Differences in Plasma MMPs and TIMPs Protein Expression and Chemotherapy Response in Patients with Tobacco- or Wood-Smoke-Induced Lung Cancer

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Key Words

Gelatinase activity • Lung cancer • Matrix metalloproteinases • Never-smokers • Tissue inhibitors of matrix metalloproteinases • Wood smoke

Abstract

Background: One of the risk factors associated with lung cancer in never-smoker patients is wood smoke exposure (WS). However, information about its clinical and molecular characteristics remains scant. **Objective:** This was to analyze – in plasma from patients with tobacco- or wood-smoke-induced lung cancer – whether the enzymatic activity and concentration of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) differ, and to determine whether there was a correlation between these indicators of the metastatic potential and the first-line chemotherapy response. **Methods:** Patients were classified according to lung cancer associated with: the smoking of tobacco (T), WS and where no association with a known risk factor (N) could be established. The gelatinase activity of plasma MMP was analyzed by radiolabeled substrate degradation and zymography assay. Protein expression of MMPs and TIMPs was evaluated by Western blot densitometry analysis. **Results:** The 26.9% WS patients had a better response to therapy in comparison with the T group (OR = 4.9, 95% CI =

1.25–20.15; $p = 0.019$). The lowest gelatinase activity was observed in WS subjects, in comparison with T and N subjects (96.7 ± 15.9 , 182.9 ± 31.5 and 163.3 ± 22.7 μg of degraded gelatin/mg of incubated plasma protein, respectively; $p < 0.025$); this enzymatic activity corresponded to MMP-2. The highest MMP-2, MMP-9, MT1-MMP and TIMP-1 plasma levels were observed in T subjects. **Conclusion:** Tobacco and wood smoke have different effects on MMP and TIMP synthesis and gelatinase activity, directly influencing lung cancer metastatic potential and chemotherapy response.

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Introduction

Tobacco has been considered the main risk factor in lung cancer pathogenesis. However, statistics show that 15% of men and 53% of women with lung cancer are never-smokers [1]. In recent years, epidemiological studies have identified several etiological factors that could be involved in lung cancer onset in never-smokers, such as exposure to radon, coal smoke, wood smoke or asbestos, as well as genetic, hormonal and viral factors [2, 3].

In developing countries, wood, dung and charcoal (defined as 'biomass fuel') are burned and used as low-cost fuel sources for cooking, heating and lighting [4]. The use

of wood as fuel, particularly in rural areas, could be a potential risk factor for lung cancer, but the evidence confirming that wood smoke induces lung cancer is still not enough [5, 6]. Nevertheless, the International Agency for Research on Cancer concluded that biomass smoke (mainly wood) is 'probably carcinogenic to humans (Group 2A)' on the basis of the studies that demonstrated molecular and cytogenetic effects in humans and in experimental animals [7]. These effects could be due to wood smoke composition that includes carcinogenic molecules such as formaldehyde, benzene, benzo(a)anthracene, benzo(a)fluoranthene, benzo(a)pyrene, chromium, nickel and chlorinated dioxins; all of them have been implicated in lung cancer pathogenesis [8].

In general terms, lung cancer in never-smokers has a different profile from that of tobacco smokers. It affects women more frequently, it shows a major incidence at a younger age, adenocarcinoma is the predominant reported histologic subtype and never-smoker patients have a better response to targeted therapy [9]. Although knowledge about lung cancer in never-smokers has substantially increased, studies evaluating the metastatic potential of this cancer are not yet available. Definition of lung cancer aggressiveness might help in treatment onset, as it is known that lung cancer in never-smokers is diagnosed at a more advanced stage compared to tobacco-smoking patients [9, 10].

In this context, several components of the extracellular matrix (ECM) are modified during the metastatic process by the matrix metalloproteinases (MMPs), a family of calcium-zinc-dependent endopeptidases [11–13]. These enzymes are synthesized as zymogens and require cleavage of the amino-terminal domain for their activation. Based on their substrate specificity, MMPs are grouped into collagenases, gelatinases, stromelysins and membrane-type MMPs (MT-MMPs). Two of them, MMP-2 and MMP-9 (gelatinases), have often been associated with the invasive potential of neoplastic cells and the aggressiveness of cancer. MMP enzymatic activity is regulated by a group of 4 specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) that bind to the active forms of these enzymes, forming tight 1:1 stoichiometric complexes [14].

On the other hand, never-smoker patients with lung cancer at advanced stages are treated as tobacco smokers with standard first-line chemotherapy (cisplatin and a third-generation drug) or second-line treatment (docetaxel and pemetrexed) [15, 16]. In some health centers, never-smokers' second-line treatment consists of the use of targeted therapy (erlotinib and gefitinib) and good re-

sponses have been observed [17]. However, targeted therapy in developing countries is too expensive; therefore, conventional chemotherapy is used more often, even though information concerning response to platin-based therapy in never-smoker lung cancer is scarce [18, 19].

Since lung cancer pathogenesis, tumor biology and clinical features are different among smoker and never-smoker patients, it is conceivable to postulate that differences in lung cancer metastatic potential and in the response to cisplatin first-line chemotherapy might also exist. Therefore, the aim of this study was to analyze whether the gelatinase activity of plasma MMP-2 and MMP-9 and the plasma concentrations of MMP-2, MMP-9, MT1-MMP, TIMP-1 and TIMP-2 in tobacco- and wood-smoke-induced lung cancer patients differ, and to determine whether these indicators of the metastatic potential correlate with the cisplatin first-line chemotherapy response.

Materials and Methods

Study Population

Seventy-eight patients with primary lung adenocarcinoma were studied. They received follow-up for the duration of their first-line chemotherapy treatment. Only these 78 patients fulfilled the criteria for the variants we wanted to examine in this work, they were staged prior to chemotherapy according to Mountain [20].

According to risk factors, subjects were divided into the following groups: (1) lung cancer associated with wood smoke exposure (WS) consisting of patients who were never-smokers but had been exposed to domestic wood smoke for a mean duration of 35 ± 22.5 years (232.35 ± 41.3 h/year; range 30–816 h/year), (2) lung cancer associated with tobacco smoking (T) including subjects who were current smokers >10 years (30.6 ± 13.9 years) with a mean smoking history of 24.82 ± 5.2 pack years (range 2.5–98 pack years) and (3) a negative group (N) that consisted of those patients with no history of tobacco, wood smoke, solvent, radon or asbestos exposure, nor a family history of lung cancer (table 1).

Each patient received 6 cycles of cisplatin alone ($n = 4$; 70 mg/m² on day 1) or in combination with paclitaxel ($n = 31$; 200 mg/m² on day 1), vinorelbine ($n = 22$; 30 mg/m² on days 1 and 7), gemcitabine ($n = 17$; 1,250 mg/m² on days 1 and 8) or docetaxel ($n = 4$; 75 mg/m² on day 1). There was a 3-week interval between each cycle. Chemotherapy was decided according to the economic status of each patient, taking into consideration that the standard treatment consists of cisplatin or carboplatin plus a third-generation drug [15]. The response to chemotherapy was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines: CR = complete response, PR = partial response, PD = progression of disease, ORR = overall response rate, i.e. CR + PR [21].

Thirty-one healthy never-smoker volunteers without WS, with normal spirometry values, no signs of infective respiratory disease and no history of atopy, allergy or asthma were actively recruited as controls.

Table 1. Clinical characteristics of lung adenocarcinoma patients

Character-istics	All patients	WS	T	N
Subjects	78	27	24	27
Gender				
Women	51	24/51	10/51	17/51
Age	61.2 ± 11.4	63.7 ± 11.8	60.3 ± 12.6	57.7 ± 9.5
Men	34.6 (27/78)	11.1 (3/27)	51.9 (14/27)	37 (10/27)
Age	58.9 ± 11.4	57 ± 22.6	59.7 ± 11.1	58.2 ± 10.9
Stage (TNM)				
IIIA	5/78	2/5	2/5	1/5
IIIB	16/78	6/16	5/16	5/16
IV	57/78	19/57	17/57	21/57

Data are presented as number of studied patients/total patients. Age is expressed in years as mean ± SD.

Informed consent was obtained from each patient and the protocol was approved by the local Ethic and Research Committees.

Venous blood samples from healthy subjects and from lung cancer patients obtained prior to therapy were collected in 5-ml lithium heparin-coated tubes. Plasma protein amounts were measured by the bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, Ill., USA) [22].

Gelatinase Activity Assay

Twenty micrograms of ³H-labeled heat-denatured type I collagen (gelatin) were incubated with 20 µg of plasma protein from each subject and TNC buffer (50 mM Tris-HCl, 0.15 mM NaCl, 20 mM CaCl₂ and 0.02% sodium azide; pH 7.4), with or without EDTA at 37°C for 24 h [23]. Labeled gelatin plus collagenase clostridium and gelatin with TNC buffer were used as positive and negative controls. Gelatinase activity was limited to EDTA-inhibitable ³H-gelatin degradation. Results were expressed as micrograms of degraded gelatin per milligram of incubated plasma protein.

Zymography Analysis

Substrate gel electrophoresis was carried out in 8% SDS-PAGE gels containing 0.1% pig skin gelatin. Twenty micrograms of plasma protein from each sample were applied per lane under non-denaturing conditions. After electrophoresis, the gels were washed in 2.5% Triton X-100 for 30 min and then incubated with TNC buffer at 37°C for 24 h. Each gel was stained in 0.05% Coomassie blue G-250 and destained in 10% methanol-10% acetic acid. The gelatinase activity was visualized as transparent bands against a blue background on the stained SDS-PAGE gelatin gels. Bands detected in the zymography assay were analyzed by densitometry, using Kodak Digital Science ID Image Analysis software. Results were expressed as densitometry units (DU).

Western Blot Assay

Western blotting was performed using 20 µg of plasma protein from each subject per lane in 8% SDS-PAGE gels under reducing conditions with 5% 2-mercaptoethanol boiled for 10 min. After

electrophoresis, proteins were transferred to PVDF membranes and blocked with 2.5% nonfat dry milk in 100 mM Tris-HCl buffer, pH 7.5 with 150 mM NaCl and 0.1% Tween 20 (TTBS buffer) and incubated for 90 min with the following monoclonal antibodies: 2 µg/ml anti-MMP-2, 4 µg/ml anti-MMP-9, 10 µg/ml anti-MT1-MMP, 2 µg/ml anti-TIMP-1 and 2 µg/ml anti-TIMP-2 (Calbiochem-Novabiochem International, San Diego, Calif., USA). Beta-tubulin (1:200) was used as loading control (Calbiochem-Novabiochem International). Unbound antibodies were removed by washing with TTBS buffer. Primary antibodies were detected with the VectaStain ABC kit (Vector Laboratories, Burlingame, Calif., USA). Bands identified in the immunoblotting assay were analyzed by densitometry and results were expressed as DU.

Statistical Analysis

The χ^2 test that combines the 2 × 2 contingency tables (EPI-INFO statistical program, version 6.04b) was used to determine the association between the response to therapy and the risk factor. The χ^2 were calculated using the Yates correction. Gelatinase activity and Western blot results were analyzed using the Mann-Whitney U test and expressed as mean ± standard error. For all results, $p \leq 0.05$ was considered significant.

Results

Lung Adenocarcinoma Incidence

Most of the patients examined were never-smokers (69.2%, 54 of 78 subjects) and 50% of them had been exposed to wood smoke (table 1). The highest WS was observed in women, while T was identified as the major risk factor in men. In the N group (27 patients), 63% were women and 37% were men. Clinical staging demonstrated that most of the patients had stage IV of the disease (73.1%).

Chemotherapy Response

The ORR for all lung cancer patients was 59% (46 of 78 subjects) (table 2). According to the risk factor, there were significant differences in chemotherapy response between the WS and the T groups (ORR = 26.9%, i.e. 21 of 78 and 12.8%, i.e. 10 of 78 patients, respectively; OR = 4.9, 95% CI = 1.25–20.15; $p = 0.019$; Yates-corrected) (table 2). The number of subjects with an ORR to treatment in the N group was close to the number of patients with a progression of the disease. The differences between them and the WS and T groups were therefore not significant, nor were those between the chemotherapy regimens and disease stages of the 3 groups.

Plasma Gelatinase Activity

Gelatinase activity was increased in all lung cancer patients compared to that in the control subjects (36.8 ± 4.8

Table 2. Chemotherapy response according to stage and risk factor

Response	CR	PR	ORR	PD
All Patients	25.6	33.3	59	41
Risk factor				
WS	16.7	10.3	26.9	7.7
T	2.6	10.3	12.8	17.9
N	6.4	12.8	19.2	15.4
Stage (TNM)				
IIIA	3.8	0	3.8	2.7
IIIB	10.3	6.4	16.7	3.8
IV	11.5	26.9	38.5	34.6

Data are expressed as % (100% = 78 patients). Response to chemotherapy was evaluated according to RECIST: CR = complete response; ORR = overall response rate (i.e. CR + PR); PD = progression of disease; PR = partial response.

Fig. 2. Gelatin substrate zymography. **a** MMP-2 gelatinase activity (65-kDa band) was detected in all cancer samples, with an increase in its intensity in comparison with control subjects. MMP-9 gelatinase activity (86-kDa band) was also identified in most of the lung cancer subjects. Molecular weight markers are listed on

μg of degraded gelatin/mg of incubated plasma protein; $p < 0.001$) (fig. 1). Regarding the cancer groups, the lowest activity was observed in WS subjects, in comparison with the T and N groups (96.7 ± 15.9 , 182.9 ± 31.5 and $163.3 \pm 22.7 \mu\text{g}$ of degraded gelatin/mg of incubated plasma protein, respectively; $p < 0.025$).

Likewise, most of the patients with an ORR had low rates of gelatinase activity (38 of 46 patients) compared with subjects with PD (27 of 32 subjects) (60.6 ± 4.5 and

Fig. 1. Gelatinase activity. MMPs gelatinase activity was increased in all lung cancer patients in comparison with control subjects ($p < 0.001$). Among risk groups, WS patients had the lowest gelatinase activity ($p < 0.025$). Bars indicate the mean of each group $\pm$ standard error.

the left. **b** The zymography densitometry analysis showed an increase in MMP-2 intensity in the T group compared with the control, WS and N subjects ($p < 0.02$). MMP-9 intensity was also increased in T patients in comparison with the other groups ($p < 0.0045$).

$288.5 \pm 22.8 \mu\text{g}$ of degraded gelatin/mg of incubated plasma protein; $p = 4.73 \times 10^{-17}$). The χ^2 test showed a correlation between ORRs and low gelatinase activity rates (OR = 0.04, 95% CI = 0.01–0.15; $p < 0.000001$; Yates-corrected).

Zymography analysis revealed the presence of a lysis band of about 65 kDa in all samples (fig. 2a). This band corresponded to MMP-2 activity. The highest intensity of this band was observed in the T group samples ($483,706.3$

Fig. 3. MMP and TIMP Western blots from lung adenocarcinoma plasma samples. Western blot assay demonstrated the presence of the MMP-2 active form (65-kDa band) in all samples assayed. The pro-MMP-2 was also detected in some samples as a 72-kDa band. The MMP-9 active form was observed in all samples as an 86-kDa

band. A 92-kDa band (pro-MMP-9) was also identified. The active MT1-MMP (55-kDa band) form was present in all samples assayed. TIMP immunoblotting revealed the presence of a 28-kDa band which corresponded to TIMP-1. A 21-kDa band, TIMP-2, was also observed in the control and lung cancer samples.

$\pm 12,778.7$ DU) compared to the N and WS subjects ($377,490.1 \pm 7,836.9$ and $236,960 \pm 52,135.3$ DU, respectively; $p < 0.02$) (fig. 2b). MMP-2 activity in all lung cancer samples was higher than that observed in the control group ($86,309.3 \pm 3,888.1$ DU; $p < 0.005$). An 86-kDa band (MMP-9 activity) was also identified in some control samples and in almost all lung cancer patients. The highest intensity of this band was observed in samples from the T group ($189,531 \pm 38,573.1$ DU) when compared with the control, WS and N subjects ($35,934.2 \pm 11,074.8$, $30,639.3 \pm 7,349.9$ and $35,867.4 \pm 11,134.2$ DU, respectively; $p < 0.0045$).

MMP-9 and MMP-2 Plasma Levels

MMP-2 Western blot demonstrated the presence of a 65-kDa band in all samples (fig. 3). This band corresponded to the active form of MMP-2. In some samples, a 72-kDa band (pro-MMP-2) was observed. The MMP-2 densitometry analysis showed a significant increase in MMP-2 plasma levels in the T and N groups compared to the control subjects ($57,907.7 \pm 5,196.9$, $57,952.1 \pm 3,798.4$ and $45,487.2 \pm 1,393.8$ DU, respectively; $p < 0.015$) (fig. 4a). There were no significant differences between WS patients and control subjects or between the lung cancer groups. MMP-9 immunoblotting revealed

the presence of the MMP-9 active form as an 86-kDa band in all samples (fig. 3). A 92-kDa band that corresponded to pro-MMP-9 was also observed in some of the control and lung cancer samples. The highest 86-kDa-band net intensity was found in the T group samples when compared to the controls ($52,950.8 \pm 6,042.2$ and $24,733.3 \pm 2,457.1$ DU, respectively; $p = 0.003$) (fig. 4a). MMP-9 protein concentration was also increased in the WS and N samples ($33,410.7 \pm 3,057.9$ and $39,420.7 \pm 3,808.5$ DU, respectively; $p < 0.04$) when compared to the control group. There were significant differences between the WS and T groups ($p = 0.005$).

Regarding chemotherapy response there were no significant differences in MMP-2 and MMP-9 plasma levels among subjects with ORR and with PD.

MT1-MMP Plasma Levels

MT1-MMP immunoblotting technique demonstrated the presence of a band with a molecular weight of about 55 kDa in most of the plasma samples (fig. 3). Densitometry analysis showed a significant increase of this MT-MMP in samples from all lung cancer groups in comparison with control subjects ($38,068.4 \pm 2,935.2$ DU; $p < 0.003$) (fig. 4a). The T group had the highest MT1-MMP plasma concentrations ($76,292.8 \pm 3,442.5$ DU) in com-

Fig. 4. Western blot densitometry analysis. **a** An increase of MMP-2 in plasma samples from the T and N groups ($p < 0.015$) is shown. MMP-9 plasma levels were increased in all cancer samples in comparison with control subjects. MMP-9 concentration was higher in the T group in comparison with the WS samples ($p = 0.005$). The analysis of the MT1-MMP bands showed that its highest plasma concentration corresponded to the T group and the

lowest to the WS patients. **b** An increase of plasma TIMP-1 levels in all lung cancer samples is shown, particularly in the T group ($p < 0.0001$). TIMP-2 concentration was also increased in plasma samples from lung cancer patients in comparison with control subjects ($p < 0.0001$). The N group had the highest concentration in comparison with the other lung cancer groups ($p < 0.025$). Bars indicate the mean of each group $\pm$ standard error.

parison with the WS and the N groups ($62,710.4 \pm 3,527.9$ and $50,167.7 \pm 2,226.4$ DU, respectively; $p < 0.015$).

Regarding the chemotherapy response, differences in plasma MT1-MMP levels among patients with ORR and with PD were not significant.

TIMPs' Plasma Concentration

In all groups, TIMP Western blot revealed a 21- and a 28-kDa band which corresponded to TIMP-2 and TIMP-1, respectively (fig. 3). There was a significant increase in TIMP-1 plasma concentration in lung cancer patients in comparison with the control group ($36,571.9 \pm 1,164.1$ DU; $p < 0.0001$) (fig. 4b). Although the highest net intensity of TIMP-1 was observed in the T group ($100,526.9 \pm 11,537.2$ DU), there were no significant differences among this group and the WS ($74,580.6 \pm 7,403.8$ DU) and N ($84,013.8 \pm 8,147.6$ DU) lung cancer groups.

TIMP-2 protein levels were increased in all lung cancer groups when compared with control subjects ($57,028.5 \pm 1,798.2$ DU; $p < 0.0001$) (fig. 4b). There was a significant increase of TIMP-2 plasma concentration in samples from the N group ($216,122.1 \pm 19,538.9$ DU) in comparison with the WS and T patients ($166,419.3 \pm 8,212.9$ and $152,756.1 \pm 12,595.9$ DU, respectively; $p < 0.025$).

Regarding the chemotherapy response and TIMPs' plasma concentration, differences among patients with ORR and with PD were not significant.

Discussion

WS has been considered a risk factor for lung cancer in never-smokers; however, information about its molecular and clinical features is scarce [24].

In this study, we found that 69.2% of the analyzed primary lung adenocarcinoma patients were never-smokers. Lung cancer incidence was higher in women than in men, particularly in the never-smoker group; probably because this group included WS patients, and women are exposed to wood smoke for more hours than men [6, 8].

We also observed that never-smokers, particularly WS patients, had a better response to cisplatin first-line therapy. In this context, Toh et al. [19] found no differences in the response to cisplatin first-line chemotherapy among tobacco smokers and never-smokers. On the contrary, Tsao et al. [18] observed that never-smokers had higher response rates and lower PD rates than smokers. The differences in these studies could be due to the different ethnic populations analyzed (Asiatic and Caucasian, respectively). Regarding targeted therapy response, it has been described that never-smoker women with lung adenocarcinoma had a better response to erlotinib or gefitinib, both epidermal growth factor receptor inhibitors, when compared to smokers. The response to these drugs has been associated with mutations found in the tyrosine kinase domain of the epidermal growth factor receptor [25]. The response to cisplatin that we observed was per-

haps also associated with this protein or to modifications in other molecules such as the multidrug-resistant gene 1 and the excision repair cross-complementing 1 protein; here, further research is necessary [26, 27].

Likewise, it is possible that the expression and activity of other proteins such as MMPs and TIMPs could be different among tobacco smokers and never-smoker lung cancer patients. Regarding these molecules, it has been pointed out that the metastatic potential of a neoplastic cell is associated with an increase in MMP expression and enzymatic activity [11, 12]. Therefore, these enzymes have been studied in many cancers looking for the possibility of a reliable prognostic factor. However, disagreement still prevails as to which MMPs are more sensitive to predict non-small-cell lung cancer progression. In this context, some authors found an association between a poor prognosis and an increase in MMP-9 tissue expression with a TIMP-1 decrease in the early stages of the disease, while others suggested that the increase in MMP-2 synthesis is a better prognostic biomarker than MMP-9 [28–30].

In our study, we analyzed the plasma concentration and gelatinase activity of MMP-2 and MMP-9. We found that the plasma enzymatic activity of these molecules was increased in lung cancer patients in comparison with control subjects, particularly in those patients with PD. Moreover, we observed a correlation between chemotherapy response and gelatinase activity but not between the plasma concentrations of MMPs and of TIMPs.

Likewise, gelatinase activity was significantly lower in the WS group in comparison with the T patients. Interestingly, the WS group showed the best response to chemotherapy, while the T group had the highest PD rates. Regarding the N group, even though plasma samples from this group showed higher gelatinase activity in comparison with WS subjects, a correlation with chemotherapy response was hard to make, because too large a variation was observed in the treatment response. This variation could be due to the undefined risk factors involved in the pathogenesis of lung cancer among these subjects.

The zymography analysis demonstrated an increase in the MMP-2 enzymatic activity in all lung cancer patients in comparison with control subjects. However, MMP-2 blood concentration was similar in the WS and control groups. On the other hand, TIMP-2 concentration was increased in cancer samples. Besides TIMP-2 inhibitory activity, this protein is able to activate proMMP-2, forming a molecular complex with MT1-MMP (MMP-14) on the cell surface [31]. Therefore, the MMP-2 activity found

in lung cancer patients is probably due to the activation process of this enzyme. Moreover, we also measured the other component of the activation complex, the MT1-MMP. This MMP was also increased in lung cancer patients, particularly in the T group. In this group, the increase in both MT1-MMP and MMP-2 could explain, at least in part, the high MMP-2 gelatinase activity found in the T patients.

Likewise, MMP-9 activity detected by zymography was increased in some patients when compared to the control group, but it was lower than MMP-2 enzymatic activity. Its plasma concentration was also lower than MMP-2. TIMP-1 concentration was also increased, and this TIMP has the function to activate proMMP-9 through the formation of a complex with MMP-3, as does TIMP-2 [32]. Therefore, the high MMP-9 and TIMP-1 levels together with the MMP-2 enzymatic activity observed in the T group could explain the high gelatinase activity found in these patients.

In summary, our results point out that there are differences in the metastatic profile and in the cisplatin first-line chemotherapy response between patients with tobacco-induced and those with wood-smoke-induced lung cancer. This implies that treatments for these 2 groups must be different. Tobacco smokers with an increase in MMP plasma concentration and in plasma gelatinase activity should receive – alone or in combination with the conventional chemotherapy – a specific therapy that interferes with MMP synthesis and activation [33, 34]. Furthermore, first-line treatment with a cisplatin base could be a good option for WS patients with low MMP plasma levels and gelatinase activity, when targeted therapy becomes unaffordable. On the other hand, predictive indexes for the second- and third-line therapy of non-small-cell lung cancer have been developed [35, 36]. According to our results, quantification of MMP-2, MMP-9, MT1-MMP, TIMP-1 and TIMP-2 plasma levels, as well as MMP-2 and MMP-9 gelatinase activity, could be used as a reliable indicator of the metastatic potential and, consequently, greatly improve predictive prognostic indexes.

Finally, as Maio [37] points out, it is difficult for a physician to choose a therapy based on affordability; however, the physician must offer each patient the most effective treatment.

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