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ORIGINAL ARTICLE

Metallic elements in lung tissues: results of a meta-analysis

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Abstract. Several studies have investigated the levels of metallic elements in the pulmonary tissues of healthy subjects, patients with lung diseases and occupationally exposed subjects. The present meta-analysis was aimed at both assessing the possible contribution of metal exposure to the development of lung diseases, including lung cancer, and evaluating systematically the role and the weight of variability factors affecting the results of such studies. A literature research covering the period 1980-2007 was conducted using the public database PubMed. A standard scoring method was elaborated with a minimum score of 5 for inclusion and evaluation. Selected papers underwent a meta-analytical assessment. Fifty-eight papers were retrieved, but 21 of them could not be admitted to further analysis, due to failure to achieve the minimum score. The main limitations of individual studies included: limited sample sizes, poor control of smoking habits and differences in subjects' ages, lung tissue topography, sampling methods, storage procedures and data analysis. Copper and zinc were the most represented elements (121.96±0.74 and 12.98±0.07 μg/g dry weight, respectively). Among toxic metals, the highest concentrations were observed for chromium and lead (2.42±0.12 and 2.14±0.04 μg/g, respectively). Tissue concentrations were similar in unaffected tissues from both controls and lung cancer patients, whereas they were lower in lung tumor samples. A considerable intra and inter-individual variability was noted. Such a variability of measures, combined with the very low metal concentrations calls for the definition and use of standardized procedures of sample collection, storage, and analysis. (www.actabiomedica.it)

Key words: Meta-analysis, metallic elements, lung, lung cancer

Introduction

Several lung diseases and in particular lung cancer are associated to the occupational-environmental exposure to toxic and carcinogenic xenobiotics, including metallic elements. The mortality increase for lung cancer following the exposure to atmospheric pollutants released with autovehicle exhausts, lubricating oils and tobacco smoke, was esteemed to be around 8% (1).

Information about the causal connections among occupational-environmental exposures and lung pathologies is often difficult to gain, as objective and reliable exposure data are not always available. This was the reason leading some studies to the assessment of the cumulative dose of metallic elements in the pulmonary tissue in different conditions.

We performed a systematic revision of literature studies dealing with the determination of metallic elements in the pulmonary tissue, with the aim to characterize the average cumulative pulmonary dose of metallic elements, as well as the main variability factors interfering with the reported measures.

Each selected publication was carefully analyzed, applying standardized methods, considering the main characteristics of both subjects and tissue samples.

Methods

We selected the studies reporting the concentrations of metallic elements in the pulmonary tissue, published from 1980 to 2007 and quoted in the

PubMed database. The search was conducted with the following terms: "trace elements and pulmonary tissue"; "lung cancer and metals"; as additional tool, the "*related articles*" option of the database was used.

For every study, we analyzed by standardized criteria the following variables: year of tissue collection; country; subjects' characteristics [age, gender, smoking habits, health status, residential history, kind of exposure (occupational, environmental, accidental), occupational history, biomarkers of exposure, if available]; sampling and storage methods (autopsy *vs* biopsy; lung topography; amount of tissue; cutting tools; fixation solutions; freezing temperatures; lyophilization methods); analytical methods (tissue digestion; analytical technique; detection limits; no. and type of metallic elements) and quality assurance procedures; data expression by wet or dry tissue; descriptive statistics (distributions; percentiles; mean±standard deviation; median; range).

The selected publications were subsequently scored according to the criterions reported in table 1 and we admitted to further analysis only the studies obtaining a higher than 5 score. Data from such studies were analyzed by the Comprehensive Meta-Analysis (CMA-Biostat) software (2) that allows comparisons among different studies after loading of descriptive data (means, standard deviations and sample sizes). The software gave the effective weight and the range of interval for every study. The meta-analysis was limited to metallic elements more often reported in different studies, including As, Cd, Co, Cr, Ni, Pb, Cu, Se, Zn. For each element, we obtained the cumulative result of the meta-analysis. The data pooling reduced the inaccuracies of single studies.

Results

We retrieved 58 papers, including: (i) studies reporting exposure data for occupational or lifestyle (tobacco smoke) risk factors (3-30) [of these, 21 were case-control studies (10-30)]; (ii) studies on subjects affected by lung cancer, including both case-control studies (53-57) and determinations on both cancerous and unaffected lung tissues (58-60); (iii) studies on autoptic lung specimens from the general population (31-52).

After evaluation of the criteria reported in Table 1, 21 papers did not reach the score of 5 required for further analysis, so the final number of evaluated studies was 37. Table 2 summarizes the main sampling procedures and pre-analytical methods applied in the selected papers. Most of the determinations involved the right upper pulmonary lobe and tissues withdrawn during autopsy. Specimens were excised (information specified in 23 papers) using blades made of ceramics, titanium, quartz, copper-beryllium alloy, glass, plastics, stainless steel and Teflon. One study (10) demonstrated a tissue contamination by stainless steel, causing confounding on Ni determina-

Table 1. Evaluation criteria for retrieved articles and relative scores

Variables	Information available	Scores
Year of tissue collection	No	0
	Yes	1
Health status	No	0
	Yes (without concentration in sub-group)	1
	Yes (with concentration in sub-group)	2
Smoking habits	No	0
	Yes (without concentration in sub-group)	1
	Yes (with concentration in sub-group)	2
Sample storage	No	0
	Yes	1
Pre-analytical treatments	No	0
	Yes (synthetic description)	1
	Yes (detailed description)	2
Detection limits	No	0
	Yes	1
Quality assurance	No	0
	Yes	1
Data distribution analysis	No	0
	Yes	1
	One parameter	0
	Two or more parameters	1
Inferential statistical analysis	No	0
	Yes	1

Table 2. Sampling, storage and pre-analytical treatments of lung tissue specimens in selected studies

Refs	Localization	Source	Conservation	Pre-analytical treatment
3	Random	A	Formalin, Na_2HPO_4	105°C for 24 h; $HClO_4$, HNO_3, H_2SO_4
10	RLL	A	Formalin, Na_2HPO_4	Washing thrice with D. W., 105°C for 19 h, acid digestion
12	RUL	A	−20°C	Crushing with mortar of quartz
14	RUL	A	Frozen-dried	Crushing with mortar of quartz
15	RUL	A	Frozen-dried	NA
16	RUL	A	Frozen-dried	NA
17	RUL	A	Frozen-dried	NA
18	RUL	A	NS	NA
19	RUL	A	Frozen-dried	H_2SO_4, H_2O_2
20	RUL	A	Frozen-dried	NA
21	RUL	A	Frozen-dried	NA
22	RUL	A	-20°C	110°C O.N., acid digestion
23	Random	A	Frozen-dried	HNO_3, $HClO_4$
25	R vs L	A	Precedent study	Oxidative digestion
26	Random	A	Frozen-dried	Oxidative digestion
28	RML	B	Frozen-dried	Homogeneization
29	All lobes	A	Formalin 4 %+ Na_2HPO_4	D.w.; 105°C for 19h; HNO_3, $HClO_4$
30	RUL	A	Frozen	Crushing with mortar of quartz
31	RLL	A	−18°C	105°C for 19h; HNO_3, $HClO_{4,}H_2SO_4$
38	RUL	A	Frozen	105°C for 19h; HNO_3, $HClO_{4,}H_2SO_4$
40	NS	B	Frozen	Homogeneization
41	LUL	A	−20C°	NA
42	RUL	A	−20C°	Lyophilization
45	All lobes	A	NA	HNO_3, $H2_SO_4$
46	UL	A	Fresh tissue	Drying, HNO_3, $HClO_{4,}$
47	UL	A	Fresh tissue	Drying, HNO_3, $HClO_{4,}$
49	RUL	A	Formalin	110°C for 12 h, HNO_3, $HClO_{4,}$
50	All lobes	A	−30°C	Drying, HNO_3, $HClO_{4,}$
52	NS	A	Frozen	Homogeneization
53	RUL	A	Formalin	HNO_3, $HClO_{4,}$
54	Random	A*, B**	Formalin	HNO_3, $HClO_{4,}$
55	RUL	A	Lyophilization	Incineration, chelation, extraction
56	RUL	A	Lyophilization	Incineration, chelation, extraction
57	All lobes	A	Fresh tissue	HNO_3, H_2SO_4
58	Random	A	Alcohol	Lyophilization
59	Random	B	−80°C	Lyophilization
60	Random	A	−20°C	Homogeneization

RLL: Right lower lobe; RUL: Right upper lobe; LUL: Lower upper lobe; UL: Upper lobe; R: Right; L: Left; RML: Right medium lobe; A=Autopsy; B: biopsy (*healthy tissue; **cancer tissue); O.N.: overnight; D.W: deionized water; NA: not available

tion. Most of the studies analyzed frozen tissues and a minority formalin fixed tissues. Tissue concentrations of metallic elements were mostly expressed by dry weight tissue. One author (25, 45, 57) reported metal levels by both dry and wet weight and concluded that concentrations expressed by dry tissue were about five times higher than those expressed by wet tissue weight. Analytical methods can affect the sensitivity of the determinations. Atomic absorption (AAS) was the most widely used technique (10, 17, 19-21, 22, 23, 25, 26, 29, 31, 42, 45, 49, 50, 53, 55-59), followed by instrumental neutron activation analysis (INAA) (12, 14-17, 19-21, 28, 40, 41, 52), inductively coupled mass spectrometry (ICP-MS) (3, 30, 38, 47) or atomic emission spectroscopy (ICP-AES) (46, 54) and energy dispersive X-ray fluorescence (EDXRF) (60). The majority of studies (12, 16, 17, 19, 21, 22, 25, 26, 29, 31, 38, 40-42, 45-47, 49, 50, 53-56, 58, 59) followed quality assurance procedures. Table 3 resumes studies including reference subjects,

Table 3. Main characteristics of reference subjects in selected studies

Refs	Source	Gender (N)	Age§	Metallic elements	Smoking*
10	GP	NA(16)	NA	Ni	No
12	GP	NA(8)	43-72	Sb, As, Cd, Cu, Cs, Cr, Co, Pb, Mn, Hg, Au, La, Mo, Fe, P, Sc, Se, Ag, Te, Sn, W, Zn	Yes
14	C	M(11)	67.5	Sb	Yes
15	C	M(14)	67.9	Cr, Co, La	Yes
16	C	M(25)	68.25	Se, Sb, As, Cd, Cr, Co, La, Pb	Yes
17	C	M(26)	68.25	Cd, Zn, Pb	Yes
18	C	M(25)	68.25	Sb, As, Cd, Co, Cr, La, Se, Pb	Yes
19-21	C	M(25)	68.25	Sb, As, Cd, Co, Cr, La, Se, Pb, Zn	Yes
22	C	M(16)	70.6	Cd,Cu, Zn	No
23	C	M(4) F(1)	59-78	Cd,Cu,Zn	Yes
25	C	M(29) F(1)	NA	Ni, Cr	No
26	C	M(29) F(1)	49	Ni, Cr	Yes
28	C	NA (4)	NA	Sc, La, Ce, Nd, Sm, Eu, Tb, Yb, Lu, Hf, Th, U	No
29	C	M(5) F(5)	1-88	Ni	No
30	C	M(11)	67.4	As, Se	Yes
31	GP	M(23) F(27)	70.6	Ni	No
38	GP	M(57) F(21)	56±20	As, Cd, Co, Cr, Cu, Hg, Pb, Mn, Ni, Sn, Zn, V	Yes
40	GP	M(10) F(4)	20-79	Se, Hg, Cs, Sc, Rb, Zn, Fe, Co, Sb	No
41	GP	M(64)	6-82	Cl, Co, Cu, Fe, K, Mn, Na, Rb, Se, Zn, As, Sb, Al, La, Ca, Mg, Cd, Mo, Cs, Sc	No
42	GP	M(61) F(39)	26-92	Ni, Cr	No
45	GP	M(14) F(1)	17-83	Ni, Cr	Yes
46	E.	M(45)	67	Ni, Cr	Yes
47	GP	M(45)	67.6±9.3	Cd	Yes
49	GP	M(1353) F(778)	30-99	Fe, Ca, Mg, Zn, Cu, Co, Ni, Pb, Cr	No
50	GP	M(12) F(5)	64	Al, Cd, Cr, Ni, Pb, Mn, Cu, Zn	Yes
52	GP	M(24)	35-60	As, Cd, Hg	No
53	GP	M(1003) F(712)	61.1	Al, Cd, Cr, Ni, Pb, Mn, Cu, Zn	No
54	C	M(43)	67	Cr	Yes
55	GP	NA(25)	NA	Cr, Ni	No
56	GP	M(50) F(37)	25-92	Cd	No
57	GP	M(8) F(11)	37-88	Cr, Ni	Yes

GP: general population; C: controls; M: male; F: female; NA: not available; PE: pulmonary emphysema; §Means (±standard deviation) or ranges of age distributions; *availability of information about smoking habits

investigated with the aim of: (i) defining reference values of metal concentration in pulmonary tissues; (ii) assessing differences as compared to either occupationally metal exposed or lung cancer subjects. Most of the studies reported gender and age distributions, but only few of them showed the distributions of metallic elements by gender and age. The information about smoking habits was available in a high number of studies; nevertheless only five of them extrapolated metal concentrations in subjects classified by smoking habits (as current smokers, not smokers, ex-smokers) or in relationship with tobacco consumption (as pack/years) (26, 38, 45, 46, 47). Table 4 resumes the main characteristics of occupationally metal exposed subjects (all males, in advanced age) investigated by cohort or case-control studies. A research group in particular published several reports (14-22) on the post-mortem lung tissues concentrations of some elements in foundry workers, collected from 1970 onward. Table 5 summarizes the main characteristics of patients investigated by studies reporting metal concentrations in lung cancer. Some studies investigated the cancerous (53-57) and others the unaffected lung tissues (58-60). Adachi and Kollemeier (53, 55) provided metal tissue concentrations in subjects stratified by lung cancer histotype.

Table 4. Main characteristics of metal exposed subjects (there were only males) in selected studies

Refs	Occupational setting	N	Age§	Metallic elements	Smoking*
3	Refinery	1	64	Ni, Co, Cu, Pb, Cr	No
10	Refinery	39	66±8	Ni	Yes
12	Foundry and refinery	21	55-76	Sb, As, Cd, Cu, Cs, Cr, Co, Pb, Mn, Hg, Au, La, Mo, Fe, P, Sc, Se, Ag, Te, Sn, W, Zn	Yes
14	Foundry	40	66.6	Sb	Yes
15	Foundry	66	68.7	Cr, Co, La	Yes
16	Foundry	76	66-71	Se, Sb, As, Cd, Cr, Co, La, Pb	Yes
17	Foundry	86	68.3±9	Cd, Zn, Pb	Yes
18	Foundry	76	66-71	Sb, As, Cd, Co, Cr, La, Se, Pb	Yes
19	Foundry	85	66-71	Sb, As, Cd, Co, Cr, La, Se, Pb, Zn	No
20	Foundry	115	68.3±9.1	Sb, As, Cd, Co, Cr, La, Se, Pb, Zn	No
21	Foundry	85	66-71	Sb, As, Cd, Co, Cr, La, Se, Pb, Zn	No
22	Foundry	32	67.4± 9.5	Cd, Cu, Zn	No
23	Plating	1	62	Cd, Cu, Zn	No
25	Foundry (6) Welding (3) Refinery (1)	10	NA	Ni, Cr	No
26	Refinery (11) Welding (3) Foundry (1)	15	NA	Ni, Cr	No
28	Mine	13	NA	Sc, La, Ce, Nd, Sm, Eu, Tb, Yb, Lu, Hf, Th, U	No
29	Refinery	15	52-91	Ni	Yes
30	Foundry	40	66.6	As, Se	Yes

N.A.: information not available; §Means(±standard deviation) or ranges of age distributions; *Availability of information about smoking habits

Table 5. Main characteristics of lung cancer patients in selected studies

Refs	Gender	Age§	Metallic elements	Smoking*
53	M(174) F(50)	63	Al, Cd, Cr, Ni, Pb, Mn, Cu, Zn	No
54	M(53)	61±10	Cr	No
55	M(5)	44-76	Cr, Ni	No
56	M(7)	44-76	Cd	No
57	M(13) F(2)	50-84	Cr, Ni	Yes
58	M(15) F(5)	62¶	Cr, Ni	No
59	M(59) F(5)	60±7	Cu, Zn, Mg, Ca, Fe	No
60	NA(16)	NA	Ca, V, Mn, Fe, Ni, Cu, Zn, Se, Cr, Ru, Sr, Pb, Hg, As, Mo, Br	No

M: male; F: female; NA: information not available; §Means(±standard deviation), ranges, or ¶median of age distributions; *Availability of information about smoking habits

Figures 1 and 2 resume the results of the meta-analysis of metal concentrations in reference subjects (C) and in lung cancer patients, in the latter case considering both unaffected (U) and cancerous (T) tissues. Zn and Cu were the most represented tissue elements (mean values of 121.96±0.74 and 12.98±0.07, respectively), followed by Cr and Pb (mean values of 2.42±0.12 and 2.14±0.04, respectively). Whereas metal concentrations were almost similar for several elements (Cr, Ni, Pb, Co, Cu, Zn) in control and unaffected tissues, they tended to be lower in tumor samples for Cu, Zn, Cr and Ni. Studies not reporting indices of data dispersion were not considered by the statistical software. They include investigations on workers occupationally exposed to metal and the relevant results are summarized in table 6.

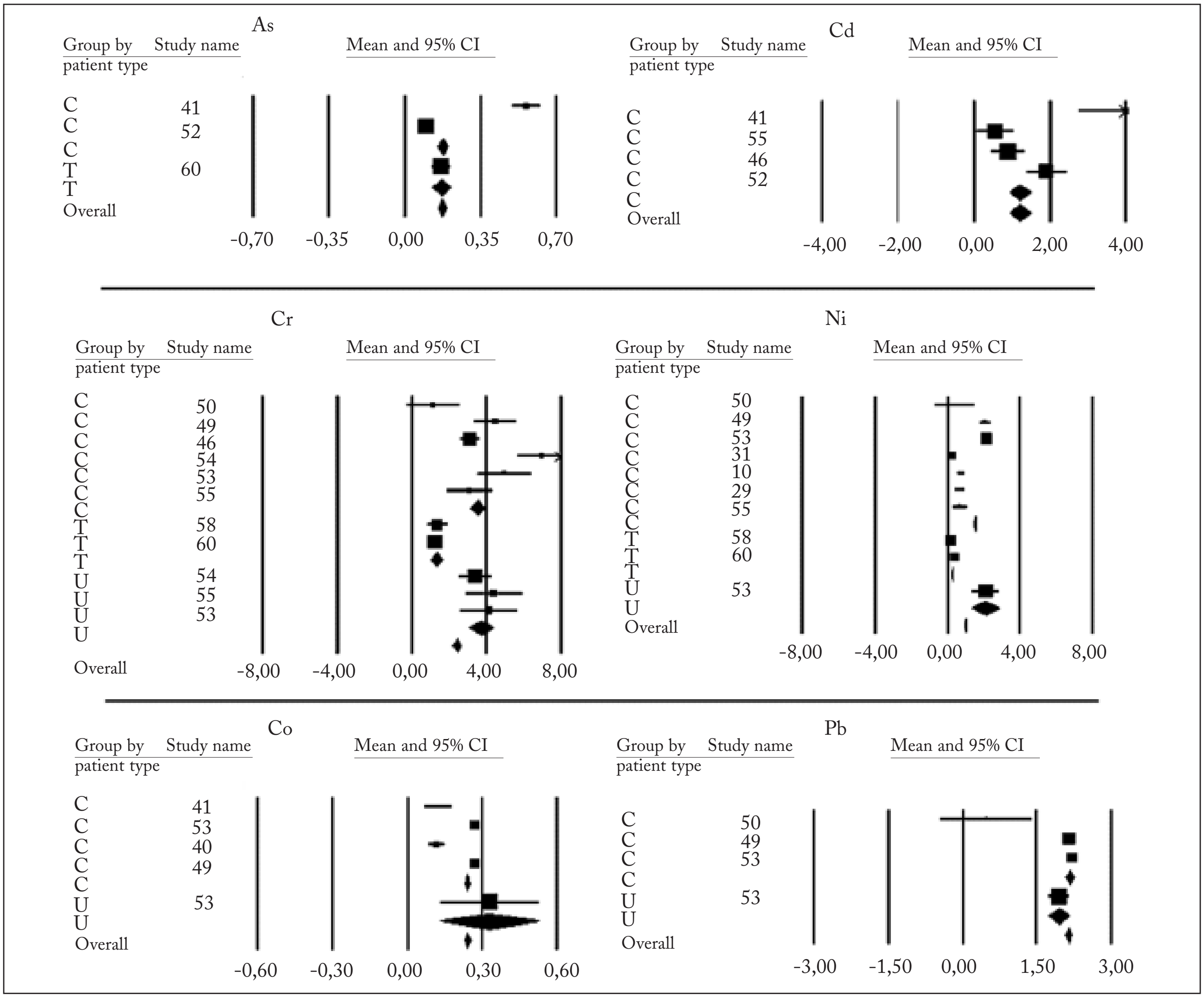

Figure 1. Meta-analytical results for As, Cd, Cr, Ni, Co and Pb (expressed as μg/g dry weight) in tissues of reference subjects (C), cancerous (T) and unaffected (U) lung tissue of subjects with diagnosis of lung cancer. The horizontal lines represent the confidence intervals of the means, the central points the arithmetic means, the diamonds the cumulative results for different groups of samples and, at the bottom, for the overall sample. The thickness of the symbols is proportional to the sample sizes

Discussion

The main aim of the study was to evaluate the levels of reported concentrations of metallic elements in the lung tissue, as factors involved into the development of lung cancer. The meta-analysis showed that the most represented elements in lung tissue were Zn and Cu. Both these transition elements play essential physiological roles including that of enzyme cofactors, in particular of the Cu/Zn superoxide dismutase, that is highly expressed in the lung tissue and is involved in the scavenging of the superoxide radical formed in the cytosol. Among elements with toxic properties, Cr and Pb showed the highest concentration levels. An interesting issue and somewhat unexpected finding was that, with the exception of As and Se, the metal (Cu, Zn, Cr and Ni) concentrations reported for tumor samples were lower than observed in tissues from both reference subjects and unaffected samples from patients suffering lung cancer. This finding can possibly rely on the cyto-histological modifications of the cancerous as compared to the healthy lung tissue and

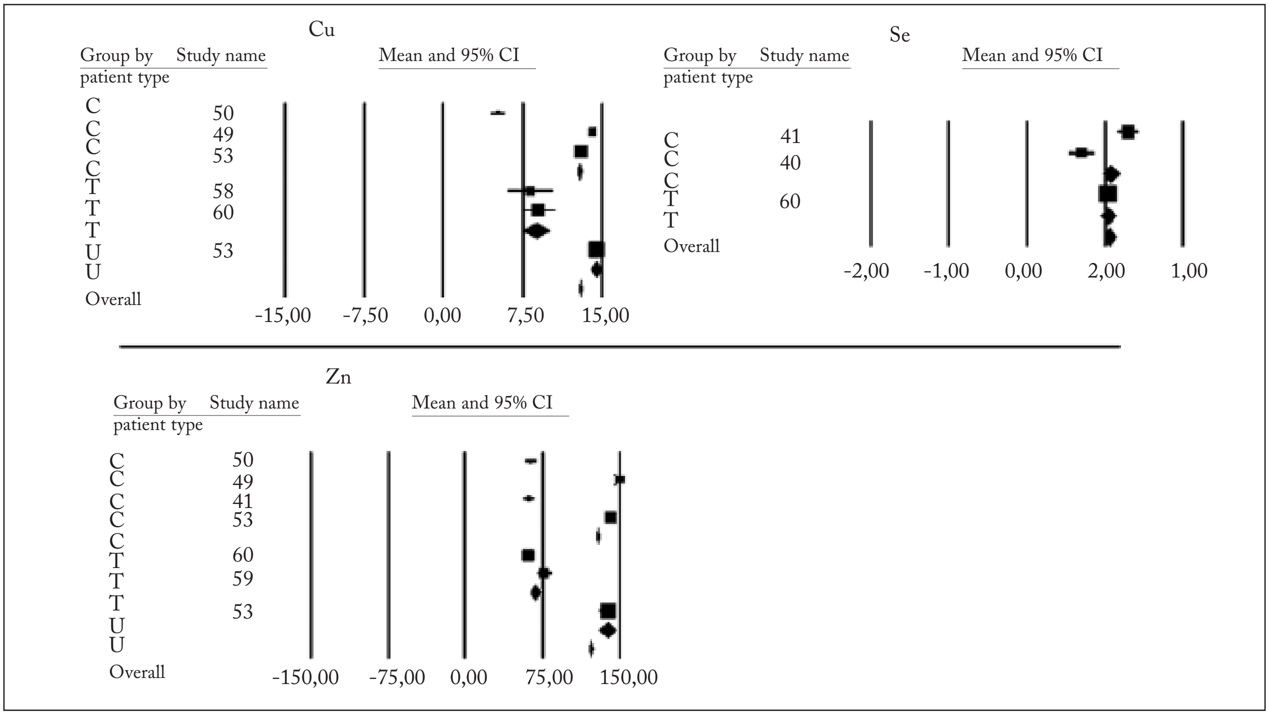

Figure 2. Meta-analytical results for Cu, Se, Zn (expressed as µg/g dry weight) in tissues of reference subjects (C), cancerous (T) and unaffected (U) lung tissue of subjects with diagnosis of lung cancer. The horizontal lines represent the confidence intervals of the means, the central points the arithmetic means, the diamonds the cumulative results for different groups of samples and, at the bottom, for the overall sample. The thickness of the symbols is proportional to the sample sizes

therefore in systematic errors of expression of the results as weight/weight. If this would hold true, then the expression of results by specific weight could limit the bias. Moreover, the cyto-histological modifications of the tumor tissue and the necrotic and proliferative phenomena characterizing its development and progression may reduce the representativeness of tumor samples with reference to past exposures to carcinogenic compounds (including metal elements).

In our analysis, we considered potentially interfering factors, such as gender, age, lifestyle (smoking habits) and occupational exposures, lung cancer and lung topography. Few studies defined the lung tissue concentration of metallic elements as a function of gender in the general population, often with inconsistent results. Andersen (31) did not find differences of Ni levels between both genders. Tsuchiyama (50) compared the concentration of 8 elements (Cd, Cr, Pb, Mn, Cu, Zn, Al, Ni) in both genders and found higher Al and Cr concentration in males, as previously shown by Takemoto (49) and Kollemeier (56), but without considering confounding factors, such as smoking habits and past occupational history for metal exposure. Kollemeier and collaborators (55, 56) found higher Cr, Ni and Cd concentrations in not occupationally exposed males as compared to females, but differences were statistically significant only among residents in highly environmentally polluted zones. In conclusion, data about the interference by gender on metal lung tissue concentrations are still inconclusive, due to the lack of proper statistical evaluations.

Even the relationship between metal concentration in lung tissue and age is still unclear in studies on general population. Kollmeier (55) evidenced a positive relationship between both pulmonary Ni and Cr concentrations and age (with average increase of 2.45% /year for Cr and 3.0% /year for Ni) but not for Cd (56). Raithel (42) confirmed the relationship between Cr (but not Ni) and age, also among occupa-

Table 6. Descriptive results of studies investigating occupationally metal exposed groups. Data are reported as means, ranges (min-max) and medians* of distributions

Metallic elements	Refs.	Occupational settings	N	Descriptive data (μg/g tissue)	
				Tissue dry wt.	Tissue wet wt.
Ni	10	Refinery	39	149.0	
	29	Refinery	10	58.5; 15.0*	
	26	Refinery	11		2.1-3.9
	3	Refinery	1	14.2	
	26	Welding	2		2.6-8.5
Cr	26	Welding	2	0.6-36.7	0.8-4.8
	23	Chrome plating	1		4.53*
	3	Refinery	1	0.18	
	26	Refinery	11	0.79-3.15	0.09-0.18
	12	Foundry	21		0.29* (0.05-11.0)
	15	Foundry	66		0.42*
	16, 18, 20	Foundry	76		0.41*
	19, 20	Foundry	85		0.45*
As	12	Foundry	21		0.05* (0.01-0.21)
	16, 17, 20	Foundry	76		0.04*
	19, 20	Foundry	85		0.03*
	30	Foundry	40		0.05*
Cd	12	Foundry	21		0.25* (0.04-1.4)
	16, 17, 20	Foundry	76		0.16*
	16, 19, 20	Foundry	85		0.16*
	22	Foundry	32		0.21* (0,03-0,86)
Se	12	Foundry	21		0.13* (0.001-1.56)
	16, 17, 20	Foundry	76		0.15*
	20	Foundry	85		0.15*
	30	Foundry	40		0.15; 0.13*
Cu	12	Foundry	21		1.04* (0.06-2.15)
	22	Foundry	32		1.17* (0.27-3.36)
	23	Plating	1		0.55
Zn	23	Plating	1	12.5	
	12	Foundry	21		3.1* (1.0-9.9)
	16, 19, 20	Foundry	85		11.5*
	22	Foundry	32		10.8* (1.70-26)

tionally metal exposed subjects (58). On a limited group of copper foundry workers, Gerhardsson (22) failed to significant relationships among Cu, Zn, Cd and age or exposure duration.

According to Kollemeier (55), tobacco smoke would contribute to the pulmonary accumulation of metallic elements because of its intrinsic metal content but also by inducing a failure in mucociliary clearance mechanism and favoring the development of pulmonary pathologies (as emphysema) that alter the lung structure and physiological clearance processes. Tobacco smoking is consistently associated to the lung accumulation of Cd and Cr, whereas its relationships with Ni and Pb lung levels are still unclear. Tsuchiyama (50) found higher pulmonary concentrations of Al, Cd, Cr, Ni, Pb and Mn among smokers with possible past occupational metal exposure, as compared to not smokers and possibly unexposed subjects. In non cancerous lung tissues, Anttila (54) measured statistically higher Cr levels in smokers, as compared to not smokers, and positive relationships among Cr levels and both smoking duration and tobacco consumption (as pack/year). The absence of these relationships in cancerous tissues, led the author to conclude for the carcinogenic role of other exposures. Alternatively, we hypothesize that this could be related to the abovementioned morphological and structural changes of tumor tissues. Both Raithel (26, 45, 57) and Andersen (10) failed to find significant correlations among Ni concentrations and smoking habits. Kollemeier (56) showed greater lung tissue Cd concentrations (about four times) in smokers, as compared to not smokers and concluded that the principal source of Cd accumulation in lung tissue was represented by tobacco smoke. He estimated that a subject smoking about 20 cigarette/day accumulates about 1.6 μg of Cd per day, as compared to about 0.25 μg/day for a resident in an industrial area exposed to air Cd concentration of about 0.05 $\mu g/m^3$.

Many authors have demonstrated the tendency of some metallic elements, particularly Cr and Ni, to accumulate in the lung of people occupationally exposed to metals. In his several studies on a cohort of ex foundry-workers, Gerhardsson (14-20) highlited significantly higher concentrations of all the investigated metallic elements (with the exception of Zn) in workers, as compared to controls. Some of the elements (Sb, Cr, Co, La) did not show the tendency to decrease after the end of exposure. The subjects were defined as exposed mainly on the basis of their general job title (foundry workers, nickel refinery workers, welders, etc), even if it is known that a same job in different contexts can give rise to different exposures. Dufreshne et al. (5) demonstrated the lung accumulation of silicates in an electrician employed in the extractive industry and of Ni in an electrician employed in a private activity.

The determination of metallic elements in the lung tissues of patients affected by lung cancer does not allow to draw any conclusive indication about their causal role in the development of the disease. Most of the studies investigating the relationships between accumulation of metallic elements and lung cancer gave rise to inconsistent results. Several studies (23, 42, 54, 58, 57) demonstrated significantly higher Cr concentrations in lung tissue from subjects with lung cancer, as compared to controls, confirmed even by Adachi (53) limitedly to males. The same author (53) determined higher Cu and lower Zn and Mg concentrations in the tissues from lung cancer patients, as compared to controls and hypothesized physiological fluctuations of the elements in the blood of these patients (61, 62). The main Cr source for subjects not occupationally exposed to metals would be represented by cigarette smoke. Akslen (58), but not Diez, (59) or Andersen (10) or Svenes (29), demonstrated significantly higher Ni concentrations in subjects suffering lung cancer, as compared to people dead for other causes (58). Kollmeier (42, 55, 56) found higher Cd, Cr and Ni concentrations in subjects with lung cancer, as compared to controls. Diez (59) showed higher Cu, Ca and Mg concentrations, and a greater Cu/Zn ratio in the cancerous tissue as compared to both the unaffected tissue of lung cancer subjects and the controls' tissue. No relationship was found among tissue metal levels and histotype or tumor staging. Drake (60) found lower levels of Cr, Pb, Cu and Zn levels in the cancerous tissues as compared to unaffected tissues. With regard to cancer histology, Adachi (53) and Akslen (58) signaled higher Cr and Ca concentrations in subjects affected by squamocellular carcinoma.

It seems that lung tumors causally related to the inhalation of carcinogens affect the central rather than peripheral part of the lung, with a prevailing localization at the bronchial bifurcations (6). Some authors reported higher Cr levels in the upper lobes, as compared to the lower ones. Both Tsuchiyama (50) and Raithel (25) found higher metal concentrations (Cu, Zn, Al, Cr, Ni, Pb, Mn in the study of Tsuchiyama and Ni and Cr in that of Raithel) in the superior/medium lobes, probably related to their higher ventilation. The analysis of the selected studies allowed us to draw important methodological information. A considerable intra and inter-individual variability in the concentration of metallic elements in the pulmonary tissues (8, 42, 45, 51) exists and this, together to the very low concentrations, asks for the definition of standardized procedures of tissue collection, storage and analysis (8), to get the most reliable tissue metal concentrations.

The following precautions should be adopted during the sampling and analytical phases: (i) use of cutting tools made of quartz, glass or titanium (avoiding steel made tools) (31); (ii) use of plastic materials for the sample packing and storage; (iii) withdrawing of samples always from the same areas; (iv) tissue desiccation can minimize the variability of different water contents. It was clearly stated by several authors the importance to specify the sampling area and to make comparisons among corresponding pulmonary areas. It is not possible, at this point, to define the best storage method for samples. Nevertheless, when the tissue is fixed in formalin, it is opportune to exclude its contamination by metallic elements. Some authors (26,45) sustain that formalin fixation can lead to an underestimation of Ni concentration, because of ionization, but others (31) showed that Ni determination was comparable on both frozen and formalin fixed tissues. Different authors underlined in their studies as the expression of the data on dry weight was the most qualified method; nevertheless, for a correct comparison, it was important to optimize and to conform the desiccation technique to fresh (frozen) or formalin-fixed tissues. As it concerns the analytical technique, it is essential that both the detection limits and the quality assurance procedures are clearly defined.

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