

Metallic elements in pulmonary biopsies from lung cancer and control subjects

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Abstract. Occupational/environmental exposure to some metallic elements is a risk factor for the development of lung diseases, including lung cancer. We aimed at investigating the levels of arsenic, beryllium, cadmium, cobalt, chromium, nickel and lead in the lung tissue of patients affected by early stage non small cell lung cancer (NSCLC). A small number of patients without a diagnosis of lung cancer were also included as control group. Lung tissue biopsies were collected from 45 NSCLC patients (both cancerous and unaffected tissues) and 8 control subjects undergoing surgery. Patients were stratified for smoking habits, histopathology and cancer sites. Metallic elements were determined in dry tissue after digestion by means of ICP-MS. Cd, Ni and Pb levels were higher in unaffected than in control tissues (0.52 *vs* 0.18 µg/g dry, *p*<0.05 for Cd; 4.49 *vs* 1.8 µg/g dry, *p*<0.05 for Ni; 0.21 *vs* 0.06 µg/g dry, *p*<0.01 for Pb). The three elements, and particularly Cd, were influenced by smoking habits; Pb levels were higher in squamocellular carcinoma than adenocarcinomas; Ni distributed in the lungs in an inhomogeneous way. This study demonstrates that the unaffected lung tissue is more representative than the cancerous tissue of the pulmonary content of metallic elements. Tobacco smoke is a main factor affecting the concentration levels of Cd, Pb, and to a lesser extent Ni in the lung tissues of NSCLC patients. The role of past environmental-occupational exposures could not be fully elucidated, due to the limited sample size and the retrospective nature of the study. (www.actabiomedica.it)

Key words: Lung cancer, cigarette smoke, arsenic, beryllium, cadmium, cobalt, chromium, nickel, lead

Introduction

Several studies have demonstrated that metallic elements and their compounds may interact with biological macromolecules, like enzymes and DNA, activating or inhibiting several biological processes that alter cellular homeostasis, thus inducing cellular damage (1-4). Carcinogenic elements may act as either genotoxic or epigenetic carcinogens (5-11). Metal-containing dust from either polluted environments or cigarette smoke is a well-acknowledged risk factor for the development of cancer (12-15). Therefore, mech-

anistic studies of metallic element carcinogenicity, as well as the biomonitoring of subjects exposed to toxic metallic compounds, are primary key-points.

The traditional biomonitoring approach, based on the determination of xenobiotics or their metabolites in blood and/or urine of exposed subjects (16), can only provide an estimate of the overall absorbed dose (17) but does not give specific information about the effective dose at the target organ level. This holds particularly true for some metallic elements, whose intake by inhalation is a recognized risk factor for the development of lung diseases, like chronic obstructive

pulmonary disease (COPD), asthma, pulmonary fibrosis and lung cancer (10, 17, 18). The availability of metal determinations in pulmonary tissue can allow the study of dose-response relationships at the target organ level. The lung tissue concentrations of metallic elements have been reported in exposed workers or lung cancer patients (reviewed by Catalani et al in this same issue). Most of the papers, however, examined post-mortem samples; in only few cases, tissue samples excised during surgical interventions were considered, even without distinction among cancerous and unaffected tissues (19, 20). Metallic elements can also be determined in biological fluids representative of airways, including exhaled breath condensate (EBC), sputum and bronchoalveolar lavage fluid (BALF), from metal exposed subjects or from patients suffering lung diseases (21-24). A recent study has measured and related the Cr levels in both the pulmonary tissue and EBC of patients with diagnosis of non small cell lung cancer (NSCLC) (25).

In this study, seven metallic elements known to cause adverse pulmonary effects were measured in both cancerous (CT) and unaffected tissues (UT) of NSCLC patients undergoing surgical intervention. Metal pulmonary levels were determined also in a small group of patients without a final diagnosis of lung cancer. Five of the investigated elements have been classified as class 1 carcinogens (carcinogenic to humans) by the International Agency for Research on Cancer (IARC), namely Chromium [Cr, as Cr(VI)] (26), Cadmium (Cd) (27), Nickel (Ni, only in non-metallic form) (26), Beryllium (Be) (27) and Arsenic (As) (28,29). IARC has recently included Lead (Pb) in class 2A (probably carcinogenic to humans) (30) and Cobalt (Co) in class 2A if associated to Tungsten (W) and in class 2B (possibly carcinogenic to humans) when alone (31, 32).

Materials and Methods

Subjects

Lung biopsies were collected in 45 NSCLC patients and 8 controls undergoing pulmonary resection at the Department of Thoracic Surgery of Parma Uni-

versity Hospital in the periods January 2003-June 2005 e January 2007-September 2007. All NSCLC patients had a stage I (IA or IB) tumor. The main patients' characteristics are reported in table 1. Among NSCLC patients, the histopathologic features were 24 (53%) adenocarcinomas (ADK), 19 (42%) squamocellular carcinomas (SCC), 1 large cell anaplastic carcinoma (2.2%), 1 undifferentiated carcinoma (2.2%). The control subjects included 3 lung metastases from other primary tumors, 5 not cancer-

Table 1. Main characteristics of the study population. The possible occupational exposure to metals was extrapolated by past occupational history and it cannot be considered as a quantitative variable.

Variables	NSCLC Patients (n=45)	Controls (n=8)
<i>Age (Years)</i>	70.0 (64.0-74.5)	64.5 (31.5-73.0)
<i>Sex</i>		
F	8 (17.8%)	2 (25.0%)
M	37 (82.2%)	6 (75.0%)
<i>Smoking habits</i>		
Current Smokers	15 (33.3%)	1 (12.5%)
Ex-Smokers	21 (46.7%)	5 (62.5%)
Non-Smokers	8 (17.8%)	2 (25.0%)
NA	1 (2.2%)	
<i>Histology</i>		
Squamous Cell Carcinoma (SCC)	19 (42.2%)	-
AdenoCarcinoma (ADK)	24 (53.3%)	-
Other forms	2 (4.4%)	
<i>Pack year (PY)</i>	41.0 (25.0-54.5)*	22.3 (1.8-29.0)
<i>Tiffeneau Index</i>	74.0 (69.0-85.0)	69.0 (61.0-94.0)
<i>Cancer Site</i>		
Upper lobes	26 (57.8%)	-
Medium right lobe/lingula	1 (2.2%)	-
Lower lobes	14 (31.1%)	-
NA	4 (8.9%)	-
<i>BPCO</i>		
Yes	13 (28.9%)	4 (50.0%)
No	30 (66.7%)	2 (4.4%)
NA	3 (37.5%)	1 (12.5%)
<i>Occupational exposure to metals</i>		
Yes	19 (42.2%)	4 (50.0%)
No	26 (57.8%)	4 (50.0%)

NA=information not available. *p<0.05 (Mann Whitney U test)

ous lung diseases. None of the enrolled patients received previous chemotherapy or radiotherapy treatments. An occupational health physician collected socio-demographic information, past occupational/environmental exposure and lifestyle data from each patient using a structured questionnaire. Two occupational health physicians evaluated questionnaires blindly and attributed past occupational exposure to metallic elements according to the reported job titles. According to the smoking habits, subjects were classified into the following subgroups: current smokers (CS), ex-smokers (ES) and non smokers (NS). CS included even subjects who stopped tobacco smoking from no more than 1 year, whereas the NS group included both subjects who had never smoked and ex-smokers from at least 15 years who smoked no more than 30 packyears (PY). All the enrolled subjects gave their written informed consent to the study that was approved by the local ethic committee.

Study design, sample collection and storage

At least two tissue fragments (about 5 mm per side, each) were excised from each patient during surgery: at least one from the cancerous mass and another from the seemingly UT, far at least 3 cm from cancerous tissue, in the same specimen. All fragments were excised far from surgical clips or staple lines, to avoid any metal contamination. Fragments were put into sterile vials (Eppendorf Int., Hamburg, Germany) and immediately stored at -80°C . The biological sampling was performed as laid down in the Declaration of Helsinki.

Metallic element analysis

Little pieces of the collected fragments of cancerous and UT (about 200 mg wet tissue) were excised by means of a glass-made cutter, in order to avoid any contamination due to the cutting maneuver, dried at 70°C for 3 hours and then weighted by an analytical balance with a sensitivity of 0.1 mg. Finally, they were digested in a solution 1:1 of hyperpure nitric acid 70% and bi-distilled water for 30 min. at 70°C .

Multi-elemental analysis was performed by means of inductively coupled-plasma mass spectrom-

eter (ICP-MS, ELAN DRC II, Perkin Elmer, Waltham, USA). As, Be, Cd, Co, Ni and Pb were analyzed using the Total Quant method, whereas for Cr the Dynamic Reaction Cell (DRC) method with ammonia was used. External calibration was performed using the calibration standard 3, stock multielement (10 $\mu\text{g}/\text{ml}$; Perkin Elmer, Waltham, USA). The accuracy of the method was determined based on the mean values obtained on certified reference material NIST 1640 (trace elements in water). The precision, calculated as coefficient of variation, was of 4-8% intra-series and 6-12% between-series. Concentration of metallic elements in lung tissues, calculated as ratio between the total amount (in μg) of metallic elements measured in the digestion solution and the weight of the digested dry tissue, is reported as $\mu\text{g}/\text{g}$ dry tissue. The detection limits (LOD), calculated as 3 standard deviations of the background signal obtained on 10 white samples, for different elements (expressed as $\mu\text{g}/\text{g}$ dry tissue) were: 0.00005 for Cd; 0.0003 for Co; 0.0008 for Pb; 0.001 for As and Be; 0.03 for Cr and 0.01 for Ni.

Statistical Analysis

Due to not-normal distribution of data and the presence of points below the LOD, we chose to perform non-parametrical statistical tests. We used the Mann-Whitney and the Wilcoxon test tests for comparisons between two independent and two dependent data sets, respectively. When more than two comparisons were performed involving both independent and dependent variables, the Bonferroni-Keppel correction was applied to the significance levels of the single tests. If more than two independent variables were involved, the Kruskal-Wallis test followed by the Dunn's test for multiple comparisons was performed. The variables' characteristics did not allow to run a multiple way analysis of variance, therefore only the main effects of the factors considered in this study were assessed. Finally, the Spearman's correlation coefficient was calculated in assessing the relationship between pairs of variables. Statistical analysis was performed using the SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) and PRISM 4.0 (GraphPad, San Diego, CA, USA) and a significant p value of 0.05 was

chosen. When the concentration was below the LOD, we leveled to the half of the LOD values (e.g. 0.001 µg/g dry tissue for Co, As, and Be; 0.005 µg/g dry tissue for Pb), to reduce mathematical artifacts in statistical analysis due to the variability of tissue weight.

Results

As expected, smoking habits in terms of Pack year (PY) values was significantly ($p < 0.05$) higher in NSCLC patients as compared to controls (Tab. 1). The levels of metallic element in cancerous and UT of NSCLC patients, as well in the controls' lung tissue are reported in table 2. We observed significantly higher levels of Ni and Cd ($p < 0.05$) and Pb ($p < 0.01$) in NSCLC subjects (UT) *vs* controls. There were also significantly higher Pb and Ni levels in cancerous *vs* control tissues and in cancerous *vs* UTs ($p < 0.05$ for both), respectively. As and Be were below the LOD in almost all samples and therefore we failed to find statistical differences between the different data subsets. The latter held true also for Co and Cr that tended to display higher concentration levels in NSCLC and control tissues, respectively.

After stratification of NSCLC subjects for smoking habits, the Cd levels were significantly reduced in NS as compared to CS and ES [$p < 0.01$ for cancerous tissues (NS *vs* CS and ES) and UTs (NS *vs* CS); $p < 0.05$ for UTs (NS *vs* ES)] (Tab. 3). For Ni and Pb, we found similar but not statistically significant behaviors (data not shown).

Looking at the correlation among variables, Cd levels in UTs were correlated with PY ($r = 0.43$, $p < 0.01$). Moreover, in unaffected/control tissues Cd was correlated with both Pb ($r = 0.48$, $p < 0.01$, figure 1) and Ni ($r = 0.55$, $p < 0.05$, figure 2), the latter being each other correlated ($r = 0.40$, $p < 0.01$, figure 3).

After stratification of NSCLC patients by histotype, we found that SCC subjects smoked significantly more than ADK patients [on average, 45.0 (30.9-68.7) *vs*. 37.0 (21.3-42.9) PY, $p < 0.05$]. The distributions of metallic element in NSCLC subjects classified by histotype showed higher Cd, Ni and Pb levels in SCC, as compared to ADK samples, but only Pb in UT was significantly higher ($p < 0.01$).

The analysis of metal distribution by cancer localization demonstrated significantly higher Ni and Co levels in the upper/medium lobes as compared to the lower ones ($p < 0.01$ and $p < 0.05$, respectively)

Table 2. Tissue concentrations of As, Be, Cd, Co, Cr, Ni and Pb in non-cancerous and cancerous lung samples from NSCLC patients and controls (medians and ranges of values are shown)

Elements (µg/g dry tissue)	Controls	Cases	
		Unaffected Tissue	Cancerous Tissue
As	0.02 (ND-0.05)	<LOD (<LOD -0.04)	0.002 (<LOD -0.04)
Be	ND (ND-ND)	<LOD (<LOD -0.02)	<LOD (<LOD -0.02)
Cd	0.18 (0.04-0.41)	0.52 (0.22-1.07)*	0.43 (0.11-0.79)
Co	0.04 (0.02-0.18)	0.07 (0.05-0.11)	0.05 (0.01-0.10)
Cr	1.00 (0.14-1.36)	0.26 (0.10-0.62)	0.33 (0.07-0.78)
Ni	1.80 (0.79-3.52)	4.49 (2.58-6.73)*#	3.60 (1.85-5.01)
Pb	0.06 (0.005-0.12)	0.21 (0.10-0.39)**	0.14 (0.05-0.38)*

LOD = Limit of detection; * $p < 0.05$ as compared to controls; ** $p < 0.01$ as compared to controls; # $p < 0.05$ as compared to cancerous tissue

Table 3. Tissue concentrations of Cd, in non-cancerous and cancerous lung samples from NSCLC patients stratified by smoking status (median and range of values are shown)

Cd (µg/g dry tissue)	Current smokers	Ex-smokers	Non smokers
Unaffected tissue	0.75 (0.23-1.30)**	0.54 (0.33-1.32)*	0.18 (0.06-0.22)
Cancerous tissue	0.79 (0.17-1.75)**	0.46 (0.21-0.66)**	0.09 (0.04-0.19)

* $p < 0.05$ as compared to non smokers; ** $p < 0.01$ as compared to non smokers

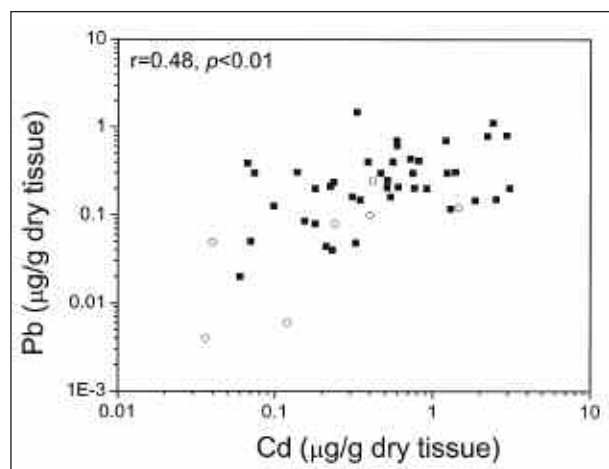


Figure 1. Correlation among concentrations of Cd and Pb in lung non cancerous tissue samples from NSCLC (■) and control (○) patients. Correlation coefficient is referred to Spearman's test and to overall points. For graphical reasons, points with Pb below LOD are not reported even if considered in the correlation

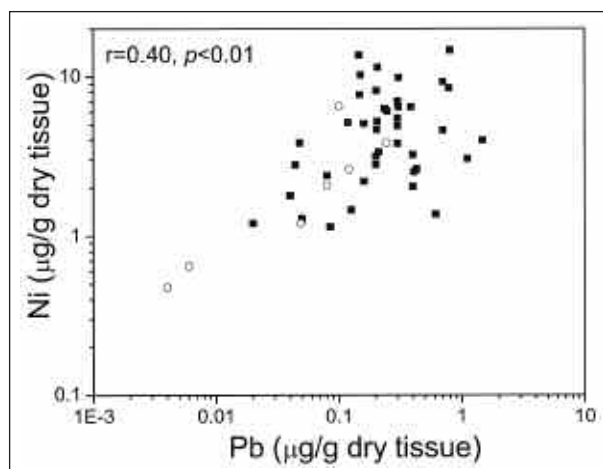


Figure 2. Correlation among concentrations of Pb and Ni in non cancerous lung tissue from NSCLC (■) and control (○) patients. Correlation coefficient is referred to Spearman's test and to overall points. For graphical reasons, points with Pb below LOD are not reported even if considered in the correlation

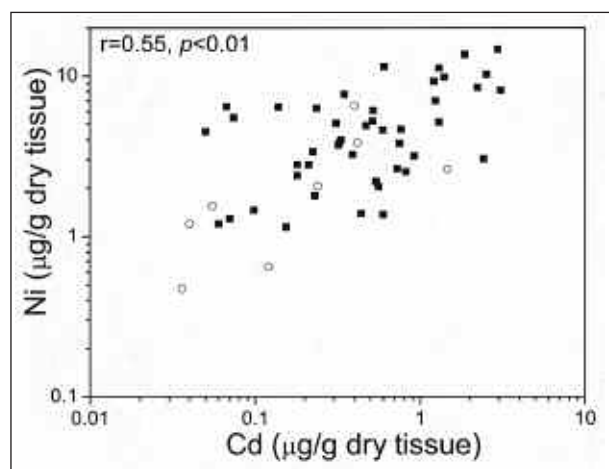


Figure 3. Correlation among concentrations of Cd and Ni in unaffected lung tissue from NSCLC (■) and control (○) patients. Correlation coefficient is referred to Spearman's test and to overall points

among UT samples, whereas no difference was observed among cancerous tissues.

Finally, NSCLC patients were stratified for possible occupational metal exposure during their past working activity. No significant differences were found among possibly occupationally exposed and unexposed NSCLC patients (data not shown). If con-

trols were, however, included into the analysis, a small difference was observed in the Cd lung tissue levels (Tab. 4). Interestingly, when the sample was stratified even for the extent of smoking habits (according to the median value of 32 packyears), the difference was still significant in the "low smoking" group only (median values of 0.67 *vs* 0.16 µg/g dry tissue in exposed and unexposed subjects, respectively).

Discussion

Our study demonstrates that Cd, Pb and, to a lesser extent, Ni levels are influenced by tobacco smoke in both cancerous and unaffected tissues of NSCLC patients, whereas other investigated metallic elements (As, Be, Co and Cr) are not. Moreover, Pb accumulates in unaffected tissues of SCC patients and Ni distribution is more inhomogeneous than that of other elements. The possible past occupational metal exposure does not seem to play a major role in affecting the lung tissue concentrations of metallic elements, as only a slight effect on Cd levels was apparent when pulling together NSCLC patients and controls.

Ni, Cd and Pb are traditionally associated to tobacco smoke. Tobacco plants absorb Ni from the

Table 4. Tissue concentrations of As, Be, Cd, Co, Cr, Ni and Pb in non-cancerous lung samples of both NSCLC and control subjects classified by past possible occupational exposure to metallic elements (medians and ranges of values are shown)

Elements ($\mu\text{g/g}$ dry tissue)	Metal exposed (n=23)	Metal unexposed (n=30)	p
As	<LOD (<LOD - 0.14)	<LOD (<LOD - 0.30)	Ns
Be	<LOD (<LOD - 1.00)	<LOD (<LOD - 0.15)	Ns
Cd	0.54 (0.06 - 3.08)	0.28 (0.04- 2.53)	<0.05
Co	0.07 (0.02 - 0.70)	0.07 (0.003 - 0.65)	Ns
Cr	0.58 (0.02 - 4.20)	0.22 (0.02 - 5.37)	Ns
Ni	3.82 (0.65 - 14.70)	3.67 (0.48 - 13.69)	Ns
Pb	0.20 (0.01 - 1.11)	0.21 (0.004 - 1.47)	Ns

LOD=limit of detection; Ns=not significant

ground and the metal is present at concentrations of about 0.5-1.5 $\mu\text{g/g}$ in leaves. Therefore, about 70 ng of Ni are contained in tobacco smoke from a cigarette and up to 30 $\mu\text{g/g}$ of Ni have been determined in the cigarette ash (33). It has been estimated that Ni absorbed from tobacco smoke could be responsible of a pulmonary dose ranging from 0.01 to 3 $\mu\text{g/g}$ of lung tissue, depending by the conditions (34). However, occupational exposure remains the main source of Ni in the airways, the contribution being one order of magnitude higher (35). Cd can accumulate into tobacco leaves up to 7 $\mu\text{g/g}$, reaching a concentration of about 3.5 $\mu\text{g/g}$ (33) in tobacco blend. Moreover, about 50% of the element can pass the cigarette filter, being transferred at relatively high concentrations into tobacco smoke (36). High Cd levels are thus measured in blood and tissues of smokers, and smoking is considered a confounding factor of Cd exposure (12, 33). Finally, the cigarette content of Pb is about 0.6-2.5 $\mu\text{g/g}$ and about 6% can pass into cigarette smoke (33). Even in this case, smoking habits is a possible confounding factor of Pb exposure.

In the NSCLC group, the Cd and Pb concentrations were not different among cancerous and unaffected lung tissues, the latter being significantly higher than in lung samples from controls. As controls smoked to a significantly lesser extent than NSCLC patients, unaffected tissue seemed to be more representative of the cumulative lung tissue dose of both the elements, as compared to the cancerous one. For Ni, the levels determined in unaffected tissues were significantly higher than measured in both the cancerous and the tissues from controls. Necrotic and proliferative phenomena, as well as neo-vascularization and

structure modifications occurring in cancerous tissue may imply modifications of the cumulative tissue dose of metallic elements, as compared to unaffected tissue.

Variable lung tissue levels of Cd, Pb, and Ni are reported in literature, due to pre-analytical (autoptic *vs* bioptic samples, dry *vs* wet tissues, subjects' characteristics, pre-analytical treatments, etc.), or analytical factors (different detection techniques) in different studies. The levels reported in this study are consistent with ranges reported in literature (37-39).

After stratification of NSCLC patients for smoking habits, Cd levels were significantly higher in any tissue from both current and ex smokers as compared to non-smokers and this could be due explained by a long persistence of the metal in the airways. Cd levels were significantly related to tobacco smoking (as PY) both in the NSCLC group and in the entire sample (NSCLC and control patients). Therefore, Cd pulmonary levels were be particularly representative of the local Cd dose deriving from inhalation of tobacco smoke. Increased Cd levels have been observed in autoptic pulmonary samples from subjects suffering lung cancer and even in these cases, a causal relationship with tobacco smoke was hypothesized (40-42). In lung tissues from foundry workers dead for lung cancer high Cd levels were observed (43), and they were correlated to the daily cigarette consumption (42).

As both Pb and Ni were increased in unaffected tissues of NSCLC patients and both the elements were significantly correlated with Cd, we hypothesize that their accumulation was related to tobacco smoking, too. This would hold particularly true for Pb that was significantly increased in SCC samples, the histotype more tightly associated to tobacco smoke (44).

However, literature data about Pb are more controversial. The element was found to be 1–2 orders of magnitude higher than controls in the lung tissue of exposed workers (42), but an accumulation of pulmonary Pb in lung cancer patients has not been definitely demonstrated (38), as well as its relationship with tobacco smoke. We were unable to find differences in Pb content among cancerous and unaffected tissues, as observed by others (45). On the other hand, our results about Ni were consistent with literature data. Raised Ni levels were found in the lung tissue of lung cancer patients (46) and higher Ni levels in the upper lung lobes have already been observed (47), as well as its inhomogeneous distribution in lung tissues from both exposed workers and lung cancer subjects (45, 48). We found that Ni was significantly higher in unaffected than cancerous tissues and in the upper lobes, as compared to the lower ones. Two previous studies did not find increased tissue Ni levels in lung cancer patients (49) or differences among cancerous and unaffected tissues (50). The sampling methods and differences in subjects' characteristics could explain these differences.

Given the relatively small sample size we simplified the classification of pulmonary lobes into upper/medium (only one subject had a cancer in the medium lobe) and lower, without taking into account the right or left side, and this can be considered a limit of the study. Our results about Ni are in partial agreement with previous reports (51, 52) showing higher concentrations of the element in the superior/middle lobes, probably in relation to their higher ventilation, as compare to the lower ones. We were unable to find any statistical difference for Cr, As and Be. Only Co was significantly higher in the upper/medium lobes than in the lower ones in unaffected tissue, but it has never been studied before and further studies are needed to support our result.

Results about Cr, whose concentrations were not influenced by tobacco smoke and were homogeneously distributed among lung lobes, are consistent with our recent study (25). A possible confounding by tobacco smoke was hypothesized by some authors (19, 41, 49, 53), but in the last years Cr concentration in cigarette smoke has fallen to undetectable levels (54, 55) and this could explain our results.

As and Be were undetectable in the majority of cases, indicating that not specifically exposed subjects don't show abnormal levels of both the elements. Raised lung tissue levels of As have been observed among smelter workers (56) but not in lung cancer patients (45). Very low As tissue levels have been found in autoptic samples from the general population (57). Be has been found at very low concentrations in lung tissue, even in mine workers exposed to Be polluted dust (42).

In agreement with previous literature, we measured very low levels of Co (57) and the element was not significantly higher in NSCLC patients (38). Pulmonary levels of the element were not influenced by age, sex, degree of lung contamination and lung pathologies, such as emphysema (39).

Several biases may have affected the concentrations of metallic elements in subjects stratified for past occupational metal exposure, due to both the retrospective assessment method (recall bias) and the limited sample size (limiting both the statistical power and the possibility to control interfering/confounding factors). Thus, we believe that our data in this regard must be interpreted very cautiously and the results must be considered as preliminary.

In conclusion, tobacco smoke is a main confounder for Cd and Pb levels in the lung tissues of NSCLC patients. Ni, that tends to distribute inhomogeneously in the lung tissue, can also be confounded by tobacco smoke, but further studies are needed to better clarify this relationship. On the other hand, Cr levels are not related to smoking habits, confirming our previous study, and also Co showed a similar trend. The very low levels observed for As and Be, with a high frequency of samples below the LOD, seem to indicate that in the absence of specific exposures, an accumulation of these elements is improbable. Occupational metal exposure extrapolated from past occupational history was not a sufficiently sensible endpoint to discriminate subjects enrolled in this study.

References

1. Harris GK, Shi X. Signaling by carcinogenic metals and metal-induced reactive oxygen species. *Mutat Res* 2003; 533: 183–200.

2. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. *Curr Med Chem* 2005; 12: 1161-208.
3. Rainbow PS. Trace metal bioaccumulation: models, metabolic availability and toxicity. *Environ Int* 2007; 33: 576-82.
4. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 2005; 26: 235-44.
5. Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF. Cadmium-induced cancers in animals and in humans. *Int J Occup Environ Health* 2007; 13: 202-12.
6. Hayes RB. The carcinogenicity of metals in humans. *Cancer Causes Control* 1997; 8: 371-85.
7. Desoize B. Metals and metal compounds in carcinogenesis. *In Vivo* 2003; 17: 529-39.
8. De Flora S. Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *Carcinogenesis* 2000; 21: 533-41.
9. Kasprzak KS, Sunderman FW, Jr., Salnikow K. Nickel carcinogenesis. *Mutat Res* 2003; 533: 67-97.
10. Navarro Silvera SA, Rohan TE. Trace elements and cancer risk: a review of the epidemiologic evidence. *Cancer Causes Control* 2007; 18: 7-27.
11. Lu H, Shi X, Costa M, Huang C. Carcinogenic effect of nickel compounds. *Mol Cell Biochem* 2005; 279: 45-67.
12. Stavrides JC. Lung carcinogenesis: pivotal role of metals in tobacco smoke. *Free Radic Biol Med* 2006; 41: 1017-30.
13. Sthos SJ, Bagchi D, Bagchi M. Toxicity of trace elements in tobacco smoke. *Inhalation Toxicology* 1997; 9: 867-90.
14. Bradley TP, Golden AL. Tobacco and carcinogens in the workplace. *Clin Occup Environ Med* 2006; 5: 117-37, x.
15. Bernhard D, Rossmann A, Wick G. Metals in cigarette smoke. *IUBMB Life* 2005; 57: 805-9.
16. Apostoli P. Elements in environmental and occupational medicine. *J Chromatogr B* 2002; 778: 63-97.
17. Mutti A, Corradi M. Recent developments in human biomonitoring: non-invasive assessment of target tissue dose and effects of pneumotoxic metals. *Med Lav* 2006; 97: 199-206.
18. Krantz A, Dorevitch S. Metal exposure and common chronic diseases: a guide for the clinician. *Dis Mon* 2004; 50: 215-62.
19. Anttila S, Kokkonen P, Paakko P, et al. High concentrations of chromium in lung tissue from lung cancer patients. *Cancer* 1989; 63: 467-73.
20. Diez M, Arroyo M, Cerdan FJ, Munoz M, Martin MA, Balibrea JL. Serum and tissue trace metal levels in lung cancer. *Oncology* 1989; 46: 230-4.
21. Goldoni M, Catalani S, De Palma G, et al. Exhaled breath condensate as a suitable matrix to assess lung dose and effects in workers exposed to cobalt and tungsten. *Environ Health Perspect* 2004; 112: 1293-8.
22. Caglieri A, Goldoni M, Acampa O, et al. The effect of inhaled chromium on different exhaled breath condensate biomarkers among chrome-plating workers. *Environ Health Perspect* 2006; 114: 542-6.
23. Murgia N, Muzi G, Dell' Omo M, et al. Induced sputum, exhaled breath condensate and nasal lavage fluid in electroplating workers exposed to chromium. *Int J Immunopathol Pharmacol* 2006; 19: 67-71.
24. Romeo L, Maranelli G, Malesani F, Tommasi I, Cazzadori A, Graziani MS. Tentative reference values for some elements in broncho-alveolar lavage fluid. *The Science of The Total Environment* 1992; 120: 103-10.
25. Goldoni M, Caglieri A, Corradi M, et al. Chromium in exhaled breath condensate and pulmonary tissue of non-small cell lung cancer patients. *Int Arch Occup Environ Health* 2008; 81: 487-93.
26. IARC. Chromium, Nickel and welding. IARC Monog Eval Carcinog Risk Hum, 49. International Agency for Research on Cancer, Lyon, France, 1990.
27. IARC. Beryllium, Cadmium, Mercury and exposures in the glass manufacturing Industry. IARC Monog Eval Carcinog Risk Hum, 58. International Agency for Research on Cancer, Lyon, France, 1993.
28. IARC. Some metals and metallic compounds. IARC Monog Eval Carcinog Risk Hum, 23. International Agency for Research on Cancer, Lyon, France, 1980.
29. IARC. Overall evaluation of carcinogenicity: an updating of IARC monographs volumes 1 to 42. IARC Monog Eval Carcinog Risk Hum, Suppl 7. International Agency for Research on Cancer, Lyon, France, 1987.
30. IARC. Inorganic and Organic Lead Compounds. IARC Monog Eval Carcinog Risk Hum, 87. International Agency for Research on Cancer, Lyon, France, 2006.
31. IARC. Chlorinated drinking water; chlorination by-products; some other halogenated compounds; Cobalt and Cobalt compounds. IARC Monog Eval Carcinog Risk Hum, 52. International Agency for Research on Cancer, Lyon, France, 1990.
32. IARC. Cobalt in hard-metals and cobalt sulphate, gallium arsenide, indium phosphide and vanadium pentoxide. IARC Monog Eval Carcinog Risk Hum, 86. International Agency for Research on Cancer, Lyon, France, 2003.
33. Chiba M, Masironi R. Toxic and trace elements in tobacco and tobacco smoke. *Bull World Health Organ* 1992; 70: 269-75.
34. Edelman DA, Roggli VL. The accumulation of nickel in human lungs. *Environ Health Perspect* 1989; 81: 221-4.
35. Torjussen W, Zachariassen H, Andersen I. Cigarette smoking and nickel exposure. *J Environ Monit* 2003; 5: 198-201.
36. Kalcher K, Kern W, Pietsch R. Cadmium and lead in the smoke of a filter cigarette. *Sci Total Environ* 1993; 128: 21-35.
37. Saratug S, Baker JR, Reilly PE, Moore MR, Williams DJ. Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. *Arch Environ Health* 2002; 57: 69-77.
38. Adachi S, Takemoto K, Ohshima S, Shimizu Y, Takahama M. Metal concentrations in lung tissue of subjects suffering from lung cancer. *Int Arch Occup Environ Health* 1991; 63: 193-7.
39. Takemoto K, Kawai H, Kuwahara T, Nishina M, Adachi S. Metal concentrations in human lung tissue, with special reference to age, sex, cause of death, emphysema and contamination of lung tissue. *Int Arch Occup Environ Health* 1991; 62: 579-86.

40. Kollmeier H, Seemann J, Wittig P, Rothe G, Muller KM. Cadmium in human lung tissue. *Int Arch Occup Environ Health* 1990; 62: 373-7.
41. Paakko P, Kokkonen P, Anttila S, Kalliomaki PL. Cadmium and chromium as markers of smoking in human lung tissue. *Environ Res* 1989; 49: 197-207.
42. Baumgardt B, Jackwerth E, Otto H, Tolg G. Trace analysis to determine heavy metal load in lung tissue. A contribution to substantiation of occupational hazards. *Int Arch Occup Environ Health* 1986; 58: 27-34.
43. Gerhardsson L, Brune D, Nordberg GF, Wester PO. Distribution of cadmium, lead and zinc in lung, liver and kidney in long-term exposed smelter workers. *Sci Total Environ* 1986; 50: 65-85.
44. Barbone F, Bovenzi M, Cavallieri F, Stanta G. Cigarette smoking and histologic type of lung cancer in men. *Chest* 1997; 112: 1474-9.
45. Drake II EN, Sky-Peck HH. Discriminant analysis of trace element distribution in normal and malignant human tissues. *Cancer Res* 1989; 49: 4210-5.
46. Kuo CY, Wong RH, Lin JY, Lai JC, Lee H. Accumulation of chromium and nickel metals in lung tumors from lung cancer patients in Taiwan. *J Toxicol Environ Health A* 2006; 69: 1337-44.
47. Raithel HJ, Ebner G, Schaller KH, Schellmann B, Valentin H. Problems in establishing norm values for nickel and chromium concentrations in human pulmonary tissue. *Am J Ind Med* 1987; 12: 55-70.
48. Svenes KB, Andersen I. Distribution of nickel in lungs from former nickel workers. *Int Arch Occup Environ Health* 1998; 71: 424-8.
49. Raithel HJ, Schaller KH, Akslen LA, Myking AO, Morkve O, Gulsvik A. Analyses of chromium and nickel in human pulmonary tissue. Investigations in lung cancer patients and a control population under special consideration of medical expertise aspects. *Int Arch Occup Environ Health* 1989; 61: 507-12.
50. Raithel HJ, Hennig F, Schaller KH. Quantitative determination of chromium and nickel in tumour and tumour-free human tissue. *J Environ Pathol Toxicol Oncol* 1989; 9: 115-26.
51. Tsuchiyama F, Hisanaga N, Shibata E, et al. Pulmonary metal distribution in urban dwellers. *Int Arch Occup Environ Health* 1997; 70: 77-84.
52. Raithel HJ, Schaller KH, Kraus T, Lehnert G. Biomonitoring of nickel and chromium in human pulmonary tissue. *Int Arch Occup Environ Health* 1993; 65: S197-200.
53. Akslen LA, Myking AO, Morkve O, Gulsvik A, Raithel HJ, Schaller KH. Increased content of chromium and nickel in lung tissues from patients with bronchial carcinoma. *Pathol Res Pract* 1990; 186: 717-22.
54. Wagner KA, McDaniel R, Self D. Collection and preparation of sidestream cigarette smoke for trace elemental determinations by graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry. *JAOAC Int* 2001; 84: 1934-40.
55. Rustemeier K, Stabbert R, Haussmann HJ, Roemer E, Carmines EL. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food and Chemical Toxicology* 2002; 40: 93-104.
56. Gerhardsson L, Brune D, Nordberg GF, Wester PO. Multielemental assay of tissues of deceased smelter workers and controls. *Sci Total Environ* 1988; 74: 97-110.
57. Garcia F, Ortega A, Domingo JL, Corbella J. Accumulation of metals in autopsy tissues of subjects living in Tarragona County, Spain. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2001; 36: 1767-86.

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