhance their interaction with platelets and/or subendothelial matrix (15). Another possibility is that depletion of vWf may be due to the production of biologically active substances either by the tumor, or in response to the tumor (*e.g.*, hyaluronic acid, inhibitory auto-antibodies or paraproteins)(14), a phenomenon usually belonging to paraneoplastic syndromes. In the absence of conclusive data, any of the above-mentioned mechanisms must be taken into consideration to explain the abnormally low levels of vWf:Ag occasionally found in advanced stages of NSCLC.

One final issue that deserves attention is the association found between low vWf:Ag and elevated CEA levels. The gene for CEA is expressed on the majority of NSCLC (16). Levels of serum CEA have been shown to generally correlate with tumor mass. Moreover, circulating tumor cells can be detected in the peripheral blood of patients with resectable NSCLC by reverse transcriptase-polymerase chain reaction (RT-PCR) of CEA mRNA, and

their incidence correlated highly with the pathological stage of disease (17). Thus, the predictive value of CEA on vWf:Ag levels might be explained by the observation that elevated CEA levels can be indicative of advanced tumor stage and possibly of the occurrence of circulating tumor cells, causing a depletion of vWf through the above-mentioned mechanisms.

We may, therefore, conclude that, contrary to the findings obtained in other human cancers, the vWf antigen levels of NSCLC patients are not substantially altered. A small subset of these patients will have a depletion of circulating vWf:Ag, probably because of a paraneoplastic process associated with an advanced stage of disease. Further studies are needed to better understand the mechanism(s) responsible for the depletion of vWf:Ag levels in NSCLC.

Acknowledgements

This study was performed under the Research Fellowship of *Tecnologie e Terapie Avanzate in Chirurgia* awarded by Tor Vergata University of Rome, Italy.

References

1 Hemostasis and Thrombosis. Basic Principles and Clinical Practice (Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN, eds). Philadelphia, Lippincott Williams & Wilkins, 2001.

2 Roselli M, Mineo TC, Martini F, Mariotti S, Ambrogi V, Spila A, D'Alessandro R, Basili S, Guadagni F and Ferroni P: Serum concentrations of soluble selectins in patients with lung cancer. Int J Biol Markers *17*: 56-62, 2002.

3 Roselli M, Mineo TC, Basili S, Mariotti S, Martini F, Bellotti AM, Ambrogi V, Spila A, D'Alessandro R, Gazzaniga PP, Guadagni F and Ferroni P: Vascular endothelial growth factor (VEGF-A) plasma levels in non-small cell lung cancer: relationship with coagulation and platelet activation markers. Thromb Haemost *89*: 177-184, 2003.

4 Zanetta L, Marcus SG, Vasile J, Dobryansky M, Cohen H, Eng K, Shamamian P and Mignatti P: Expression of von Willebrand factor, an endothelial cell marker, is up-regulated by angiogenesis factors: a potential method for objective assessment of tumor angiogenesis. Int J Cancer *85*: 281-288, 2000.

5 Byrne GJ, Ghellal A, Iddon J, Blann AD, Venizelos V, Kumar S, Howell A and Bundred NJ: Serum soluble vascular cell adhesion molecule-1: role as a surrogate marker of angiogenesis. J Natl Cancer Inst *92*: 1329-1336, 2000.

6 Gadducci A, Baicchi U, Marrai R, Del Bravo B, Fosella PV and Facchini V: Pretreatment plasma levels of fibrinopeptide-A (FPA), D-dimer (DD), and von Willebrand factor (vWF) in patients with ovarian carcinoma. Gynecol Oncol *53*: 352-356, 1994.

7 Gadducci A, Baicchi U, Marrai R, Facchini V, Del Bravo B, Foselle P and Fioretti P: Pretreatment plasma level of fibrinopeptide-A (FPA), D-dimer (DD), and von Willebrand factor (vWF) in patients with operable cervical cancer: influence of surgical-pathological stage, tumor size, histologic type, and lymph node status. Gynecol Oncol *49*: 354-358, 1993.

8 Röhsig LM, Damin DC, Stefani SD, Castro Jr. CG, Roisenberg I and Schwartsmann G: von Willebrand factor antigen levels in plasma of patients with malignant breast disease. Br J Med Biol Res *34*: 1125-1129, 2001.

9 Blann AD, Gurney D, Wadley M, Bareford D, Stonelake P and Lip GY: Increased soluble P-selectin in patients with haematological and breast cancer: a comparison with fibrinogen, plasminogen activator inhibitor and von Willebrand factor. Blood Coagul Fibrinolysis *12*: 43-50, 2001.

10 Damin DC, Rosito MA, Gus P, Roisemberg I, Bandinelli E and Schwartsmann G: Von Willebrand factor in colorectal cancer. Int J Colorectal Dis *17*: 42-45, 2002.

11 Mills PJ, Parker B, Jones V, Adler KA, Perez CJ, Johnson S, Cohen-Zion M, Marler M, Sadler GR, Dimsdale JE and Ancoli-Israel S: The effects of standard anthracycline-based chemotherapy on soluble icam-1 and vascular endothelial growth factor levels in breast cancer. Clin Cancer Res *10*: 4998-5003, 2004.

12 O'Donnell J, Boulton FE, Manning RA and Laffan MA: Amount of H antigen expressed on circulating von Willebrand factor is modified by ABO blood group genotype and is a major determinant of plasma von Willebrand factor antigen levels. Arterioscler Thromb Vasc Biol *22*: 335-341, 2002.

13 Sukhu K, Poovalingam V, Mahomed R and Giangrande PL: Ethnic variation in von Willebrand factor levels can influence the diagnosis of von Willebrand disease. Clin Lab Haematol *25*: 247-249, 2003.

14 Michiels JJ, Budde U, van der Planken M, van Vliet HH, Schroyens W and Berneman Z: Acquired von Willebrand syndromes: clinical features, aetiology, pathophysiology, classification and management. Best Pract Res Clin Haematol *14*: 401-436, 2001.

15 Suter CM, Hogg PJ, Price JT, Chong BH and Ward RL: Identification and characterisation of a platelet GPIb/V/IX-like complex on human breast cancers: implications for the metastatic process. Jpn J Cancer Res *92*: 1082-1092, 2001.

16 Goldenberg DM, Sharkey RM and Primus FJ: Carcinoembryonic antigen in histopathology: immunoperoxidase staining of conventional tissue sections. J Natl Cancer Inst *57*: 11-22, 1976.

17 Kurusu Y, Yamashita J and Ogawa M: Detection of circulating tumor cells by reverse transcriptase-polymerase chain reaction in patients with resectable non-small-cell lung cancer. Surgery *126*: 820-826, 1999.

Received November 25, 2004
Accepted December 14, 2004