ORIGINAL ARTICLE

Residual fibre lung burden among patients with pleural mesothelioma who have been occupationally exposed to asbestos

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ABSTRACT

Objectives To evaluate the lungs asbestos fibres concentration in participants with malignant pleural mesothelioma (MPM) who have been occupationally exposed.

Methods The lung samples were obtained from pleuropneumonectomies or autopsies of 271 male MPMs. The lung samples were examined through scanning electron microscopy. Retrospective assessment was used to assess for asbestos exposure. This study includes 248 MPMs with an occupational exposure defined as either 'definite' or 'probable' or 'possible'.

Results The participants had finished working in asbestos exposure conditions more than 20 years ago (on average 26.1±11.0 years). The fibre burden resulted with a geometric mean equal to 2.0 (95% CI 1.6 to 2.4) million fibres per gram of dry lung tissue. The burden was higher among participants employed in asbestos textiles industry and in shipyards with insulation material, if compared with construction workers or non-asbestos textile workers or participants working in chemicals or as auto mechanics. 91.3% of MPMs had a detectable amount of amphibole fibres. A strong lung clearance capability was evident among workers exposed to chrysotile fibres. Owing to that, the 1997 Helsinki Criteria for occupational exposure were reached in <35% of cases among participant working in construction, in metallurgical industry, in chemical or textile industry and among those performing brake repair activities.

Conclusions The MPM cases are now occurring in Italy in participants who ceased occupational asbestos exposure decades before the analysis. A large majority still shows a residual content of amphibole fibres, but given the lung clearance capability, attribution to occupational exposure cannot rely only on fibres detection.

What this paper adds

- ► The residual asbestos fibre lung burden was investigated in a large set of malignant pleural mesothelioma cases related to asbestos occupationally exposed workers, whose exposure ceased several years before the tissue analysis.
- ► Given the time passed between the exposure and the tissue analysis, the burden of lung fibres represents only partially the cumulative exposure, since only amphibole fibres persist in the lungs, whereas chrysotile fibres largely disappear, due to the difference in lung clearance rates of these two asbestos fibres types.
- ► Considering the fibre burden in patients with a work history as asbestos occupationally exposed workers, a significant amount of fibre burden was detected in a large number of these patients, but in some cases it was below the threshold proposed to be considered as significant of occupational exposure.
- ► The pre-eminent importance of gathering information on occupational asbestos exposure, in the framework of a health surveillance system, is stressed.

INTRODUCTION

Asbestos is a term, encompassing different silicate minerals. Their properties made asbestos a widely used material. In several applications and products a mixture of different type of fibres was used.

Consumption of asbestos has been high in Italy, reaching a peak in the 1970s and lasting longer than in other industrialised countries.[1] Restrictions on the use of crocidolite were introduced in 1986, in accordance with European Union (EU) Directives, and in 1992 any new use of asbestos was banned.

Malignant pleural mesothelioma (MPM), an aggressive cancer of the membranes lining the lungs, is strongly associated with inhalation of all types of asbestos fibres, and occurs after a long latency (median 44.6 years among males; 45.2 years among females).[1]

Despite the ban, in Italy the incidence of MPMs is still increasing.[2]

The content and types of asbestos fibres retained in the lungs are the result of a dynamic process, which is not yet completely understood, which includes their deposition, accumulation, transposition, biotransformation and clearance.[3 4]

Amphibole fibres are biopersistent, although they are eliminated from the lungs at a relatively slow rate.[5 6] Chrysotile fibres are less durable so that the amount of retained chrysotile fibres mostly reflects recent exposures.[7 8]

The amount and characterisation of fibres through an electron microscope (either by applying Scanning – SEM or applying Trasmission – TEM) in the lungs of patients with malignant mesothelioma (MM) with occupational exposures[9 10] and studies

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comparing the retained asbestos fibres concentration in individuals with known exposures against patients with MM with improbable asbestos exposures suggested a likely relation between the lung fibre burden amount and the risk of MM. There have also been attempts to establish threshold values for a causal attribution between MM occurrence and the lung fibre burden amount.[4 11 12]

The opinion of the groups convened at the Finnish Institute of Health created a reference document. The first Helsinki Criteria stated that analysis of lung tissue for asbestos fibres can provide data to supplement the occupational history, and that a threshold value of 'over 1 million amphibole fibres (>1 μm) per gram of dry lung tissue as measured by electron microscopy in a qualified laboratory' can 'identify persons with a high probability of exposure to asbestos dust at work' with no mention on the type of electron microscopy to be used.[11] The updated criteria modified the statement as follows: a 'history of significant occupational, domestic or environmental exposure will suffice for attribution'; the threshold suggested for attribution should be based on 'a lung fibre count exceeding the background range for the laboratory in question'. The 'higher clearance rates for chrysotile' is mentioned as a caveat.[13]

The analysis through an electron microscope is based on a technique well established,[14] but all aspects, ranging from the preparation of the sample to the analysis, may influence the results.[15]

We present the results of the residual lung burden of asbestos fibres among MPMs occurring nowadays in Italy for workers who ceased asbestos exposure because of the 1992 ban. The combination of the retrospective exposure assessment and the residual amount and type of asbestos fibres was analysed to provide insights on the link between lung fibre burden and occupation performed, overall and in each job sector.

MATERIALS AND METHODS

Participants under study

During the period 2001–2014, lung tissue samples from 316 MPM cases were provided for asbestos fibre analysis to a single laboratory.

The samples were collected from patients who underwent pleuropneumonectomy at chest surgery units or were examined postmortem in areas of North East Italy (Province of Brescia and Mantua, Lombardy; Province of Padua and Venice, Veneto; Province of Gorizia and Trieste, Friuli Venezia Giulia). All MMs occurring in these areas have been investigated by Regional Centres of the Italian National Mesothelioma Registry (ReNaM), and eventually classified for diagnostic certainty and asbestos exposure, following national guidelines.[16]

The presentation is restricted to 271 'definite' MPMs (121 cases derived from surgery, 150 from autopsies), which means morphological and usually immunohistochemical staining features typical of MM, as judged by the referring pathologist, which occurred in participants classified as asbestos occupational exposed workers.[16]

Occupational exposure to asbestos

Information on asbestos exposure was derived from face-to-face interviews with the individuals or with the relatives, if the individuals were severely ill, refusing or dead.

Interviews were conducted by trained staff using a predefined questionnaire.[16] A chronological description of all jobs lasting at least 6 months was requested. The questionnaire collects information about residence conditions, the presence of asbestos-containing materials at home, and the occupations of persons with whom the patients lived. Additional information is usually gathered through the employment records of the National Insurance Institute (Istituto Nazionale Previdenza Sociale; INPS), enquiries about the workplaces where the patients were employed, and contacts with coworkers.

Exposure to asbestos was assessed by the hygienist of the local Mesothelioma Registry and classified according to the circumstances as occupational, household, environmental, hobbies, unlikely or unknown.

Table 1 Asbestos exposure parameters applied in the JEM (a) and definition of the variables used to assess occupational asbestos exposure (b)

(a) Asbestos exposure parameters used in the JEM		
Parameters for each single task		
	Per cent	**Weight (α)**
Probability of exposure		
Possible	<30	0.15
Probable	30–70	0.5
Certain	>70	0.85
Intensity of exposure	ff/mL	Weight (β)
Low	<0.1	0.05
Medium	0.1–1	0.55
High	1–10	5.5
Very high	>10	20
Parameters for the entire occupational history		
Frequency of prevalent exposure	**Per cent**	**Weight (η)**
Occasional	<50	0.25
Continuous	>50	0.75
Type of asbestos prevalent exposure	**Per cent**	**Weight (λ)**
Indirect	<50	0.25
Direct	>50	0.75
(b) Definition		
Asbestos fibres	Analyses through a scanning electron microscope equipped with an X-ray fluorescence microanalyser: fibres counted when length >1 μm and aspect ratio 3:1; identification as commercial asbestos (amosite, crocidolite, anthophyllite and chrysotile) or tremolite structures (available for 271/271 cases).	
CEI	The sum of the product of each performed task for probability and intensity, and overall weighted for frequency and type of exposure (available for 207/271 cases).	
Duration of work	Years spent in each job sector (available for 246/271 cases).	
AFE	Age at the first job involving asbestos exposure (available for 270/271 cases).	
TSLE	Years between the availability of tissue sample and the end of the last job involving asbestos exposure (available for 270/271 cases).	

AFE, age at first exposure; CEI, Cumulative Exposure Index; JEM, job-exposure matrix; TSLE, time since first exposure.

If multiple activities have been performed during life, the person was assigned to the one carrying the highest contribute to the cumulative asbestos exposure (see job sectors in table 1).

The collection of the activities performed enabled us to calculate a Cumulative Exposure Index (CEI).[17 18] In a first assessment, each job and activity performed was ranked into semiquantitative parameters related to the exposure probability (possible, probable, certain) and according to the exposure intensity in ff/mL (low, medium, high and very high) (see online supplementary appendix).[19] Then, for each participant, the jobs and activities have been rated for the prevalence of asbestos exposure (occasional or continuous) and the type of asbestos contact (direct: activities implying a manipulation of asbestos fibres/materials; indirect: exposures due to an environmental contamination at the workplace).

Thus, CEI is defined as:

$$\mathrm{CEI(k)} = \left[\sum_{j=1}^{n} (\alpha_j(k) \times \beta_j(k) \times d_j(k)\right] \times \eta(k) \times \lambda(k) \quad (1)$$

where the exposure related to the kth worker is the sum of the duration (d) related to n jobs, each one weighted for probability (α) and intensity (β); the resulting sum was multiplied by the prevalent frequency (η) and the type (λ) of asbestos contact (parameters and details in table 1).

Lung fibre burden measurement

Lung tissue was selected from tumour-free parenchyma and, whenever possible, samples from different lung lobes were taken and stored in a formaldehyde solution.

The lung samples from the same patient were pooled together and freeze dried. About 50 mg of freeze-dried lung was completely ashed at low temperature with an oxygen plasma asher (Emitech K1050X) to remove organic matter. The ash was suspended in a solution made of 20 mL distilled water and 20 mL isopropyl alcohol vigorously shaken; the solution was filtered using a polycarbonate filter (0.2 μm pore size). The filter was ashed at low temperature (at the same power settings as above) and the ash suspended in 48 mL distilled water plus 2 mL ethyl alcohol. Two polycarbonate (0.2 μm pore size) filters were prepared, the first with 10 mL and the second with 20 mL of solution. The filter with the best load of particles was used for the analysis. Analysis was carried out using an SEM equipped with an X-ray fluorescence microanalyser at 12 000 magnification (Leica Stereoscan 420 + Oxford PentaFETx3 and ZEISS EVO 40 + Oxford XMAX). Fibres were counted and measured when corresponding to the following criteria: (1) length >1 μm and aspect ratio 3:1; (2) identification as asbestos of commercial origin (amosite, crocidolite, anthophyllite) and chrysotile or as tremolite structures.

Fibre concentration is reported as million fibres per gram of dry lung tissue (Mff/g dt).

With the microanalysis system, fibres with a diameter >0.13 μm were clearly identifiable, while fibres with a diameter <0.05 μm were visible, but not identifiable, and were not counted. However, the number of unidentified fibres was not higher than the analytical sensitivity of the method (0.1 Mff/g dt).

For fibres with a diameter between 0.05 and 0.13 μm, the X-ray signal-to-noise ratio is too low to be distinguished over the background for the peaks of Na and Mg. In this class of fibre diameter, the distinction between the two main types of commercial amphibole asbestos (amosite and crocidolite) was not possible and they were all grouped as commercial amphibole asbestos. Elongated fibre-like tremolite structures were separately counted and included in the estimation of the lung fibre burden.

The SEM analysis stopped after the positive counting of 30 asbestos fibres or after the accomplished test of a number of microscopic fields sufficient to warrant a detection limit (DL) at about 0.3 Mff/g dt.

The analytical method was validated according to ISO guide 33 using the BCR665 and BCR666 Certified Reference Materials. The method resulted adequate both in in specificity and in sensitivity.

Values lower than the DL have been handled as the half of the DL of that analysis.

The analyst had no knowledge of the exposure of the participants examined. A more detailed description has been published.[20]

Statistical analysis

Descriptive statistics (mean, geometric mean (GM), median or percentages and SD, IQR, 95% CI) were used to summarise the data.

Wilcoxon signed-rank and Kruskal-Wallis tests were used to investigate differences across categorical variables. Spearman's correlation assesses the association between fibres and CEI, by reason of asymmetric distribution of the fibre content. Tests of statistical significance were two-sided with a significance level at 0.05. In the figures, the reported values are in a logarithmic scale, base 10. The statistical analysis was carried out with the software STATA 12.

RESULTS

The main characteristics of the participants under study are reported in table 2, separating the participants by the time period of first asbestos occupational exposure: 66.4% of participants started exposure in the period 1950–1970, 14% and 19.6% before or after.

The MPMs from Veneto and Brescia were more heavily represented (65.3%). Only 25 MPMs (9.2%) were residents outside the Regions of North East of Italy.

In total 248 of the 271 (91.5%) were males; average age was 68.4 years (SD 9.1; range 45–92). Epithelial was the prevalent histological subtype (59.8%), and it was predominant among the samples derived from a surgical intervention (71.9%).

Overall, the lung burden of asbestos fibres was well over 1 Mff/g dt (CI 95% 1.6 to 2.4), higher among the participants who started work in the decades 1950–1970. The total burden was higher among necroscopic samples (GM 2.2 vs 1.7 Mff/g dt).

The commercial amphibole fibres were clearly more represented, while the chrysotile fibres were definitively less: moreover, the latter were even of lower value among participants whose exposure started before the 1960s (table 2). Tremolite fibres were only marginally detected.

Time since first exposure (TSLE) had a mean of 26.1 years, with the highest values among participants who started asbestos occupational exposure before the 1950s (36.5 years).

In table 3 and figure 1, additional features of the MPMs are summarised, separated by the activity performed.

Participants with MPMs can, in a large majority, be classified as normal or skilled workers (blue collars); only 11 participants worked as technical employees; however, they were sharing the same work environment as the blue collars.

Males were predominant in all considered jobs, except in the asbestos and non-asbestos textile industry, where 83.3% and 69.3% of MPMs examined occurred in women.

Table 2 Main characteristics of the participants by period of first exposure

	Period of first exposure				
	<1950 (n=38)	1950–1960 (n=72)	1960–1970 (n=108)	>1970 (n=53)	Total (n=271)
Residence (% (n))					
Veneto	31.6 (12)	50.0 (36)	33.3 (36)	43.4 (23)	39.5 (107)
Brescia	36.8 (14)	20.8 (15)	25.9 (28)	24.5 (13)	25.8 (70)
Friuli Venezia Giulia	31.6 (12)	13.9 (10)	23.2 (25)	13.2 (7)	19.9 (54)
Mantua	0.0 (0)	9.7 (7)	5.6 (6)	3.8 (2)	5.6 (15)
Elsewhere	0.0 (0)	5.6 (4)	12.0 (13)	15.1 (8)	9.2 (25)
Gender					
Male; (% (n))	79.0 (30)	94.4 (68)	91.7 (99)	96.2 (51)	91.5 (248)
Age at death					
Years (mean±SD)	80.0±5.9	70.6±6.7	65.0±6.4	63.0±10.1	68.4±9.1
Histology (% (n))					
Sarcomatous	23.7 (9)	4.2 (3)	11.2 (11)	7.5 (4)	10.0 (27)
Epithelial	47.4 (18)	66.6 (48)	58.3 (63)	62.3 (33)	59.8 (162)
Mixed	18.4 (7)	15.3 (11)	18.5 (20)	18.9 (10)	17.7 (48)
Undefined	10.5 (4)	13.9 (10)	13.0 (14)	11.3 (6)	12.5 (34)
Source of lung tissue (% (n))					
Surgery	2.6 (1)	47.2 (34)	49.1 (53)	62.3 (33)	44.7 (121)
Necroscopy	97.4 (37)	52.8 (38)	50.9 (55)	37.7 (20)	55.3 (150)
Asbestos fibres Mff/g dt					
GM (95% CI)	1.6 (1.0 to 2.9)	2.1 (1.4 to 3.2)	2.3 (1.6 to 3.1)	1.7 (1.1 to 2.5)	2.0 (1.6 to 2.4)
Percentage of amphibole					
Median (1st to 3rd quartile)	91.5 (43–100)	90 (64–100)	86 (47–100)	92.0 (67–100)	90 (56–100)
Percentage of chrysotile					
Median (1st to 3rd quartile)	0 (0–10)	5 (0–18)	3 (0–33)	3 (0–21.5)	2.5 (0–20)
Percentage of tremolite					
Median (1st to 3rd quartile)	0 (0–9)	0 (0–9.5)	0 (0–12)	0 (0–6)	0 (0–9)
CEI ff/mL-years					
GM (95% CI)	0.9 (0.3 to 2.4)	0.6 (0.3 to 1.2)	0.7 (0.4 to 1.2)	0.4 (0.2 to 1.0)	0.6 (0.4 to 0.9)
AFE					
Years (mean±SD)	16.1±4.0	18.6±5.3	21.6±6.6	26.4±9.4	20.9±7.4
TSLE					
Years (mean±SD)	36.5±12.3	25.1±10.5	24.9±9.9	22.4±8.7	26.1±11.0

AFE, age at first exposure; CEI, Cumulative Exposure Index; GM, geometric mean; TSLE, time since first exposure.

The asbestos exposure intensity was associated with tasks and jobs using the criteria found in the literature:[19] high intensities were deemed to have occurred among participants with MPMs, who worked in the asbestos cement industry, with insulation material, and asbestos textile industry, with a percentage of 81.8%, 80% and 66.7%, respectively. This percentage decreases to 34.4% if considering shipyard workers, although 4.7% of this group is supposed to have experienced very high direct exposures. The jobs performed have been considered to have implied a low intensity of asbestos exposure in more than 55% of participants with MPMs who worked in building construction, foundry industry, chemical industry, non-asbestos textile and brake repair.

Duration of work varied among job sectors: 11.1 years among participants in asbestos textile industries; longer than 26 years among those in non-asbestos textile industries (26.5 years) and construction of industrial installations (26.2 years).

An elevated percentage of amphibole fibres was seen when a high total amount of asbestos fibres was found. These were participants with MPM who were working in asbestos textile, in asbestos cement production and installation, in shipbuilding and ship repair (GM 22, 13.1, 5.4 Mff/g dt, respectively).

The activities related to the lowest asbestos counts were among participants with MPMs who worked in body shops and brake repair, in chemicals production, in non-asbestos textile industries, and in building construction (GM 0.6, 0.8, 0.9, 1 Mff/g dt, respectively).

A fibre count exceeding 100 Mff/g dt was found in nine participants with MPMs (2 women). These participants have been employed in asbestos cement production, in shipyards and in recycling jute sacks previously containing asbestos.

Only in a few cases (9 participants corresponding to 3.3% of the total) was the asbestos fibres amount below the DL, even if all of them have been judged to have been certainly exposed to asbestos.

The fibre burden increased according to our estimate of asbestos exposure: participants classified with a 'certain' occupational exposure had the highest value (GM 2.4 Mff/g dt), in comparison with that classified with a 'probable' and 'possible' (GM 0.7 Mff/g dt) occupational exposure (Wilcoxon test $p<0.001$).

A limited number of participants with MPM (n=27) who performed heterogeneous activities (ie, goldsmiths, glass makers, dental technicians or truck drivers) was included in the category 'other activities'.

Table 3 Main characteristics of the MPM cases by job sectors, ordered by percentage of MPM which meet the 1997 Helsinki Criteria (amphiboles >1 Mff/g dt) for attribution to an occupational exposure

	Asbestos textile n=6	Asbestos cement n=11	Shipyard n=64	Construction of industrial plant n=45	Railway carriage n=9	Insulation n=10	Foundry industry n=14	Building construction n=54	Chemical industry n=11	Non-asbestos textile n=13	Brake repairers n=7	Other activities n=27
Gender (% (n))												
Male	16.7 (1)	100.0 (11)	95.3 (61)	97.8 (43)	100.0 (9)	100.0 (10)	100.0 (14)	98.2 (53)	100.0 (11)	30.7 (4)	100.0 (7)	85.2 (23)
Highest probability of exposure (% (n))												
Definite (category 1)	100.0 (6)	100.0 (11)	98.4 (63)	91.1 (41)	100.0 (9)	100.0 (10)	85.8 (12)	72.2 (39)	90.9 (10)	30.8 (4)	85.7 (6)	77.8 (21)
Probable (category 2)	–	–	1.6 (1)	2.2 (1)	–	–	7.1 (1)	3.7 (2)	–	23.1 (3)	–	3.7 (1)
Possible (category 3)	–	–	–	6.7 (3)	–	–	7.1 (1)	24.1 (13)	9.1 (1)	46.1 (6)	14.3 (1)	18.5 (5)
Highest intensity of exposure (% (n)) (ff/mL)												
Low (<0.1)	32.3 (2)	9.1 (1)	9.4 (6)	53.3 (24)	22.2 (2)	10.0 (1)	92.9 (13)	70.4 (38)	63.6 (7)	100.0 (13)	57.1 (4)	85.2 (23)
Medium (0.1–1)	–	9.1 (1)	51.5 (33)	31.1 (14)	44.5 (4)	10.0 (1)	7.1 (1)	18.5 (10)	18.2 (2)	–	42.9 (3)	7.4 (2)
High (1–10)	66.7 (4)	81.8 (9)	34.4 (22)	15.6 (7)	33.3 (3)	80.0 (8)	–	11.1 (6)	18.2 (2)	–	–	7.4 (2)
Very high (>10)	–	–	4.7 (3)	–	–	–	–	–	–	–	–	–
Duration of work (years)												
Mean±SD	11.1±8.8	18.4±9.6	21.5±15.4	26.2±20.9	18.1±12.8	18.9±13.4	19.1±12.8	22.0±19.0	18.6±11.9	26.5±21.7	19.3±21.8	23.3±16.8
Asbestos fibres (Mff/g dt)												
GM (95% CI)	22.0 (1.5 to 32.4)	13.1 (2.6 to 65.8)	5.4 (3.6 to 7.9)	1.8 (1.2 to 2.7)	3.3 (0.9 to 12.1)	2.2 (0.6 to 8.4)	1.5 (0.7 to 3.1)	0.9 (0.7 to 1.2)	0.8 (0.5–1.2)	0.9 (0.5 to 1.6)	0.6 (0.3 to 1.5)	0.8 (0.4 to 1.4)
Percentage of amphiboles												
Median (1st to 3rd quartile)	89 (70–100)	100 (92–100)	97 (84–100)	91 (73–100)	100 (86–100)	37 (10–95)	83 (26–100)	84 (50–100)	67 (40–87)	74 (43–100)	37 (13–67)	90 (56–100)
Amphiboles >1 Mff/g dt (%)	83.3*	81.8*	76.5*	60.0*	55.6*	50.0	42.8	31.5	18.2	7.7	0.0	18.5

*Percentage higher than 50%.
GM, geometric mean; MPM, malignant pleural mesothelioma.

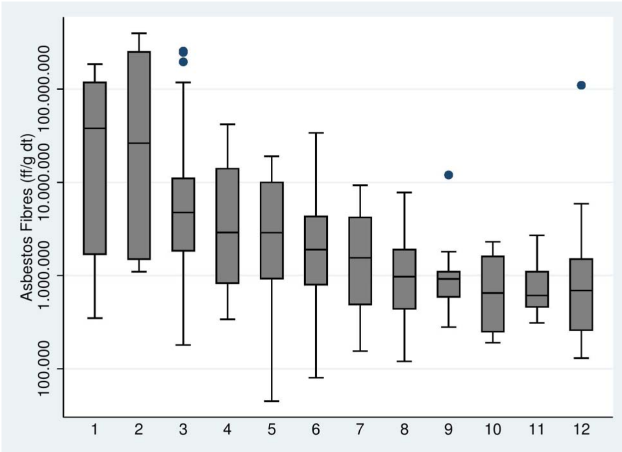

Figure 1 Residual asbestos fibre burden among the MPMs by activity performed* in a log10 scale. *1=Production and installation of asbestos cement; 2=asbestos textile; 3=shipbuilding and repair; 4=production and repair of railway carriages; 5=insulation; 6=industrial plant construction and maintenance; 7=foundry industry; 8=building construction; 9=non-asbestos textile; 10=automobile mechanics; 11=chemical industry; 12=other activities. ff/g dt, fibres per gram of dry lung tissue; MPM, malignant pleural mesothelioma.

Overall, amphibole fibres were detected in 88.6% of the lung samples, and chrysotile fibres in 55.4%. The percentage of participants with MPM which fulfils the 1997 Helsinki Criteria would be met only in 48.3% of the cases of this study. The percentage rises to 75% when considering only participants who worked in the construction or repair of railway carriages, in asbestos cement industries and shipyards, but it results in <20% when considering only participants who worked in chemical industries, in brake repair and in non-asbestos textile activities.

In 17 participants, the lung content showed no presence of commercial amphiboles, just the presence of chrysotile and/or tremolite.

The association between the lung fibre burden and CEI was discrete (Spearman's $\rho=0.57$; $p<0.01$). The correlation with CEI was higher for amphibole fibres (Spearman's $\rho=0.58$, $p<0.01$) than for chrysotile fibres (Spearman's $\rho=0.14$, $p=0.19$) (figure 2).

Among participants with MPMs presenting <1 Mff/g dt, the CEI values were below 0.1 ff/mL-years, while participants with more than 20 Mff/g dt (figure 2) showed a CEI>10 ff/mL-years.

DISCUSSION

This is the largest study currently available in Italy related to the asbestos fibres burden and characterisation in the lungs of participants with MPM who have been occupationally exposed to asbestos. It adds knowledge about the asbestos exposure occurring in several job sectors.[10 21 22]

The analysis has been carried out by means of an SEM in a single laboratory as part of a controlled laboratory test, using a standardised and consistent methodology, under blind conditions.

The SEM magnification used allowed the identification of asbestos fibres longer than 1 µm and with a diameter >0.05 µm. An underestimation of chrysotile fibres may still have occurred, because it is known that chrysotile fibres retained in the lungs may be smaller than these dimensions. The underestimation cannot be quantified, because there are no Certified Reference Materials of lung tissue containing chrysotile fibres, but the uncertainty is of at most 0.1 M ff/g dt.

The retrospective assessment was performed taking advantage of the standardisation of methodologies applied to all newly occurring MMs in the areas under study, and was complemented with a qualitative effort. The regional centres involved are knowledgeable about what has occurred in their areas in terms of industries and occupations that present risks of asbestos exposure.

We acknowledge some limitations. Our study involves MPMs that represent a specific sample. In fact, we have a prevalence of epithelial type due to the fact that pleuropneumonectomies were performed among those affected by this subtype. Besides, the higher asbestos fibre burden detected among necroscopic samples suggests that necroscopies had been carried out by coroners when an occupational disease was suspected.

Our sample reflects only partially the broad range of activities in which asbestos has been used. However, this bias does not affect the relationship between occupational asbestos exposures and the lung fibre burden.

Parenchymal asbestosis was stated to be present in a small percentage of the participants with MPM under investigation, based on radiology or tissue pathology. A larger percentage of participants had diagnosis of pleural plaques. No effort has

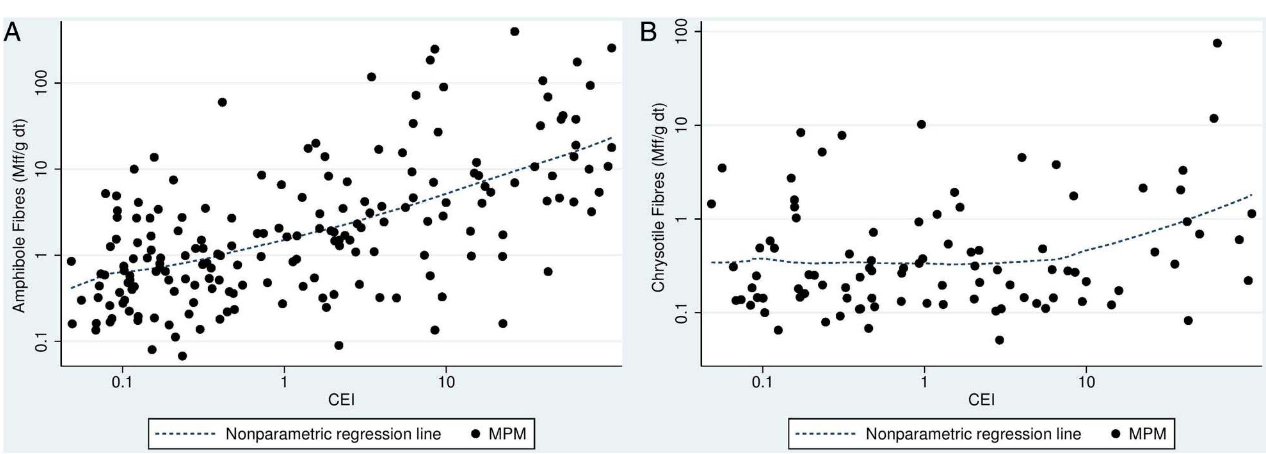

Figure 2 Residual asbestos fibre burden and CEI in a log10 scale by type of asbestos: amphibole (A) and chrysotile (B). Non-parametric regression in dashed line. CEI, Cumulative Exposure Index; Mff/g dt, million fibres per gram of dry lung tissue; MPM, malignant pleural mesothelioma.

been undertaken to validate these diagnoses and we have not considered the fibre burden in relation to asbestos diseases other than MPM.

The study is restricted to MM classified as 'definite'. The clinical records of the patients have been retrieved and ranked for certainty, applying predefined criteria. The histological diagnoses of MPM were not reviewed; however, the diagnoses of the participants under study are prone to the lowest probability of misclassification. Pleuropneumonectomies have been performed in a restricted number of highly specialised centres of thoracic surgery. Very experienced pathologists examined the specimen. Here, autopsies have been carried out by coroners to obtain the highest certainty that a participant was affected by an MPM and to establish the cause of death.

We attempted to compute a cumulated index of the occupational asbestos exposure and thus to correlate the fibre burden with an estimate of the asbestos exposure. However, our estimates on intensities are mainly based on values derived from the literature.

The main result was that a fibre count over the DL was detected in more than 96% of participants with MPM considered to have been occupationally exposed. The fibre count increases with the CEI implying that heavier cumulative asbestos exposures resulted in higher counts of asbestos fibres. This relationship was strengthened with the presence of amphibole fibres.

Mesotheliomas may occur at relatively low exposures to asbestos, as it is highlighted in the fraction of MPMs with low fibre counts or even below the DLs.

The fibre content of participants with MPM was estimated at least 20 years after the ban of any new use of asbestos, and shows high percentages of amphibole fibres consistent with the high amphibole fibres' biopersistence in the lungs and with the chrysotile fibres[23] dissolution process. As a consequence of the lung clearance, all the activities involving almost entirely chrysotile exposure (ie, non-asbestos textile workers, brake repair and, among 'other activities', dental technicians and glass workers) resulted in a low number of residual asbestos fibres and a limited portion of these cases could be attributed to asbestos occupational exposure according to the 1997 Helsinki Criteria.

The results by fibres type, among the participants with MPMs separated by activity, deserves some comments. We have already commented on the fibre burden of participants with MPM among workers of some specific activities,[24–27] but now the number of participants analysed is larger, and allows a comparison between activities.

The difference in lung fibre burden detected among participants with MPM employed in production of asbestos cement products versus participants with MPM working in building construction: the residual amount of fibres is 13 times higher among the former than the latter (GM 13.1 vs 1 Mff/g dt). However, the exposure source is the same for both workers' categories: plain and corrugated sheets, tubes and pipes, with a composition of about 80% of Portland cement and asbestos, mainly chrysotile, and, to a very lesser extent, crocidolite and amosite. The percentage of crocidolite was stated at 10–15% of total asbestos when producing sheets, and proportionally higher, up to 30%, in the production of high-pressure pipes. Amosite was an additive used in very small amounts. In contrast, the percentage of amphibole burden detected in the lungs is largely different from the percentage known to be present in the products to which the participants were exposed.

The Italian asbestos cement industries could have purchased chrysotile mined at Balangero (Italy), which was free from contamination of amphiboles,[28] or imported chrysotile mined in other countries, virtually all contaminated from tremolite. In this occupational sector, exposure was mostly direct and continuous and measurements of breathable fibres showed that some jobs have involved a very high exposure level.[29 30]

Among construction workers, it is common to perform just that single job throughout their working lives, moving to different employers, often in small enterprises.[27] The manipulation of asbestos cement products is occasional and happens only when mechanical tools are used, that is, during cutting, perforation or dismantling sheets. In these cases, high asbestos exposures are likely to occur. Workers involved in insulation activities with asbestos have not been included among our classification of construction workers.

As a result, only 20% of construction workers would be considered asbestos occupationally exposed following the 1997 Helsinki Criteria. This low percentage creates a loop of negative consequences. The widespread persistence of asbestos cement in buildings is continuing to extend the risk of MPM among construction workers performing maintenance and refurbishing. Whereas, as in other countries,[31 32] the construction sector[33] ranks first for prevalence of new cases of MPM, the recognition of these MPM as occupational disease is deficient.[27]

The very high percentage of amphibole fibres in the lungs of participants with MPMs employed in construction or repair of railroad wagons is the consequence of exposures due to the state railways requirement to have these wagons insulated with sprayed crocidolite in the late 1950s. This required a deinsulation at the beginning of the 1980s.[34]

The values of fibre burden among participants with MPM who manufactured non-textile asbestos are the lowest by occupational type, and the low variability among measurements suggests that exposure intensity differences between activities or between different industries were modest. The surveillance system on mesothelioma shows a high occurrence of MPM among women, who constituted the majority of the workforce in that activity, and suggests why asbestos exposure occurred: 'Textile machines (ring spinning, twisting, warping, winding, looms) used until the 1990s were without exception equipped with asbestos-lined mechanical brakes. The heavy action required produced relatively rapid wear of the linings and the dust produced was spread into the atmosphere'.[35]

The lung burden among participants with MPM working in auto mechanics exposed to asbestos during brake repair, who exhibited the lowest fibre burden and the lowest percentage of amphibole fibres, is in line with the knowledge that chrysotile was the component of brakes and suggests caution on the opinion of absence of risk for MPM among auto mechanics.[36 37]

Occupational exposure to talc was stated very infrequently by investigation of participants with MPM and the finding of tremolite fibres in the lungs is better explained by its contamination of chrysotile ore.

Only in a few participants with MPM did the lung content not reveal amphibole fibres. Among these participants, the fibre concentration was low with a predominant proportion of chrysotile fibres and a minor proportion of tremolite. These MPMs reported to have performed activities involving exposure to asbestos that we definitively considered due to chrysotile, such as glass makers and goldsmiths, but the low residual fibre content plays against a clear occupational attribution.

The percentage of cases which would comply with the previous Helsinki Criteria is unsatisfactory for a proper causal attribution. The attribution increases from about 50% to 88.9%, if a lung clearance rate of 12% for amphibole fibres for each year elapsed since exposure ceased is applied: this value was suggested in previous studies.[38]

Our findings confirmed previous knowledge of high fibre counts among participants with MPM who were employed in activities well known for intense asbestos exposures, and stated the importance of factors, such as the differences in asbestos exposures by occupation, job tasks, periods at work and types of asbestos used.

A large literature has been published to correlate the patients' occupations and the asbestos burden detected in the lungs of participants who developed MPM or other lung diseases (cancer, asbestosis, pleural plaques). The studies varied for the technique used (TEM vs SEM, different amplification) and on fibres counted (all detectable vs >5 μm). Roggli and Sharma[37] summarised the results of the published studies on asbestos fibers (AF) (>5 μm) analysed by SEM among participants with lung diseases related to asbestos by occupations. The highest values of AF have been observed among workers working in insulators industries and shipyards, showing amosite as the predominant fibre, whereas construction workers and car mechanics had a clearly lower median content of AF.

A comparison with previous studies is impaired by the differences in preparation for the analysis, magnification and counting of fibres. This caveat also applies with the analysis carried out when a TEM is applied to detect fibres.[39 40] To make it possible, we followed the suggestions that: 'A systematic approach to counting fibers of all dimensions and analysis of lung fiber burdens need to be used, as described by the European Respiratory Society (De Vuyst et al, 1988). The criteria used to define and count asbestos fibers need to be stated explicitly. Some investigators only count fibers longer than 5 μm; however the majority of asbestos fibers in human tissue samples are shorter than 5 μm (Dodson and Atkinson, 2006)'.[4]

CONCLUSIONS

This study offers an evaluation of the residual asbestos fibre content in the lungs of participants with MPM who had an occupational exposure to asbestos. The lung burden of participants with MPM has for the most part been investigated several decades after the asbestos exposure ceased, because the participants left work and asbestos has been banned, allowing for biotransformation and clearance of asbestos fibres which reached the lungs.

Our analysis was not limited to fibres above 5 μm: it makes visible a consistent portion of fibres often not considered and allows comparison of the fibre content among participants with MPM occupationally exposed in several industrial occupations. This study helps to characterise and rank the intensity of exposures that occurred in Italy in different occupational activities.

High exposures occurred in some industrial sectors, resulting in high values of fibres in the lungs of the workers; however, in several participants with MPM, the limited amounts of asbestos fibres found may be still considered as a suggestion of the occupational exposure. In fact, the surveillance system on mesothelioma[33] strongly suggests that an occupational asbestos exposure has occurred, but the amount of retained fibres does not fit with the values suggested in the literature for attribution to asbestos occupational exposure. The attribution may be partially improved taking into account the time since the last exposure.

Starting in the middle of the 1970s, several countries banned any new use of asbestos and in some the use of crocidolite was reduced or banned, so that fibre burden measurements will be prospectively more and more determined in participants with a long time lapse since their occupational exposures ceased.

Among MPMs who have been exposed mainly or exclusively to chrysotile asbestos, the rapid dissolution of these fibres in lung impairs an attribution based only on lung burden.

Our findings show an agreement with the more recent Helsinki proposition[13] that a 'history of significant occupational, domestic or environmental exposure will suffice for attribution to asbestos occupational exposure' in case of MPMs.

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Contributors EM and PG contributed to all aspects of conception, study design, data collection and analysis, data interpretation, manuscript preparation and critical revision. AS perfomed fibre analyses and contributed to data interpretation, manuscript preparation and critical revision. PGB contributed to data collection, data interpretation and a critical revision of the manuscript.

Competing interests EM and PGB have been requested as expert witnesses for the office of public persecutor or for the office of the judge in criminal trials on asbestos diseased workers.

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REFERENCES

1 Marinaccio A, Montanaro F, Mastrantonio M, *et al*. Predictions of mortality from pleural mesothelioma in Italy: a model based on asbestos consumption figures supports results from age-period-cohort models. *Int J Cancer* 2005;115:142–7.
2 Girardi P, Bressan V, Merler E. Past trends and future prediction of mesothelioma incidence in an industrialized area of Italy, the Veneto Region. *Cancer Epidemiol* 2014;38:496–503.
3 Lippmann M. Effects of fiber characteristics on lung deposition, retention, and disease. *Environ Health Perspect* 1990;88:311–17.
4 Broaddus VC, Everitt JI, Black B, *et al*. Non-neoplastic and neoplastic pleural endpoints following fiber exposure. *J Toxicol Environ Health B Crit Rev* 2011;14:153–78.
5 Berry G. Models for mesothelioma incidence following exposure to fibres in terms of timing and duration of exposure and the biopersistence of the fibres. *Inhal Toxicol* 1999;11:111–30.
6 Churg A, Wright JL. Persistence of natural mineral fibres in human lungs: an overview. *Environ Health Perspect* 1994;5:229–33.
7 Albin M, Pooley FD, Strömberg U, *et al*. Retention patterns of asbestos fibres in lung tissue among asbestos cement workers. *Occup Environ Med* 1994;51:205–11.
8 Churg A. Deposition and clearance of chrysotile asbestos. *Ann Occup Hyg* 1994;38:625–33, 424–5.
9 Tuomi T, Huuskonen MS, Tammilehto L, *et al*. Occupational exposure to asbestos as evaluated from work histories and analysis of lung tissues from patients with mesothelioma. *Brit J Ind Med* 1991;48:48–52.
10 Tossavainen A, Karjalainen A, Karhunen PJ. Retention of asbestos fibres in the human body. *Environ Health Perspect* 1994;102(Suppl 5):253–5.
11 Asbestos, asbestosis, and cancer: the Helsinki criteria for diagnosis and attribution. *Scand J Work Environ Health* 1997;23:311–16.
12 Roggli VL, Sanders LL. Asbestos content of lung tissue and carcinoma of the lung: a clinicopathologic correlation and mineral fiber analysis of 234 cases. *Ann Occup Hyg* 2000;44:109–17.
13 Wolff H, Vehmas T, Oksa P, *et al*. Asbestos, asbestosis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations. *Scand J Work Environ Health* 2015;41:5–15.
14 De Vuyst P, Karjalainen A, Dumortier P, *et al*. Guidelines for mineral fibre analyses in biological samples: report of the ERS Working Group. European Respiratory Society. *Eur Respir J* 1998;11:1416–26.
15 Paoletti L, Martinelli C, Camilucci L, *et al*. The determination of asbestos fiber concentration by scanning electron microscopy: a comparison of the results obtained by different laboratories. *Med Lav* 1996;87:386–93.
16 Nesti M, Adamoli S, Ammirabile F, *et al*. *Guidelines for the identification and definition of malignant mesothelioma cases and the transmission to Ispesl by Regional Operating centres. Monograph*. Rome, Italy: ISPESL, 2003. http://www.ispesl.it/dml/leo/download/RenamGuidelines (access 12 Oct 2015).

17 Van Oyen SC, Peters S, Alfonso H, *et al*. Development of a Job-Exposure Matrix (AsbJEM) to estimate occupational exposure to asbestos in Australia. *Ann Occup Hyg* 2015;59:737–48.
18 Offermans NS, Vermeulen R, Burdorf A, *et al*. Comparison of expert and job-exposure matrix-based retrospective exposure assessment of occupational carcinogens in the Netherlands Cohort Study. *Occup Environ Med* 2012;69:745–51.
19 Lacourt A, Gramond C, Rolland P, *et al*. Occupational and non-occupational attributable risk of asbestos exposure for malignant pleural mesothelioma. *Thorax* 2014;69:532–9.
20 Barbieri PG, Mirabelli D, Somigliana A, *et al*. Asbestos fibre burden in the lungs of patients with mesothelioma who lived near asbestos-cement factories. *Ann Occup Hyg* 2012;56:660–70.
21 Dodson RF, Williams MG, Corn CJ, *et al*. Asbestos content of lung tissue, lymph nodes, and pleural plaques from former shipyard workers. *Am Rev Respir Dis* 1990;142:843–7.
22 Paci E, Zappa M, Paoletti L, *et al*. Further evidence of an excess of risk of pleural malignant mesothelioma in textile workers in Prato (Italy). *Br J Cancer* 1991;64:377–8.
23 Donaldson K, Oberdörster G. Continued controversy on chrysotile biopersistence. *Int J Occup Environ Health* 2011;17:98–9.
24 Barbieri PG, Somigliana A, Tironi A. Lung asbestos fibre burden in textile workers with malignant mesothelioma. *Med Lav* 2010;101:199–206.
25 Barbieri PG, Somigliana A, Festa R, *et al*. Pulmonary concentration of asbestos fibres in steel workers with pleural mesothelioma. *G Ital Med Lav Ergon* 2010;32:149–53.
26 Barbieri PG, Somigliana A, Lombardi S, *et al*. Asbestos fibre lung burden and exposure indices in asbestos-cement workers. *Med Lav* 2009;100:21–8.
27 Merler E, Bressan V, Somigliana A. Mesothelioma in construction workers: risk estimate, lung content of asbestos fibres, claims for compensation for occupational disease in the Veneto Region mesothelioma register. *Med Lav* 2008;100:120–32.
28 Pira E, Pelucchi C, Piolatto PG, *et al*. Mortality from cancer and other causes in the Balangero cohort of chrysotile asbestos miners. *Occup Environ Med* 2009;66:805–9.
29 Oddone E, Ferrante D, Cena T, *et al*. Studio di mortalità in una fabbrica per la produzione di manufatti in cemento-amianto in provincia di Pavia. *Med Lav* 2014;105:15–29.
30 Magnani C, Ferrante D, Barone-Adesi F, *et al*. Cancer risk after cessation of asbestos exposure: a cohort study of Italian asbestos cement workers. *Occup Environ Med* 2008;65:164–70.
31 Järvholm B, Englund A. The impact of asbestos exposure in Swedish construction workers. *Am J Ind Med* 2014;57:49–55.
32 Rolland P, Gramond C, Lacourt A, *et al*. Occupations and industries in France at high risk for pleural mesothelioma: a population-based case-control study (1998–2002). *Am J Ind Med* 2010;53:1207.
33 Marinaccio A, Binazzi A, Branchi C, *et al*. *Il Registro Nazionale dei Mesoteliomi (ReNaM), IV Rapporto. Monograph*. Rome, Italy: INPS, 2012:107–90. http://www.inail.it/internet_web/wcm/idc/groups/internet/documents/document/ucm_085815.pdf (access 12 Oct 2015).
34 Pavone VL, Scarnato C, Marinilli P, *et al*. Mortality in a cohort of railway rolling stock construction and repair workers in Bologna. *Med Lav* 2012;103:112–22.
35 Chiappino G, Pellissetti D, Moretto O, *et al*. Asbestos risk in the textile industry: braking systems on machinery used until the 1990's. *Med Lav* 2005;96:250–7.
36 Finley BL, Pierce JS, Paustenbach DJ, *et al*. Malignant pleural mesothelioma in US automotive mechanics: reported vs expected number of cases from 1975 to 2007. *Regul Toxicol Pharmacol* 2012;6:104–16.
37 Roggli VL, Sharma A. Analysis of tissue mineral fiber content. In: Oury TD, Sporn TA, Roggli VL, eds. *Pathology of asbestos-associated diseases*. 3rd edn. New York, NY: Springer, 2014;:53–92.
38 Barone-Adesi F, Ferrante D, Bertolotti M, *et al*. Malignant pleural and peritoneal mesotheliomas in former miners and millers of crocidolite at Wittenoom, Western Australia. *Occup Environ Med* 2004;61:912–16.
39 Tuomi T. Fibrous minerals in the lungs of mesothelioma patients: comparison between data on SEM, TEM, and personal interview information. *Am J Ind Med* 1992;21:155–62.
40 Dodson RF, Hammar SP, Poye LW. A technical comparison of evaluating asbestos concentration by Phase-Contrast Microscopy (PCM), Scanning Electron Microscopy (SEM), and Analytical Transmission Electron Microscopy (TEM) as illustrated from data generated from a case report. *Inhal Toxicol* 2008;20:723–32.