Original Article

Asbestos burden in lungs of non-occupationally exposed women from Broni (Pavia, Italy): a postmortem SEM-EDS study

Silvia Damiana Visonà[1#], Silvana Capella[2,3#], Paola Borrelli[4], Simona Villani[5], Cristina Favaron[6], Zhyldyz Kurzhunbaeva[7], Claudio Colosio[8,9], Elena Belluso[2,3]

[1]Department of Public Health, Experimental and Forensic Medicine, Unit of Legal Medicine and Forensic Sciences, University of Pavia, Pavia, Italy; [2]Department of Earth Sciences, University of Torino, Torino, Italy; [3]Interdepartmental Center for Studies on Asbestos and other Toxic Particulates "G. Scansetti", University of Torino, Torino, Italy; [4]Department of Medical, Oral and Biotechnological Sciences, Laboratory of Biostatistics, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy ; [5]Department of Public Health, Experimental and Forensic Medicine, Unit of Biostatistics and Clinical Epidemiology, Pavia University, Pavia, Italy; [6]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy; [7]Department of Health Sciences, Course of Research Doctorate in Public Health Sciences, University of Milan, Milan, Italy; [8]Department of Health Sciences, University of Milan, Milan, Italy; [9]Occupational Health Unit, Santi Paolo e Carlo Hospital, Milan, Italy

Contributions: (I) Conception and design: SD Visonà, S Capella, E Belluso, C Colosio; (II) Administrative support: SD Visonà; (III) Provision of study materials or patients: SD Visonà; (IV) Collection and assembly of data: SD Visonà, S Capella, C Favaron, Z Kurzhunbaeva; (V) Data analysis and interpretation: SD Visonà, S Capella, P Borrelli, S Villani; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Silvia Damiana Visonà, MD, PhD. Department of Public Health, Experimental and Forensic Medicine, Unit of Legal Medicine and Forensic Sciences, University of Pavia, via Forlanini 12, 27100 Pavia, Italy. Email: silviadamiana.visona@unipv.it; visona.silvia@gmail.com.

Background: In Italy the incidence of malignant mesothelioma (MM) among women is remarkably high, due to the several contexts in which women had been exposed to asbestos. However, very few studies in literature focus on the inorganic lung content in women. The aim of this retrospective, observational study is to investigate the asbestos lung burden, in terms of concentration, dimensions and type of asbestos, in 42 women who died from MM and had been non-occupationally exposed to asbestos during the activity of the asbestos-cement plant located in Broni (Pavia, Northern Italy) where mainly chrysotile, crocidolite and amosite were used.

Methods: Lung samples taken during forensic autopsies have been digested using sodium hypochlorite and filtered through a cellulose-ester membrane. The filter was examined using a scanning electron microscope and the chemical composition of the fibers was analyzed using an electron dispersive spectroscopy. The number of detected inorganic fibers, asbestos fibers and asbestos bodies (ABs) were normalized to 1 gram of dry tissue.

Results: In six samples no asbestos has been detected. Overall, the most represented kind of asbestos was amosite, followed by crocidolite, tremolite/actinolite asbestos and chrysotile. The concentration of all inorganic fibers was significantly higher in women with environmental and household exposures compared with those with only environmental exposure ($P=0.025$), as well as the concentration of asbestos fibers ($P=0.019$) and ABs ($P=0.049$). We found a significant correlation between the concentration of asbestos fibers and the duration of exposure ($rho =0.413$, $P=0.008$), as well as with the latency of MM ($rho =0.427$, $P=0.005$). The distance of the residential address from the factory and the time spent daily in contact with asbestos did not influence the lung asbestos burden.

Conclusions: These results suggest the relevance of the lung clearance of asbestos, regarding mainly chrysotile. As a consequence, although scanning electron microscopy -energy dispersive X-ray spectroscopy (SEM-EDS) is considered the most reliable tool for assessing previous exposure to asbestos, its results should be interpreted with caution, especially in a legal context. In addition, our data confirm the relevance of environmental and household exposure in determining asbestos concentration in lungs and highlight the importance of household exposure.

Keywords: Asbestos; mesothelioma; non-occupational exposure; scanning electron microscopy -energy dispersive X-ray spectroscopy (SEM-EDS)

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Introduction

Malignant mesothelioma (MM) is a highly aggressive neoplasm, arising from serosal linings of pleura, peritoneum, pericardium, or tunica vaginalis of testis, whose causal attribution to previous asbestos exposure is well known and established. MM has a typically long latency, generally 30 to 50 years, between the beginning of exposure and the onset of the disease (1). Due to this characteristic, the MM cases currently observed are related to asbestos exposures that occurred decades ago. In Italy, asbestos extraction, use, and commercialization were banned in 1992 with the Italian Law 257/92. Oddone *et al.*, in a recent epidemiological study, predicted that the mortality of pleural MM is reaching its peak in the current years (specifically, between 2020 and 2024) and then a plateau is expected, followed by a slow decrease in the following decades (2). The peak of peritoneal MM, instead, had been reached in 2014–2016 for men and 1999–2011 for women and its mortality is currently decreasing (3). It is interesting to note that, according to Oddone's forecasts, a high number of cases of pleural MM are expected in the next 20 years (about 26,000) (2). The man-to-women ratio, which showed a continuous increase between 1970 and 2014, is expected to remain stable in the future (2). On these bases, it is clear that, even though in most European countries the use of asbestos was banned between the years 1990 and 2000, asbestos-related diseases (ARDs) and especially MM still represent a major public health problem in both sexes.

MM has been historically associated with occupational exposure to asbestos, occurring mostly in men, while in women it is more often linked with household contacts with asbestos workers or neighborhood exposure deriving from nearby industries (4). However, other domestic sources of asbestos exposure should be considered, as before the asbestos ban (in Italy, 1992) asbestos artifacts were largely present in equipment for ironing, hair dryers, kitchen supplies etc. The sex-related differences in asbestos exposure and its role in MM causation are not well understood. Previous studies on women reported a diagnosis of MM mostly due to environmental and/or household exposure to asbestos (5-7). Moreover, this exposure has always been believed to be less intense than in men, who are, instead, exposed mainly occupationally. In a recent review, Attanoos *et al.* states that, as female MM patients often show concentration of asbestos in lungs below the background level, most cases are likely to be due to alternative causes, such as exposure to other minerals or radiation, they could be idiopathic or related to germline bap-1 mutations (8). On the other hand, as demonstrated by epidemiological data collected by Italian National Mesothelioma Registry, anthropogenic environmental and household exposures (AEH) to asbestos are responsible for a remarkable proportion of MM cases in Italy, respectively of 4.9% and 4.4% of all MM cases, most of which observed in the areas adjacent to asbestos-cement industries (9). Also, Ferrante *et al.* observed a significant increase in MM incidence and mortality in women exposed to asbestos in Casale Monferrato (Alessandria, northern Italy), where an important asbestos factory was located, due to household exposure (that involves direct contact with an asbestos worker and/or the cleaning of contaminated clothes) (10). It has recently been reported that Italy presents a high incidence of

Highlight box

Key findings

- Non-occupational asbestos exposure can determine high levels of asbestos in lungs.
- Household exposure is especially remarkable in relation to asbestos lung burden.
- Chrysotile clearance influence the results of lung content analysis using SEM-EDS.

What is known and what is new?

- Malignant mesothelioma (MM) in women is associated with non-occupational asbestos exposure, a younger age at diagnosis, a better survival and a more common epithelioid histology.
- The women here analyzed, exposed to asbestos environmentally or through a family member, show high levels of asbestos in lungs.
- SEM-EDS results is influenced by asbestos clearance in lungs.

What is the implication, and what should change now?

- Environmental and familial exposures are relevant in determining asbestos burden in lungs and should be addressed in prevention programs for MM.
- SEM-EDS analysis of lung content, considered the gold standard in the evaluation of previous asbestos exposure, should be carefully interpreted.

MM among women, due to several contexts, mostly related to the former activity of asbestos industries, in which women had been exposed to asbestos. The data collected from the National Mesothelioma Registry showed that in Italy 28% of MM occur in women, with a gender ratio (female/male) of 0.4 (7). Until now, few studies systematically investigated the lung content in non-occupationally exposed individuals (11,12), and only one, to our knowledge, focused on the asbestos lung content in women with environmental and/or household exposure (5). Such studies also suggest the importance of non-occupational exposure in determining asbestos burden in lungs.

MM in women is associated with a younger age at diagnosis, a better survival and a more common epithelioid histology (13).

The present study focuses on a series of deceased women who lived in Broni, a small town located on the hills of the Pavia Province, where the Fibronit plant, involved in the production of asbestos-cement, was active. Raw products were a mixture of chrysotile, crocidolite and small amounts of amosite (14,15). The plant was active between 1932 and 1993, employing about 2,700 men and 700 women who resided prevalently in Broni or in the surrounding. Until the 1980s, no air filtration systems were present, nor other safety measures were adopted in the factory. The details of the production of the factory have been described elsewhere (6,14,16).

The aim of this study is to characterize the inorganic fiber burden, the asbestos fibers and asbestos bodies (ABs) concentration, the dimensions and type of detected asbestos, in women who died from MM, taking into account the type of asbestos exposure—anthropogenic environmental exposure (AEE) *vs.* AEH—during the activity of the above-mentioned asbestos-cement plant. Secondly, the association between the amount of time spent daily in contact with asbestos and asbestos lung content and the association between asbestos concentration and duration, latency and survival are explored. We present this article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1061/rc).

Methods

Population, study design and setting

The study population was selected among subjects who died with ARDs and had been exposed to asbestos in Broni (PV). Among the subjects for which a forensic autopsy followed by histopathological exam has been performed between 2005 and 2018, subjects with the following characteristics have been selected for the analysis:

- ❖ Female sex;
- ❖ Death due to MM;
- ❖ Anthropogenic environmental asbestos exposure (AEE) or AEH. For an explanation see the paragraph "variables".

The diagnosis of MM, already known before death, has been confirmed postmortem by immunohistochemistry according to guidelines in effect at the time (17-20). During the forensic autopsy, in each case, the whole lungs were collected, formalin-fixed, and stored for further examination. All the women investigated have been exposed to asbestos during the activity of the asbestos-cement factory which was operating from 1932 to 1993 in Broni (PV).

A retrospective cohort design was used. The study period was from 2005 to 2018.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of IRCCS Policlinico San Matteo (Pavia, Italy) (No. 20180060636) and individual consent for this retrospective analysis was waived.

Variables, data sources and measurement

The endpoints: assessment of inorganic fibers in lungs

The concentration of inhaled asbestos fibers, the dimensions and type of asbestos fibers were the endpoints. Not only asbestos, but all the inorganic fibers that fulfilled the definition of "regulated" fiber [length ≥5 µm, width <3 µm, aspect ratio greater than or equal to 3:1 (21)], but also fibers shorter than 5 µm, classified separately as short fibers, and ABs contained in 0.25 grams of wet lung (inferior lobe of right lung) were investigated by scanning electron microscopy -energy dispersive X-ray spectroscopy (SEM-EDS) in order to obtain the concentrations of total inorganic fibers, asbestos fibers, and ABs (*Figure 1*), as well as the concentrations of the various types of asbestos [chrysotile (*Figure 2*), amosite (*Figure 3*), crocidolite (*Figure 4*), tremolite-actinolite asbestos (*Figure 5*), anthophyllite asbestos] in lung samples of the selected subjects. The inorganic fibers were also measured and chemically analyzed.

The method, already described elsewhere (16,22) consists of chemical digestion (using sodium hypochlorite) of 0.25 grams of formalin-fixed lung parenchyma and filtration of the suspension through a cellulose-ester membrane

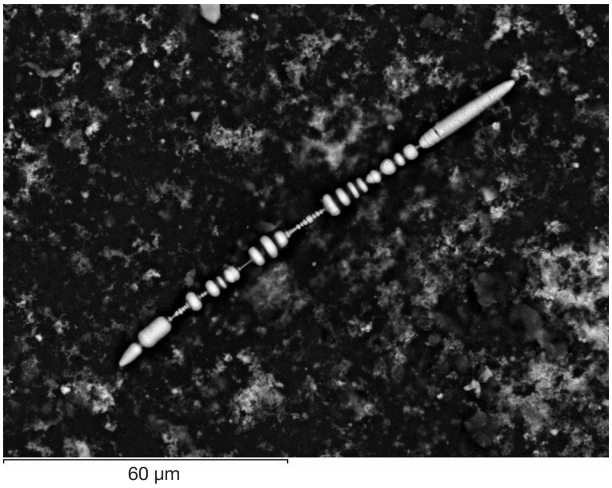

Figure 1 An example of an SEM image (backscattered electrons) of an asbestos body. SEM, scanning electrode microscope.

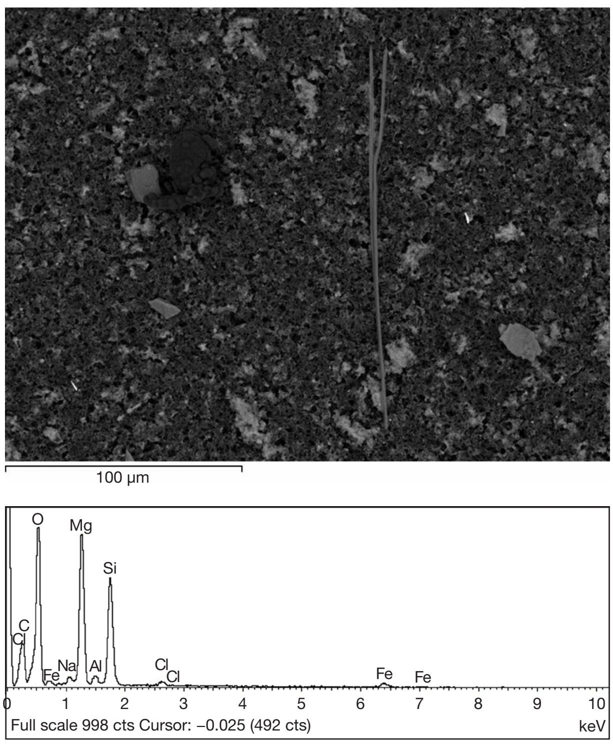

Figure 2 An example of an SEM image (backscattered electrons) of a chrysotile/asbestiform antigorite fiber, with the corresponding EDS spectra. Sodium (Na) and chlorine (Cl) peaks are related to the NaCl that is present in the background. SEM, scanning electrode microscope; EDS, energy dispersive X-ray spectroscopy.

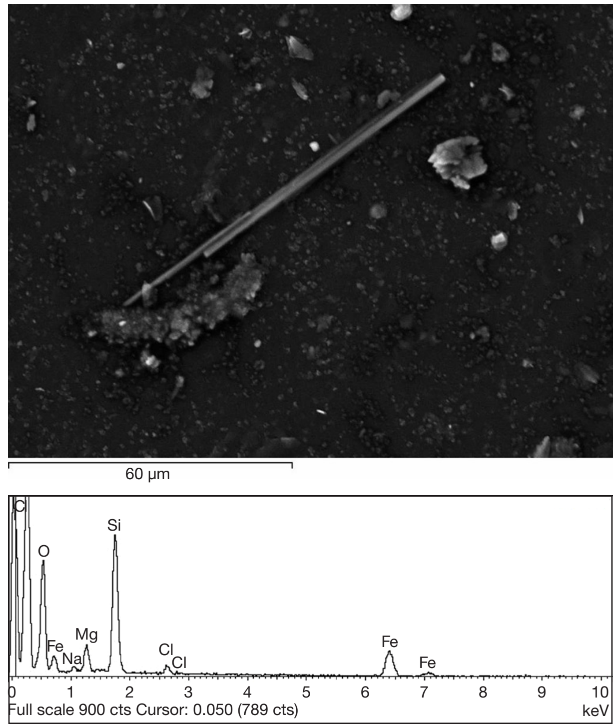

Figure 3 An example of an SEM image (backscattered electrons) of an amosite fiber, with the corresponding EDS spectra. Sodium (Na) and chlorine (Cl) peaks are related to the NaCl that is present in the background. SEM, scanning electrode microscope; EDS, energy dispersive X-ray spectroscopy.

(Millipore, Darmstadt, Germany) with a diameter of 25 mm and a pore size of 0.45 μm.

Afterwards, the filter, dehydrated and pasted on a pin-stub using a carbon tape, was examined by SEM. The observation was performed on an area of 2 mm^2 of filter at 4,000× using backscattered electrons.

The fiber chemical composition was analyzed using an EDS, Oxford Inca Energy 200, equipped with an INCA X-act SDD detector (Oxford Instruments NanoAnalysis, Bucks, UK).

The number of detected inorganic fibers, asbestos fibers and ABs were normalized to 1 gram of dry tissue, as indicated by international guidelines (23,24), reporting concentration in terms of the burden of inorganic fibers, asbestos, and ABs per gram of dry lung tissue weight: ff/gdw.

The analytic sensitivity of the method can be identified in 3,000 ff/gdw, that corresponds to the minimum content detectable (the lung content corresponding to one fiber counted).

Moreover, the "background" concentration of asbestos

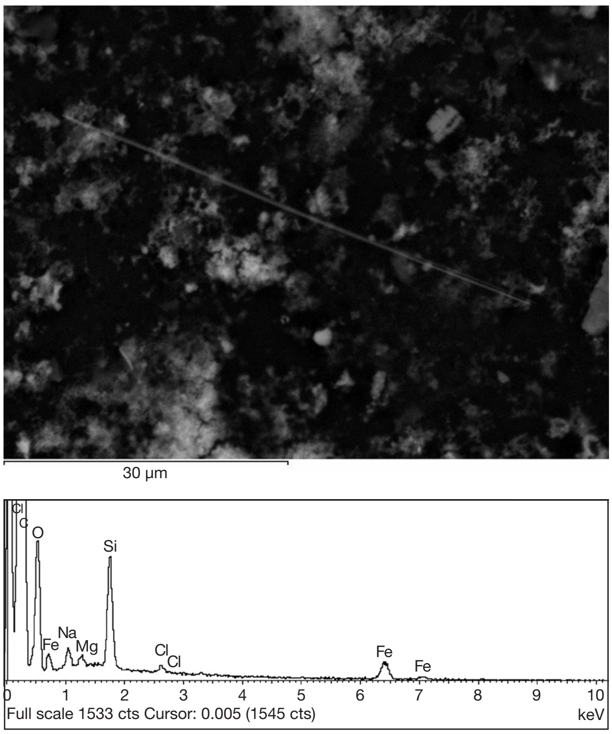

Figure 4 An example of an SEM image (backscattered electrons) of a crocidolite fiber, with the corresponding EDS spectra. Chlorine (Cl) peak is related to the NaCl that is present in the background, while sodium (Na) is related partly to the fiber composition and partly to the background.

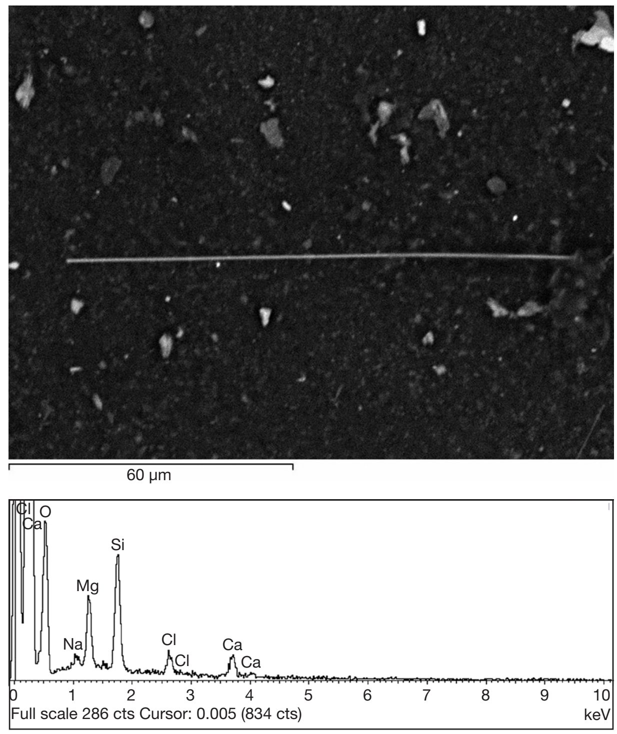

Figure 5 An example of an SEM image (backscattered electrons) of a tremolite/actinolite fiber, with the corresponding EDS spectra. Sodium (Na) and chlorine (Cl) peaks are related to the NaCl that is present in the background.

in lung tissue (that is the concentration of asbestos that everyone can randomly encounter but does not increase significantly the risk of MM) had been identified in our laboratory as below 100,000 ff/gdw. This threshold has been found by performing the same analysis on a series of 50 healthy subjects, who died from traumatic causes, aged more than 60 and with a negative history of asbestos exposure and lung disease.

To identify the different types of inorganic fibers, we compared the EDS spectra with a database internal to the laboratory.

Since the technique here used does not allow unequivocal identification of certain minerals with similar chemical composition and analogous morphology, it is not possible to distinguish chrysotile from asbestiform antigorite, and tremolite asbestos from actinolite asbestos. Therefore, we used, respectively, the following mineral group names: chrysotile/asbestiform antigorite and tremolite/actinolite asbestos.

Other variables

The following variables were used in the present work:

- ❖ Type of asbestos exposure (AEH, AEE). As all the women here investigated had AEE, but only a number of them had household exposure, we divided them in two groups: those with only AEE and those with both AEH. The term "AEE" is used for indicating people who lived in an area with air-dispersed asbestos from the asbestos-cement plant (25,26), in order to make clear the difference from the exposure to natural sources of asbestos (this last identified as "natural environmental exposure"). The "anthropogenic environmental and household exposure" is used for women who lived in the same area and lived together with an asbestos worker.
- ❖ Amount and daily exposure: living and working at Broni (LW-Broni) and otherwise (i.e., living at Broni and working in different place or not living in Broni and working in Broni).

Table 1 Type of MM and exposure of enrolled women

Variables	N=42
Histological type of MM	
Epithelial	31 (73.8)
Sarcomatoid	6 (14.3)
Biphasic	5 (11.9)
Type of exposure	
AEE	28 (66.7)
AEH	14 (33.3)
Latency (years)	49 (42.0–68.0)
Duration of exposure (years)	29 (19.0–50.0)
Survival time since diagnosis of MM (months)	14.0 (10.0–22.0)
Time since end of exposure (years)	18.0 (16.0–22.0)

N (%) or median (IQR) are shown when appropriate. MM, malignant mesothelioma; AEE, anthropogenic environmental exposure; AEH, anthropogenic environmental and household exposure; IQR, interquartile range.

- Distance in meters of the residential address and the asbestos cement plant. In case of multiple changing of address during the subject's life the mean distance was used.
- Duration of asbestos exposure.
- Histological type of MM: epithelial, sarcomatoid and biphasic.
- Time of diagnosis of MM.
- Time of death.
- Time between the end of exposure and death.

Data sources

Except the lung fibers, all the variables were extracted from the archive of Unit of Legal Medicine and Forensic Sciences of the University of Pavia from 2000 to 2018.

Statistical analysis

Quantitative variables were summarized as the mean with standard deviation if the distribution was normal, and with the median, 25th, and 75th percentiles if not. To verify normality, the Shapiro-Wilk test was used. To evaluate differences in quantitative variables across groups of exposure to asbestos (AEE *vs.* AEH) and the amount and daily exposure, was performed using a non-parametric unpaired *t*-test (Mann-Whitney test). The evaluation of differences between histological types of MM, an analogous non-parametric test of analysis of variance (Kruskal-Wallis test) was applied, followed by the appropriate post-hoc test if significant. The relationships among quantitative variables were tested using Spearman's correlation coefficient (rho). A P value less than 0.05 was considered significant. All analyses were performed using STATA 17® (StataCorp LLC., College Station, TX, USA).

Results

Among a total of 188 subjects who died from ARDs and for which a forensic autopsy followed by histopathological exam has been performed, 46 were females and died from MM. Three of these women had only occupational asbestos exposure or household exposure and one had no reported exposure. Therefore, 4 out of 46 women have been excluded from the analysis.

About 2/3 of women enrolled in the study were environmentally exposed to asbestos (AEE) and the histological type of MM was classified as epithelial in around 3/4 of cases (*Table 1*). The median time of latency was 49 years, while the survival time in at least 50% of women was 14 months. The median time elapsed between the end of exposure and death was 18 years.

Inorganic fibers in lungs by type of exposure

Among the whole series of 42 women, six cases showed no asbestos in the investigated lung sample (five of them had AEE and one had AEH), of which three subjects did not show any other kind of inorganic fibers. ABs were found in 13 cases, whereas short fibers were observed in 29 subjects.

Overall, the most represented kind of asbestos was amosite (43% of the total detected asbestos), followed by crocidolite (31%), tremolite/actinolite asbestos (24%) and chrysotile/asbestiform antigorite (2%).

The median concentration of inorganic fibers, asbestos fibers, ABs and short fibers, their dimensional characteristics in the two exposure groups are reported in *Table 2*. The concentration of inorganic fibers and of asbestos was significantly higher in women with both exposures (AEH) compared with those with only AEE, while that of ABs showed a similar pattern but the excess was borderline significant. No significant difference in fibers dimensions have been detected according to the kind of exposure.

In the AEE group, amosite was the most represented kind of asbestos (being the 42% of the total asbestos)

Table 2 Median and IQR of each variable regarding the inorganic lung content in the two groups of exposure considered for the statistical analysis (anthropogenic environmental, anthropogenic environmental and household)

Variables	Type of exposure			
	AEE (n=28)	AEH (n=14)	MW test	P value
Fibers per gram of dry weight lung tissue (ff/gdw)	44,387.2 (20,478.2–71,859.9)	92,700.6 (44,471.4–161,409.9)	−2.24	0.025
Asbestos fibres per gram of dry weight lung tissue (ff/gdw)	20,751.1 (7,452.5–28,970.7)	42,998.1 (21,784.9–73,979.5)	−2.31	0.019
Asbestos bodies per gram of dry weight lung tissue (ABs/gdw)	0.0 (0.0–0.0)	2,228.3 (0.0–13,178.5)	−2.01	0.049
Short fibers per gram of dry weight lung tissue (sff/gdw)	7,804.2 (0.0–14,493.6)	5,166.4 (2,944.3–17,704.3)	−0.59	0.563
Mean length of all fibers (μm)	18.3 (14.9–22.3)	16.7 (14.8–22.1)	0.42	0.682
Mean width of all fibers (μm)	0.8 (0.6–0.9)	0.7 (0.4–0.9)	0.70	0.488
Mean length of asbestos fibers (μm)	20.6 (11.6–26.0)	17.9 (12.3–24.2)	0.60	0.556
Mean width of asbestos fibers (μm)	0.5 (0.3–0.6)	0.6 (0.4–0.9)	−1.02	0.310
Mean length of all short fibers (μm)	3.9 (0.0–4.2)	4.1 (3.7–4.5)	−0.948	0.343
Mean width of all short fibers (μm)	0.6 (0.0–0.7)	0.6 (0.4–0.7)	−0.70	0.488

Data are presented as median (interquartile range). AEE, anthropogenic environmental exposure; AEH, anthropogenic environmental and household exposure; MW test, Mann-Whitney test.

Table 3 Median and IQR of the concentration of each type of asbestos in the two groups of exposure considered for the statistical analysis (anthropogenic environmental, anthropogenic environmental and household)

Variables	Type of exposure			
	AEE (n=28)	AEH (n=14)	MW test	P value
Chrysotile/asbestiform antigorite per gram of dry weight lung tissue (ff/gdw)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.445	0.570
Crocidolite per gram of dry weight lung tissue (ff/gdw)	0.0 (0.0–7016.2)	11,172.6 (4,456.7–20,852.6)	−2.65	0.007
Amosite per gram of dry weight lung tissue (ff/gdw)	5,843.8 (0.0–19,520.6)	15,502.6 (8,852.1–43,715.2)	−1.83	0.070
Tremolite/actinolite per gram of dry weight lung tissue (ff/gdw)	3,684.3 (0.0–8,676.4)	6,970.6 (2,944.3–16,813.5)	−1.44	0.154

Data are presented as median (interquartile range). AEE, anthropogenic environmental exposure; AEH, anthropogenic environmental and household exposure; MW test, Mann-Whitney test.

followed by crocidolite (30%), tremolite/actinolite asbestos (25%) and chrysotile/asbestiform antigorite (3%). In the AEH group, amosite represented the 45% of the total detected asbestos, followed by crocidolite (32%), tremolite/actinolite asbestos (23%) and chrysotile/asbestiform antigorite (1%).

Concerning the kind of asbestos, only the crocidolite concentration was found significantly higher in women with AEH exposure compared to only AEE, while the concentration of chrysotile/asbestiform antigorite, amosite and tremolite/actinolite asbestos did not show any significant difference (*Table 3*).

The concentration of inorganic fibers, of asbestos and of ABs was not significantly different between the women that

Table 4 Median and IQR of each variable regarding the inorganic lung content in the two groups of daily exposure (women that used to spend most of their daily life exposed to asbestos vs. those who spent only part of their daily life in a condition that implied asbestos exposure)

Variables	Daily exposure			
	LW-Broni (n=29)	Otherwise (n=7)	MW test	P value
Inorganic fibers per gram of dry weight lung tissue (ff/gdw)	52,113.9 (30,583.6–85,749.0)	24,396.6 (5,220.9–65,354.7)	1.42	0.165
Asbestos per gram of dry weight lung tissue (ff/gdw)	26,682.9 (9,702.9–50,853.6)	9,613.0 (0.0–43,968.9)	1.32	0.193
Asbestos bodies per gram of dry weight lung tissue (ABs/gdw)	0.0 (0.0–4,456.7)	0.0 (0.0–0.0)	0.835	0.488
Short fibers per gram of dry weight lung tissue (sff/gdw)	4,845.9 (0.0–15,140.5)	0.0 (0.0–7,994.4)	1.49	0.140
Mean length of all fibers (μm)	17.3 (4.8–20.7)	17.9 (7.0–25.0)	0.02	1.000
Mean width of all fibers (μm)	0.8 (0.6–1.0)	0.6 (0.3–1.6)	0.780	0.449
Mean length of asbestos fibers (μm)	20.2 (13.5–24.2)	14.1 (0.0–38.8)	0.801	0.437
Mean width of asbestos fibers (μm)	0.6 (0.4–0.8)	0.3 (0.0–0.5)	2.52	0.009
Mean length of all short fibers (μm)	3.9 (0.0–4.3)	0.0 (0.0–4.5)	0.614	0.556
Mean width of all short fibers (μm)	0.6 (0.0–0.7)	0.0 (0.0–0.9)	0.615	0.556

Data are presented as median (interquartile range). LW-Broni = women who spent most of their daily life exposed to asbestos; Otherwise = spent only part of their daily life in a condition that implied asbestos exposure. LW-Broni, living and working at Broni; MW test, Mann-Whitney test.

Table 5 Median and IQR of the concentration of each type of asbestos in the two groups of daily exposure (women that used to spend most of their daily life exposed to asbestos vs. those who spent only part of their daily life in a condition that implied asbestos exposure)

Variables	Amount and daily exposure			
	LW-Broni (n=29)	Otherwise (n=7)	MW test	P value
Chrysotile/asbestiform antigorite per gram of dry weight lung tissue (ff/gdw)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	−0.709	0.445
Crocidolite per gram of dry weight lung tissue (ff/gdw)	4,456.7 (0.0–13,450.8)	4,806.5 (0.0–8,714.0)	0.335	0.761
Amosite per gram of dry weight lung tissue (ff/gdw)	7,124.9 (0.0–22,016.9)	4,806.5 (0.0–19,985.9)	0.507	0.636
Tremolite/actinolite per gram of dry weight lung tissue (ff/gdw)	4,851.4 (2,944.3–16,813.5)	0.0 (0.0–0.0)	2.60	0.006

Data are presented as median (interquartile range). LW-Broni = women spent most of their daily life exposed to asbestos; Otherwise = spent only part of their daily life in a condition that implied asbestos exposure. MW test, Mann-Whitney test.

used to spend most of their daily life exposed to asbestos (because they lived in Broni and worked in the same town or were housewives) and those who spent only part of their daily life in a condition that implied asbestos exposure (because they lived in Broni and worked elsewhere or vice versa) (*Table 4*).

No differences in fiber length have been pointed out according to how much time a day the subjects were exposed to asbestos, whereas the asbestos fibers were significantly wider in women exposed for the whole day compared to those exposed for a lower amount of time (*Table 4*).

The concentration of tremolite/actinolite asbestos was found to be significantly higher in those who were exposed for the whole day compared to women exposed for only a part of their day (*Table 5*), while the other kind of asbestos did not show any significant difference.

Finally, a significant positive correlation between the duration of exposure and the concentration of asbestos

Table 6 Spearman's rank correlation coefficients between the variables regarding the inorganic lung content and the chronologic variables considered

Variables	Duration of exposure (years)		Latency (years)		Survival (months)		Time since end of exposure (years)	
	rho	P value	rho	P value	rho	P value	rho	P value
Asbestos fibers per gram of dry weight lung tissue	0.413	0.008	0.427	0.005	0.072	0.655	–0.186	0.249
Asbestos bodies per gram of dry weight lung tissue	0.216	0.179	0.237	0.139	0.125	0.439	0.164	0.311
Mean length of asbestos fibers	0.238	0.143	0.101	0.538	–0.241	0.137	–0.206	0.207
Chrysotile/asbestiform antigorite fibers per gram of dry weight lung tissue	–0.204	0.205	0.130	0.423	0.017	0.913	–0.050	0.757
Crocidolite fibers per gram of dry weight lung tissue	0.340	0.032	0.233	0.147	–0.068	0.676	–0.115	0.477
Amosite fibers per gram of dry weight lung tissue	0.336	0.033	0.398	0.011	0.163	0.313	–0.156	0.334
Tremolite/actinolite fibers per gram of dry weight lung tissue	0.294	0.065	0.272	0.088	–0.074	0.646	–0.120	0.460

fibers in lungs, as well as between the latency and the concentration of asbestos fibers in lungs (*Table 6*), have been observed. Moreover, the concentration of crocidolite resulted to be positively correlated with the duration of exposure and amosite concentration was positively correlated with duration of exposure and latency (*Table 6*).

Possible differences in lung content (in terms of concentration and type of inorganic fibers, asbestos fibers and their dimensions and ABs) according to the histological type of MM have been investigated, without finding any significant result (Table S1). Analyzing the histologic type according to the other variables (latency, duration of exposure and survival since diagnosis) a significantly longer survival in subjects with epithelioid MM compared to sarcomatoid and biphasic/desmoplastic MM was found.

Discussion

In this study, we investigated the characteristics of inorganic fibers contained in samples of lung tissue of a series of women non-occupationally exposed to asbestos during the activity of the asbestos-cement plant located in Broni (Pavia, Italy).

In sum, we found that, despite the well-known history of environmental and/or household exposure, not in all cases asbestos fibers and ABs have been detected at SEM-EDS investigation. Regarding the type of asbestos, amosite was found at the highest concentration, followed by crocidolite, tremolite/actinolite and chrysotile/asbestiform antigorite. Interestingly, the concentration of all inorganic fibers, of asbestos and ABs was significantly higher in women with AEH compared with those with only AEE. Moreover, the concentration of asbestos showed a positive correlation with duration of exposure (this correlation was observed also for crocidolite and amosite) and latency (this correlation was observed also for amosite). The concentration of asbestos in lungs was not different according to the daily time of exposure and the histological type.

The present study has two main limitations.

First, the subjects of the study have been retrospectively extracted from the archive of the Unit of Legal Medicine of Pavia University among those for whom a forensic autopsy was ordered by the Prosecutor in a context of a penal trial. Therefore, they do not represent the totality of women exposed to asbestos in Broni who died from MM. Notwithstanding, a selection bias is unlikely as, in the considered period of time, a forensic autopsy has been performed for most subjects who died from MM in the competence area, as the notification to the Prosecutor is mandatory in case of deaths related to occupational diseases. Furthermore, if the Prosecutor decided not to perform the forensic autopsy, the decision was based on motivations not related to the lung fiber burden (5).

The second limitation concerns the relatively low amount of lung tissue examined under SEM-EDS, as only one sample for each subject has been analyzed. Yet, on the basis of previous research (22), a lung sample taken as above explained is representative and provides the best cost-efficacy ratio. Moreover, to make sure about the suitability of the samples, in three cases we performed the analysis on two different samples from the same subject, obtaining overlapping results.

Despite the above-described limitations, the series is sufficiently large to draw sound conclusions, as the statistical analysis confirmed; indeed, this is among the largest series of women for whom the lung content has been systematically analyzed using SEM-EDS.

Moving to the interpretation of the above-summarized results, in 14% of cases, all of which had documented exposure to asbestos (AEE or AEH) the asbestos concentration was below the detection limit. This is consistent with what was observed in our previous research, conducted on a mixed-sex population exposed to asbestos in Broni, in which we detected no asbestos in around 19% of the analyzed subjects (16,27). This is in line with previous literature, as other authors observed several cases of MM without any asbestos in lungs detected at SEM-EDS (28).

In order to explain this finding, the following hypotheses can be considered.

(I) The lungs of these subjects never contained asbestos. This is extremely unlikely, as they had documented exposure in a setting where high amounts of asbestos were air-dispersed. In addition, they died from MM and, even though MM not related to asbestos exposure has been reported (8), this does not seem to be the case, given the known history of exposure which was documented in the context of a penal trial.

(II) Asbestos was present but not detected with the used technique. Also, this explanation can be ruled out, as the hypothesis that thin chrysotile fibers can be missed by SEM-EDS is unlikely considering that in some cases of the present series chrysotile has been detected and classified with certainty.

(III) The asbestos inhaled by those subjects has been completely cleared from their lungs. This hypothesis represents the most likely explanation, as the rapid clearance of chrysotile is well known and characterized in literature (29). In studies on animal models the half-life of chrysotile has been estimated as 90 days (30). Lung clearance regards also amphiboles, which are, however, much more biopersistent compared to chrysotile: studies on animal models estimated a half-life of crocidolite and amosite in lungs of, respectively, 50 and 18 months (31). Moreover, in the present series, a long period of time elapsed between the end of exposure and death (more than 22 years).

This finding suggests that the asbestos lung content is subjected to deep changes with the passing of time after the end of exposure, especially regarding chrysotile. Therefore, SEM-EDS evaluation should be interpreted carefully, especially in a legal context, where the causal attribution of ARDs depends on this assessment, as the lung content at the moment of death, even though considered the most reliable tool for assessing previous exposure (23), does not exactly reflect the actual amount of asbestos which was inhaled by the subject during life.

Interestingly, ABs have been detected in only 30% of the investigated women. The technique here used (SEM-EDS) allows to analyze a lower amount of lung tissue compared to the optical microscopy (24), so the amount of ABs reported here might have been underestimated. However, as in several cases we found a concentration of asbestos above the threshold considered indicative of previous exposure (19) but no ABs, it is worthy to underline that ABs concentration does not always reflect the asbestos lung burden.

Therefore, we think that, in order to determine previous asbestos exposure, it is always preferable to perform SEM-EDS investigation in order to detect and count asbestos fibers rather than optical microscopy to visualize ABs.

The next consideration regards the type of asbestos detected in the present series. We know that at the asbestos cement plant located in Broni the most used asbestos types were chrysotile and crocidolite. Also, amosite was used, but in small amounts, as an additive (14).

Interestingly, the results of the lung content analysis did not reflect these data about the asbestos containing materials production at the factory. Chrysotile/asbestiform antigorite was detected in only 2% of cases. This is not surprising, as it is well known that, compared to amphibole asbestos, chrysotile is much less biopersistent (29). This characteristic is due to the different crystalline structure of chrysotile, which is subjected to dissociation of magnesium from the fiber's surface in the acid lung microenvironment (29). For this reason, chrysotile is more easily fragmented and phagocytized by the lung macrophages and removed from the alveoli through the lymphatic stream (29). In our previous work conducted on a series of subjects exposed in the same setting (mainly males) we detected no chrysotile at all (27). The scarce presence of chrysotile is in line with a previous study about lung content in female cases of MM (4).

The current scientific evidence suggests that the carcinogenic potency of chrysotile asbestos is lower compared to amphibole asbestos in humans (25,26,29,32), due to its rapid clearance from lungs. However, the conclusion that the epidemiological evidence for lung cancer strongly supports a difference in carcinogenic potency between chrysotile and amphiboles has been questioned by a meta-analysis by Lenters *et al.* (33).

Surprisingly, in one of the four cases in which chrysotile was detected, the time since the end of exposure was 49 years, the highest among the entire series. Even though this is a single case, it may suggest that the capability to clear chrysotile (and, generally, asbestos) from lungs might be subjected to individual variability, and this could be a possible clue in the research about the MM individual susceptibility. Anyway, we must take into account that asbestos removed from lungs is gradually translocated towards the pleura, where asbestos exerts its carcinogenic potential (34). Therefore, studies about pleural asbestos content are necessary to clarify this concept.

The lack of detection of chrysotile (in spite of documented exposure), in many cases, likely reflects a less important role in causing MM, as indicated by the greater potency for amphiboles in MM causation as compared to chrysotile, well-known and confirmed by recent studies (35,36).

The most detected kind of asbestos in the present study was amosite, followed by crocidolite, tremolite/actinolite asbestos and finally chrysotile. This finding is in agreement with Barbieri *et al.*, who found amosite as the main kind of asbestos in 15 women, 8 of which were exposed in Broni (5). According to the literature data about the production of the plant in Broni (14), amosite was used in small quantities, whereas chrysotile and crocidolite were the most utilized kind of asbestos. However, it is extremely difficult to determine the exact amounts of the different asbestos used at a given time. This finding suggests that probably, at the plant located in Broni, more amosite was used with respect to what was declared and reconstructed according to the production data.

Furthermore, we found non-commercial asbestos (tremolite/actinolite asbestos): the most likely explanation is that the women here investigated have been exposed to talc-containing products (known to be contaminated with tremolite/actinolite asbestos), considering the large industrial use of talcum at the time (37). This was observed also in our previous study conducted on a mixed-sex series (27). However, non-commercial amphiboles are also contaminants of some chrysotile ores (38), so this source is possible, even if less likely considering the geographic setting of the study.

In women with both exposures (AEH) the amount of asbestos was significantly higher than in those with AEE alone, suggesting that household exposure represents a major source of asbestos exposure, that can significantly increase the asbestos amount in lungs, even in a context where AEE was heavy, since the emissions of the asbestos-cement plant in the surroundings area, never measured in 1960s and 1970s, are supposed to have been extremely intense (6,39). This finding is consistent with what was stated by Marsh *et al.* using epidemiological data (40) and with what was observed by other authors (5,38), who pointed out that familial exposure can lead to very high lung fiber burdens, comparable to occupational exposure. Therefore, this result confirms that non-occupational exposure is not synonymous to low-level exposure (41).

Unexpectedly, no significant differences in lung concentration of asbestos were detected according to how much time a day the woman spent exposed to asbestos. Even though the time spent daily in contact with asbestos is an important parameter in the retrospective assessment of asbestos exposure (42), in this series it did not seem to influence the asbestos burden in lungs. However, it must be considered that the exposure context of the present series is specific: the environmental exposure in Broni was so heavy that the daily time of exposure necessary to reach a high asbestos burden in lungs may be much lower compared to other settings of AEE.

Interestingly, no correlation between the distance of the house or workplace from the plant and the amount of asbestos in lungs has been observed. This means that the dispersion of large amounts of asbestos from the plant involved a wide area, as confirmed by the case of a woman resident in Albaredo Arnaboldi, 10 km far from Broni. She had a concentration of asbestos of 8,490 ff/gdw, very similar to what was observed in women who lived only a few hundred meters far from the plant. This result, apparently unexpected, is consistent with epidemiological data about the population around Broni, that showed an increased relative risk of MM not only in the town of Broni but also in the surrounding municipalities, especially for women (6,39). Yet, it must be considered that before 1993 asbestos was largely used in products of daily use and asbestos containing materials were widely installed. Moreover, the Fibronit plant was not the only firm that manufactured asbestos. Therefore, this subject might be exposed to other sources of asbestos, unknown (or forgotten).

In the present series, the prevalent MM histological subtype was epithelioid, consistently with other case series (4,43). As already known (4), this histological type was significantly associated with longer survival. We investigated possible relationships between MM subtype and exposure characteristics (kind of exposure, latency, duration, asbestos burden and asbestos types observed in lungs), without finding any significant correlation, similarly to what was previously reported for mixed-sex series (27,43). On the contrary, Leigh *et al.*, investigating a series of 226 cases of MM in Australia, found a statistically significant relationship between lung fiber content and histological type of MM, observing lower asbestos concentration in epithelial MM compared to mixed and sarcomatous, which showed the highest asbestos burden (44).

As expected, we found a positive correlation between the amount of asbestos in lungs and the duration of exposure. Even though this correlation was quite weak, it is in line with what is logically predictable and with what was previously observed by other authors (45,46). This result is in contrast with our previous research (16), where we found the absence of this correlation, possibly explained by the clearance, that may compensate for the accumulation of new fibers. Probably in women, this ratio between accumulation and clearance presents some differences compared to males. The same can be hypothesized regarding the observed correlation between asbestos concentration on lungs and latency. In fact, the latency period includes duration of exposure and time since the end of exposure. Therefore, this correlation reflects the equilibrium between the accumulation and the clearance of asbestos, which seems to present sex-based differences. Those correlations regarded, in particular, amosite and crocidolite, corroborating the above explained hypothesis of a high biopersistence of these kinds of asbestos compared to chrysotile and to the other types of amphibole asbestos. Our data, instead, do not confirm that higher asbestos burdens in lungs can cause an earlier onset of MM, as suggested by Dragani *et al.* (47).

Conclusions

In the present study, we systematically analyzed the inorganic lung fiber burden (counting, measuring, and classifying regulated asbestos and inorganic fibers as well as those shorter than 5 µm) in 42 women who have been non-occupationally exposed to asbestos during the activity of the Fibronit factory, located in Broni (Pavia, Italy).

The obtained data confirm the relevance of non-occupational asbestos exposure in determining asbestos concentration in lungs, and highlight the importance of household exposure, that, if added to anthropogenic environmental one, significantly increases the asbestos lung concentration compared to environmental exposure alone.

Moreover, our results imply that the postmortem assessment does not exactly reflect the actual amount of asbestos which was inhaled by the subject during life (due to asbestos clearance). Therefore, although SEM-EDS is considered the most reliable tool for assessing previous exposure to asbestos, the results provided by this tool should be interpreted with caution, especially in a legal context.

In addition, it must be underlined that Broni was a specific scenario of asbestos exposure, as around the plant, which was located very close to the city center, the air dispersion of asbestos was very intense (6,39). Yet, this setting was similar to what is still the reality in some countries (e.g., Russia, Central Asia countries). Besides this, in Italy, people are still suffering from the consequences of exposure (not only occupational) that occurred decades ago, and the MM epidemic is expected to continue in the next years, as demonstrated by the forecasts about the future incidence and mortality due to MM (2).

Taken together, the results presented offer a novel perspective on the characteristics of inorganic lung content in non-occupationally exposed women. Indeed, very few papers in literature focus on the sex-related differences in asbestos lung concentration. If compared with our previous work about lung content in Broni inhabitants and Fibronit workers (16,27), this focus on women offers new insights about possible differences between sexes in the efficiency of lung clearance of asbestos, regarding both chrysotile and amphiboles asbestos, and offers clues for further research.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1061/coif). C.C. and E.B. acted as experts in trials and litigations concerning asbestos (not related to the subjects of this study). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of IRCCS Policlinico San Matteo (Pavia, Italy) (No. 20180060636) and individual consent for this retrospective analysis was waived.

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Table S1 Median and IQR of each variable regarding the inorganic lung content in the three groups of histological type of MM considered for the statistical analysis

Variables	Epithelial (n=31)	Sarcomatoid (n=6)	Biphasic/desmoplastic (n=5)	KW test; P value
Inorganic fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	52,113.9 (24,396.6–98,703.9)	43,351.9 (20,214.3–53,112.8)	77,534.2 (44,471.4–79,071.0)	0.544; 0.761
Asbestos fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	25,167.4 (6,819.3–61,929.8)	13,703.6 (8,085.7–27,367.0)	29,075.3 (26,682.9–35,142.7)	0.893; 0.541
Asbestos bodies per gram of dry weight lung tissue (ABs/gdw), median (IQR)	0.0 (0.0–4,245.0)	0.0 (0.0–8,852.1)	0.0 (0.0–13,178.5)	0.718; 0.698
Short fibers per gram of dry weight lung tissue (sff/gdw), median (IQR)	7,994.4 (0.0–15,140.5)	15,829.3 (0.0–17,704.3)	0.0 (0.0–4,623.1)	3.914; 0.141
Mean length of all fibers (μm), median (IQR)	18.7 (14.8–21.4)	16.1 (14.6–26.4)	17.4 (16.7–22.1)	0.446; 0.800
Mean width of all fibers (μm), median (IQR)	0.7 (0.6–1.1)	0.8 (0.4–0.8)	0.8 (0.6–0.9)	0.938; 0.625
Mean length of asbestos fibers (μm), median (IQR)	19.9 (11.7–24.9)	23.4 (15.5–30.0)	19.0 (16.7–24.2)	0.512; 0.774
Mean width of asbestos fibers (μm), median (IQR)	0.6 (0.4–0.7)	0.5 (0.2–0.6)	0.6 (0.5–0.8)	0.457; 0.795
Chrysotile/asbestiform antigorite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.527; 0.466
Crocidolite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	3,562.5 (0.0–13,450.8)	5,376.4 (0.0–6,841.8)	4,845.9 (4,806.5–8,894.3)	0.246; 0.884
Amosite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	13,070.9 (0.0–22,597.8)	5,442.3 (0.0–8,852.1)	8,894.3 (4,845.9–13,178.5)	0.817; 0.664
Tremolite/actinolite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	3,607.5 (0.0–10,194.5)	4,447.1 (2,950.7–13,683.5)	8,894.3 (4,392.8–9,246.1)	1.151; 0.562

IQR, interquartile range; MM, malignant mesothelioma; KW test, Kruskal-Wallis test.

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