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ASBESTOS LUNG BURDEN DETERMINATION IN AN URBAN POPULATION FROM MILAN, ITALY.
ANALYSIS OF A NECROSCOPIC SERIES FROM 2009 TO 2011.

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INTRODUCTION

The term asbestos is a commercial term rather than a true mineralogic definition [1] and refers to a group of naturally occurring mineral fibers, basically divided into two subgroups: the serpentine subgroup and the amphiboles subgroup.

To the serpentine subgroup belongs only the chrysotile (white asbestos), a hydrated magnesium silicate with a wavy and filamentous morphology of its elementary bundles, a marked tendency for its elementary bundles to split into single and shorter fibrils and a $Mg_3Si_2O_5OH_4$ chemical formula.

The amphiboles subgroup is composed by the varieties crocidolite (blue asbestos, with a $Na_2Fe_3^{++}Fe_2^{+++}Si_8O_{22}OH_2$ chemical formula), amosite (brown asbestos, with a Fe-$Mg_7Si_8O_{22}OH_2$ chemical formula), tremolite (chemical formula $Ca_2Mg_5Si_8O_{22}OH_2$), actinolite (chemical formula $Ca_2MgFe_5Si_8O_{22}OH_2$) and anthophyllite (chemical formula Mg-$Fe_7Si_8O_{22}OH_2$). All the amphiboles share a typical straight morphology with parallel sides and do not show nor splayed ends neither a marked longitudinal splitting tendency. Crocidolite and amosite varieties are referred together as the Commercial Amphiboles, while tremolite, actinolite and anthophyllite represent the Non-Commercial Amphibole series.

Asbestos deposits occur in four types of rocks (alpine-type ultramafic rocks, stratiform ultramafic intrusions, serpentine limestone and banded ironstones) showing a well-defined geographic distribution: chrysotile deposits locate mainly in Quebec, Rhodesia, Russia, China, Italy and USA; crocidolite deposits in South Africa, Australia, Colombia and Rhodesia; amosite and actinolite deposits in South Africa and India; tremolite deposits in the Mediterranean region, Pakistan and South Korea; anthophyllite deposits in Finland and USA [2].

Because of its extraordinary chemical and physical properties, asbestos has been very intensively exploited in either the pre-industrial and the industrial age. In modern times the first attempts at serial asbestos mining were done in the Urali region from 1720, in Quebec from 1886 and in South Africa from 1910. In Italy the industrial asbestos mining started in the Valtellina district in 1866 and in the small town of Balangero -near Turin- in 1923 [3]. Chrysotile, crocidolite and amosite are the true commercial varieties of asbestos (chrysotile alone representing worldwide the 90-95% fraction of the commercial asbestos [4,5]), mainly consumed in textile industry, construction industry, shipyard activities, insulation activities and friction materials industry. World production of asbestos peaked in 1975 at about 5.09 million metric tons and then decreased (about 4 million metric tons in 1990 and less than 2 million metric tons in 2000) [6] mainly because of the supervening legislative bans rather than the technical abandon of the mineral. In Italy the crocidolite ban took place in 1986, while the definitive ban on all the asbestos varieties was imposed in 1992.

National and international bans on asbestos were introduced because of the cumulative evidences about the relevant asbestos pathogenicity for humans: in 1890 the inhalation of asbestos fibers was first related to the development of a non-specific lung disease, while in 1907 some human deaths were positively linked to asbestos exposure [7]; in 1924 W.E. Cooke described an asbestos-related lung fibrosis and named it asbestosis [8]; in the Thirties and Forties the first reports about lung cancer in asbestos workers were published [9,10] and the hypothesis about the asbestos carcinogenicity for exposed workers began to circulate [11]; in 1960 Wagner at al reported several cases of malignant mesothelioma among asbestos miners from South Africa [12].

Asbestos fibers are nowadays considered an etiologic factor for pleural plaques and other benign pleural diseases, asbestosis, malignant mesothelioma and lung cancer in all its 4 major subtypes [7,13-18]. At the present time conflicting opinions do exist about the etiologic role of asbestos fibers for other neoplastic diseases such as the gastrointestinal cancer, pancreatic cancer, renal cell cancer, laryngeal cancer, ovarian cancer and leukemia/lymphoma [19]. Asbestos-related diseases show a long latency period (mean interval of 30-40 years for mesothelioma [20-22]) and asbestos-induced neoplastic diseases are therefore still increasing despite the introduced bans: asbestos pathology and asbestos clinical medicine are thus still actual topics. The latency between occupational asbestos exposure and the diagnosis of asbestos-related cancer also explains the actual focus on asbestos by the law courts and consequently by the forensic pathology [23,24].

After the asbestos ban the pattern of asbestos exposures was expected to gradually change with a predominant shift from the typical massive occupational and paraoccupational exposures (the first and the second waves of asbestos exposure) to the milder environmental exposures both from antropic and natural sources (the third wave of asbestos exposure) [6].

The current guidelines for the clinical and pathological diagnosis of asbestos-related diseases were stated in January 1997 in the Helsinki Document [25]. The

Helsinki Document first underlined that lung analyses for asbestos fibers can provide data to supplement and integrate the occupational history data and then stated that an Electron Microscope amphibole fibers count over 1,000,000/g dry (fibers > 1 μm in length) or an Electron Microscope long amphibole fibers count over 100,000/g dry (fibers > 5 μm in length) or an Asbestos Bodies count over 1,000/g dry or an Asbestos Bodies count over 1/ml of bronchoalveolar lavage fluid identify persons with a high probability of exposure to asbestos dust at work. Focusing on mesothelioma, the Helsinki Document briefly reminded that non all mesotheliomas are asbestos-induced cancers and stated that such a cancer can be related on a probability basis to asbestos exposure in the presence of a lung fiber count clearly exceeding the reference values of that laboratory or in the presence of a reliable history of asbestos exposure. Focusing later on lung cancer, the Helsinki Document stated that a 2-fold risk of lung cancer is related to an amphibole fibers load over 5,000,000/g dry of lung tissue (fibers > 1 μm in length) or to a long amphibole fibers load over 2,000,000/g dry of lung tissue (fibers > 5 μm in length) or to an Asbestos Bodies count equal to 5,000-15,000/g dry or to an Asbestos Bodies count equal to 5-15/ml of bronchoalveolar lavage fluid.

Helsinki statements about the asbestos ascription of lung cancers have been vigorously debated [26-31] and opposite to the articulated Helsinki Criteria stands the hypothesis of asbestos-induced lung onchogenesis only in the presence of an asbestosis background. According to the concept that an asbestos-related disease (and especially an asbestos-related cancer) cannot be automatically labelled as an occupational disease, the Helsinki Document also recommended in its introductive section that each laboratory should establish its own reference values from the general population and that the median values for occupationally exposed populations should be substantially above the reference values.

In 1998 the European Respiratory Society Task Force further developed some Helsinki issues and published the guidelines for the mineral fiber analyses in biological samples [32]. According to the European Respiratory Society guidelines, each laboratory should create its reference values for lung burden of mineral fibers by sampling up to five different subgroups from the general population: the subgroup A composed by individuals from rural areas with no identifiable occupational exposure to asbestos; the subgroup B composed by individuals from urban areas with no identifiable occupational exposure to asbestos; the subgroup C composed by individuals from areas with asbestos deposits in the soil; the subgroup D composed by individual with identifiable non-occupational exposure to asbestos; the subgroup E composed by individual with identifiable occupational exposure to asbestos. Surgical or necroscopic samples are needed to properly analyze such subgroups from the general population. Surgical series and necroscopic series show different advantages and suffer different limitations: surgical series offer small amount of lung tissue but allow the researchers to establish a thorough occupational history for every single case, while necroscopic sample series offer great amount of lung tissue but do not allow the researchers to establish a thorough occupational history for every single case. For necroscopic series lacking occupational histories assessment, it is recommended by the European Respiratory Society guidelines a preliminary exclusion of cases with clinical or pathological diagnosis of diseases possibly related to asbestos exposure.

The reference values used by the 6 laboratories participating the European Respiratory Society Asbestos Task Force for urban individuals without known occupational exposure to asbestos (subgroup B) are about 1,000,000-4,000,000 ff/g dry of lung tissue for total fibers and about 150,000-300,000 ff/g dry of lung tissue for long fibers (fibers > 5 μm in length).

The lung content of both coated and uncoated asbestos fibers is a widely accepted index of the lifetime cumulative exposure to asbestos [33-38]. Human exposure to asbestos mainly happen through the inhalation of single fibers or fiber bundles and the asbestos lung burden is to be considered as the result from the dynamic balance between the fibers deposition (the fibers access to the deep lung being crucially affected by the respiratory function and the pulmonary anatomy of the subject) and the fibers clearance (the fibers egress from the deep lung being crucially affected by the alveolar macrophage function and the lymphatic drainage to the pleural space and the main lymphatic circulation) [14,39]. Also the morphological and chemical features of the airborne fibers crucially affect their deposition to clearance rate within the lower airways and the deep lung. Chrysotile bundles regularly stop in the upper airways [40,41] while single chrysotile fibers accessed to the alveolar zones rapidly tend to leach magnesium and to split into small fibrils: chrysotile consequently shows a low biopersistence compared to amphiboles [42-44] and the estimation of the lung burden of asbestos fibers is therefore a better index for amphibole exposition then for asbestos exposition as a whole [45]. Chrysotile has a pulmonary half-life of months, while amphiboles can remain in the lung parenchyma for decades [14,46-48]. However some authors suggest that two different sets of inhaling chrysotile fibers do exist, the former being represented by smaller and easy-clearing fibers (fibers cleared within weeks or months very unlikely to be detected by lung burden estimations) and the latter being represented by longer fibers prone to penetrate the thin alveolar walls and to fix perpetually in the subepithelial interstitium of the lung (“harsh” fibers more likely to be detected by lung burden estimations) [49,50]. It seems however that for chrysotile fibers a lower persistence inside the lung parenchyma combines with a higher persistence around the black spots in the pleural space [51].

Smoking is considered to inhibit asbestos clearance from the lung parenchyma [52,53].

Coated asbestos fibers (also called Asbestos Bodies, AB) form in the deep lung when a mixed layer of iron-protein-mucopolysaccharide material covers a single resident asbestos fiber [54-57]. Coated fibers usually represent a small fraction of the total asbestos content within the lungs at a single point in time [58-61] and the precise amount of this fraction depends on either host-dependent and host-independent variables [62]. Among the host-independent variables the most important seem to be the fiber dimensions (thick fibers longer than 10-20 μm more likely to be coated [58,63]), the fiber surface features (smooth fibers less likely to be coated [64]) and the fiber chemical composition (amphibole fibers more likely to be coated than chrysotile fibers [56,65-72]), while among the host-dependent variables the most relevant is the genetic susceptibility to be a poor AB-former versus a rich AB-former [67,73]. The lung AB burden (the product of a suggested balance between AB formation and AB breakdown [74,75]) is then to be considered as a ultra-mediated index of the lifetime cumulative exposure to asbestos [76], two identically exposed individuals turning out to be very different in AB count just because of different genetic tendencies in forming AB. Especially in the case of a low AB count [77], an isolated AB estimation is therefore not reliable in analyzing the lifetime cumulative exposure of a patient and it needs to be followed by the estimation of the burden of uncoated asbestos fibers [76,78]. Moreover the Asbestos Bodies belong to the wide group of Ferruginous Bodies (FB group comprehensive of true AB and bodies other than AB) and the estimation of the AB content of the lungs is usually performed using the traditional light microscope: this inexpensive but barely morphological technique shows very well all the FB in the lung digest but does not allow the analyst to make a definite chemical distinction between true AB and other bodies [79,80]. Nonasbestos FB may form -with limited peculiar morphology- on talc fibers, sheet silicates (up to 20% of the total FB burden in some series from the general population) [66], carbon fibers [81,82] (up to 90% of the total FB burden in series from the general population [56]), metal oxides (mainly titanium bodies [83]), ManMade Mineral Fibers [84-86], diatomaceous earth [66,82] and erionite fibers [87].

Asbestos fibers quantification in lung tissue is typically performed by light (coated fibers counting) or electronic (uncoated fibers counting) microscopy on lung digested samples [88]. Samples to be analyzed should be representative of the whole lungs and the preliminary samples selection should warrant a wide mapping of all the accessible tissue [89]: necroscopic samples (usually wide and bilateral) are therefore more informative than surgical samples (usually small and monolateral). Lung dissolution can be accomplished either by wet chemical digestion or low-temperature ashing. The wet chemical digestion is usually performed through the potassium hydroxide procedure or the sodium hypochlorite procedure [90]. Once the dissolution of the lung sample is complete, the inorganic residue may be vacuum-filtered and collected on 0.2-0.45 μm pore size polycarbonate membranes. The polycarbonate membrane can be then analyzed in Light Microscopy (traditional Light Microscopy or Contrast Phase Light Microscopy), Scanning Electron Microscopy or Transmission Electron Microscopy. AB counting can be reliably performed using LM at a magnification 100x-400x and the results for an individual analysis can be reported as number of AB per gram of wet tissue or better as number of AB per gram of dry tissue. AB counting assays performed on case series should also record the fraction of cases featuring at least one AB in the LM analysis. SEM and TEM instruments should always be coupled with an EDXA system for the chemical characterization of all the detected fibers and their results should be reported as number of asbestos fibers (both coated and uncoated) per gram of dry tissue with specific estimations about chrysotile, Commercial Amphiboles and Non-Commercial amphiboles. SEM and TEM analysis should also quantificate the lung burden of non-asbestos fibers. For SEM and TEM analysis it is mandatory a preliminary statement about the so-called counting rules, with a special focus on the analytical sensitivity and on the dimensional parameters of the fibers to be considered and counted [89].

The microscopic estimation of the asbestos lung content allowed both the researchers and the clinicians to get a better comprehension about the asbestos pathogenicity and to correlate the occurrence of various fiber-related lung diseases with the cumulative fiber burden within this target tissue [43,88,91,92]. Over the last decades many works have been published about the relationship between asbestosis occurrence and lung asbestos burden [93-97] (heavy lung asbestos burden in the vast majority of cases with median values well above the 1.000 AB/g dry cut off and the 1,000,000 ff/g dry cut off [89]), benign pleural diseases occurrence and lung asbestos burden [35, 98-101] (patients with pleural plaques having considerably smaller asbestos burden than patients with asbestosis and slightly smaller burden than patients with mesothelioma [89]), mesothelioma occurrence and lung asbestos burden [35,93,97,102-105] (patients with mesothelioma having smaller asbestos burden than patients with asbestosis [89]; existence of a subgroup of mesothelioma patients with a mean asbestos content in lung tissue indistinguishable from a reference population [20,93,106-108]) and lung cancer occurrence and lung asbestos burden [93,94,104,109,110] (asbestos-related lung cancers showing histological asbestosis and/or an asbestos content in the lung very similar to patients with asbestosis [89]). Other works have rather investigated the correlation between some kinds of well-known occupational asbestos exposure and the lung asbestos burden [89]: asbestos insulators [111], asbestos manufacturers [112-114], shipyard workers [115] and power plants workers [116] show the greatest median burdens of asbestos in lung tissue with values well above the Helsinki cut offs for non-trivial exposure; molten metal workers, construction workers [21], and chemical refinery workers [117,118] show asbestos median burdens smaller than the previous groups but nonetheless above the Helsinki cut offs for non-trivial exposure; railroad workers [115,119] and brake repair workers [120-123] show median burdens of lung asbestos greater than the general population and very close to the Helsinki cut offs for non-trivial exposure. An increased risk of developing asbestos-associated diseases has been reported among household contacts of asbestos workers too: the asbestos lung content from the relatives of asbestos workers (mainly workers from the first group identified above) is very similar to that of molten metal workers, construction workers and chemical refinery workers and it is then greater than that of the general population [89,124,125].

The determination of the asbestos lung burden from the general population is a crucial work in either an epidemiological and a clinical perspective. Such a background reference allows in fact the researchers to distinguish dose-dependent from dose-independent asbestos-related diseases and to stratify dose-dependent asbestos-related diseases and also allows the clinicians to properly investigate the etiology of any single disease hypothetically connected with asbestos exposure. Reference values from the general population are also very important for the forensic pathologists dealing with asbestos-related claims. Every laboratory involved in routine asbestos estimations should therefore define its reference evidences from the general population and should strictly compare to these evidences all the results coming from clinical or forensic routine analyses [25,32].

In Appendix 1 and 2 the results from main studies about AB lung counting and asbestos fibers lung counting in the general population are summarized. As detailed in the Appendixes, some of the studies directly focused on general population analyses, while others used general populations as control populations versus main case-series having asbestos-related diseases or known occupational asbestos exposure. In the Appendixes only results referring to the general population are reported.

STUDY AIMS

The present work aims at describing the asbestos lung burden in a sample of the general population constituted by subjects resident in the city of Milan. The study sample includes a necroscopic series selected from the 2009-2011 routine practice at the Institute of Forensic Medicine of Milan.

The performed asbestos burden investigation was both qualitative and quantitative.

We examined both the Asbestos Bodies lung prevalence and the asbestos fibers lung burden from the selected sample and we analyzed the influence by age, gender, residential district, birthplace and smoking habit. We focused on asbestos fibers type and metrics and we were also able to evaluate other inorganic fibers lung burden .

As stated in the 1998 Report from the European Respiratory Society Asbestos Task Force, the creation of reliable reference values is a multistep task and studies on necroscopic series represent just one of the pivotal steps: the present work dealt with a 55-cases wide necroscopic populations according to the European Respiratory Society recommendations for the subgroup 2B (individuals from urban areas with no identifiable occupational exposure).

MATERIALS AND METHODS

In the period running from January 2009 to August 2011 55 cases were selected from the necropsy routine at the Institute of Forensic Medicine of Milan-Italy.

The experimental population was composed by 30 males (10 cases ≤ 30 years old, 10 cases > 30 and ≤ 60 years old and 10 cases > 60 years old) and 25 females (5 cases ≤ 30 years old, 11 cases > 30 and ≤ 60 years old and 9 cases > 60 years old).

Criteria for the cases to be enrolled in the experimental population were the caucasoid race, the stable residence in Milan-Italy and the exitus from an endogenous pathology or from a major blunt trauma. Such inclusion criteria were established after an epidemiological survey of the 2006-2008 routine necroscopic practice at the Institute of Forensic Medicine of Milan. The only exclusion criterion was the occurrence of ante-mortem or post-mortem diagnosis of any of the asbestos-related pathologies (pleural adhesions, lung fibrosis, pleural plaques, pleural and peritoneal mesothelioma, lung cancer).

Some anamnestic notes about global health status, tobacco smoking habit (non-smoker *versus* previous smoker *versus* current smoker class I: 1-10 cigarettes/die, class II: 10-20 cigarettes/die or class III: > 20 cigarettes/die) and ante-mortem job occupations were obtained for every case through a brief interview and/or a phone call with the relatives of the selected subject. A subject was considered to be a former smoker after at least a 6 months period of complete ante mortem smoking abstinence. For every case in the population was also preliminarily recorded its exact Milan-residence address. For the purpose of the present study, the Milan topography was considered to be composed by four main sectors: the North West (zone 7 + zone 8), the North East (zone 2 + zone 3 + zone 9), the City Centre (zone 1) and the South (zone 4 + zone 5 + zone 6).

Every case underwent a complete judicial autopsy and every necroscopic procedure comprised a standardized pulmonary sampling allowing a wide lungs mapping [32,78]: five samples free from macroscopic abnormalities were cumulatively obtained from both the lungs, one 10 g subpleural sample coming from each pulmonary lobe. In agreement with the traditional lung mapping for forensic pathology, the upper lobe samples were obtained from the apical region while the lower lobe samples were obtained from the dependent region.

The lung samples from the same case were pooled together [72,78], half the pooling being 10%-formalin fixed and half the pooling getting a temporary 2-3 °C refrigeration. The samples undergone the 2-3 °C refrigeration step were subsequently prepared for the Ferruginous Bodies counting (counting to be performed by traditional Light Microscopy), while the samples undergone the 10%-formalin fixation procedure were subsequently prepared for the asbestos fibers analysis (analysis to be performed by Scanning Electron Microscopy).

Methods employed for the Ferruginous Bodies (FB) analysis

The samples to be analyzed for the FB counting were prepared according to a simplified version of the wet digestion technique described by Mitha and Pooley in 1993 [126]: 1 g of wet lung composed by 5 discrete samples (each sample coming from a different lobe and each weighing about 0.20 g [127]) was chemically digested in a 20 ml solution of commercial bleach until no macroscopic residue was detectable anymore. The sodium hypochlorite digestion step usually needed 24-48 hours to be complete. After the sodium hypochlorite digestion was completed, 5 ml of a 30% hydrogen peroxide solution were added to the original solution for a time of 4-6 hours.

The whole digestion procedure was accomplished in a disposable plastic vial at a constant ambient temperature of 20-22 °C and did not feature any centrifugation or shaking manoeuvre. The Authors decided to avoid heating or shaking on the digesting solution to minimize the risk about artificial FB breakdown [128,129]. After the two-steps lung digestion was completed, the whole solution was vacuum-filtered through a 0.2 µm pore size polycarbonate Millipore® membrane. After sufficient drying, the unstained membrane was directly mounted on a typical histological slide with coverslip and it was then completely explored at both the 100x and the 400x magnifications in bright field LM. The LM analysis was collegially performed by two distinct professional pathologists (one forensic pathologist and one pathologist with a 35-years experience in pulmonary pathology) and all the cases showing one or more FB underwent a further independent LM test by an experienced FB analyst [130]. After the independent confirmation by the experienced FB analysis, only the morphologically typical Asbestos Bodies (AB) were counted. AB counting results were first reported as number of AB/wet lung gram [131,132], but every case with a positive AB count had a further laboratory procedure (1 g of wet lung composed by pieces of tissue adjacent to the original five 0.20 g digested samples was dried to constant weight at 60 °C [133]) to calculate its own lung wet to dry weight ratio and then to allow AB estimations also as AB/dry lung gram [32,134,135]. All the cases with a positive AB counting showed a wet to dry ratio very close to 10, thus allowing a simple multiplicative conversion from the AB/g wet count to the AB/g dry count.

The LM analysts had no knowledge about SEM analyses results or about the intrinsic variables of each testing case. The experienced LM analyst was generally asked to check some filters for morphologically typical AB.

With the notable exception of the confirmation LM test on cases having a FB count ≥ 1, the whole technical and analytical procedure for the AB counting was performed at the Institute of Forensic Medicine of Milan.

The whole technical procedure was performed in strict agreement with the routine forensic laboratory guidelines against artificial contamination of human samples [78].

Methods employed for the asbestos fibers analysis

According to the method [136,137] described by Wang et al in 2000, the formalin-fixed pool composed by five lung samples was sequentially put for 24 hours in a bidistilled-water bath to remove the formalin matrix and then freeze dried [138]. 50 mg of the freeze-dried sample underwent a complete decomposition through a Emitech® K1050X low-temperature oxygen-plasma asher [139] (15 hours of 100 W and 0.06 mbar ashing) and the ashes were sequentially suspended in a solution of 20 ml distilled-water, 20 ml isopropyl alcohol and 20 ml hexane, vigorously shaken for a few minutes and vacuum-filtered on a 0.2 µm pore size polycarbonate Millipore® membrane. After filtering and drying, the polycarbonate membrane collecting the residue was completely decomposed by a second low-temperature oxygen-plasma ashing (15 hours of 100 W [140] and 0.06 mbar ashing) and the ashes were suspended in 50 ml of 3% distilled-water solution of ethylic alcohol. A 10 ml and a 20 ml sample from that solution were finally vacuum-filtered on two distinct 0.2 µm pore size polycarbonate Millipore® membranes, thus producing a “light” membrane and a “heavy” membrane for the SEM analysis (the selection for the best membrane to analyze was later performed by the SEM analyst during a preliminary few fields SEM analysis).

A 5 mm^2 section of both the polycarbonate membranes underwent a gold-coating procedure.

After the preliminary SEM selection of either the light or the heavy membrane, each case from the population got a 12000x SEM analysis on a gold-coated 5 mm^2

section of the selected filter. All the fibers (coated and uncoated asbestos fibers + non-asbestos fibers) longer than 1 μm and with an aspect ratio ≥ 3:1 were counted, the fiber completely locating inside the tested microscopic field counting as 1 and the fiber just partially locating inside the tested microscopic field counting as ½. The SEM analysis stopped after the positive counting of 30 asbestos fibers or after the accomplished test of a number of microscopic fields sufficient to warrant a detection limit at about 300.000 fibers/g dry (200-250 fields in cases not showing any asbestos fibers). The SEM identification of a true fiber (a corpuscle with two straight parallel sides and an aspect ratio ≥ 3 : 1) was mainly morphological, while the distinction between asbestos and non-asbestos fibers was either morphological and chemical. The chemical composition of every encountered fiber was investigated by the Energy Dispersive X-rays Analysis-system [141] connected with the SEM. The encountered fibers were alternatively grouped as asbestos fibers (further divided into chrysotile, Commercial Amphiboles - amosite and crocidolite- and NonCommercial Amphiboles -tremolite, anthophyllite and actinolite-), talcum fibers, titanium-containing fibers and inorganic fibers other than asbestos. No attempt was made to differentiate true asbestos fibers from cleavage fragments. With very few exceptions, every encountered asbestos fiber was measured for length and width.

The counting results were expressed as number of fibers per g of dry tissue (ff/g dry) together with the 95% Confidence Interval (95% CI) and the Detection Limit (DL) values. The DL was defined case by case as the upper boundary of the 95% Poisson CI for a SEM zero count. The cases not showing any asbestos fiber in the SEM analysis were then best defined as having an asbestos-fibers concentration lower than the half of the analytical sensibility (AS). This value was calculating as 1/2 x AS = 1/6 x DL, 1/6 being the multiplicative factor for the minimal hypothesis of just a half fiber in the SEM count. For the successive median values calculations, the cases having a SEM count lower than the 1/2 x AS value were considered as having a SEM count equal to the 1/2 x AS value itself [62].

All the SEM analyses were performed by the same experienced analyst. The SEM analyst had no knowledge about the LM analyses results or about the intrinsic variables of each testing case.

The whole technical and analytical procedure for asbestos fibers count was performed at the ARPA Lombardy Laboratories in Milan. ARPA Milan Laboratories represent the official Regional Centre for asbestos fibers analysis from both atmospheric and human samples and regularly cooperate with the Mesothelioma Registers from the northern Italian regions [142,143].

Statistical analyses

Standard descriptive statistics (means, Standard Deviations [SDs], medians, Interquartile-Ranges [IQRs], and proportions) have been used to summarize data. For normally distributed data, Student's t-test and Fisher's exact test were used to investigate potential differences in the variable distributions. Global and type-specific fiber burden showed asymmetric distribution and were log-transformed to approximate normality. Consistently, differences in the fiber burden distribution among strata of categorical variables of interest were investigated using nonparametric tests (Wilcoxon-Mann-Whitney Test and Kruskal-Wallis Test).

The association between fiber burden and age at death was also evaluated with linear models by regressing the log-transformed fiber burden variable over age at death. Effects are therefore expressed as percent change in fiber burden per 1-year increase in age at death.

For fiber length and diameter geometric means and corresponding 95% Confidence Intervals (95% CIs) were reported. Differences in fiber dimensions across fiber types were evaluated using one-way ANOVA. All tests of statistical significance were two sided. Statistical analyses were performed using Stata/MP 11.1 (Stata Corporation, College Station, TX).

RESULTS

The experimental population was composed by 55 cases, 30 males and 25 females. The mean age from the whole experimental population was 44,9 ± 20,4 years (range 18-83 years). In great detail, 15 cases (10 males and 5 females) belonged to the ≤ 30 years old class, 21 cases (10 males and 11 females) to the 30-60 years old class and 19 cases (10 males and 9 females) to the > 60 years old class.

The mean age did not significantly differ between genders (42,5 ± 20,6 years for males; 48 ± 20,2 years for females).

All the cases were residents in Milan: 19 cases lived in the Southern zone, 15 cases in the North Eastern zone, 14 cases in the North Western zone and 7 cases in the City Centre. Twenty-six cases from the whole population were born in Milan (mean age 40,7 ± 18,7 years), while 29 cases were not (mean age 49 ± 21,1 years). 4 out of the 29 cases born outside Milan were born abroad. According to the tobacco smoking habit, the cases from the experimental population were alternatively divided into the non-smokers subgroup and the smokers subgroup. Reliable notations about the smoking habits were not available in 6 cases. 19 subjects were never smokers and 4 were former smokers. The smokers subgroup was composed by 26 cases and was further divided into the 1st smoking class (6 cases usually smoking less than 10 cigarettes/die), the 2nd smoking class (6 cases usually smoking 11-20 cigarettes/die) and the 3rd smoking class (14 cases usually smoking more than 20 cigarettes/die).

Table 1 reports the general features of each study subject, whereas in Table 2 the results of the microscopic analyses (Light Microscope and Scanning Electron Microscope analyses) performed are illustrated.

Morphologically typical Asbestos Bodies were found in the 14.5% of cases (8 cases). The Asbestos Bodies prevalence was 0% in the ≤ 30 years old subgroup, 9.5% in the 30-60 years old subgroup and 31.6% in the > 60 years old subgroup (p = 0,034). For the positive cases, the range of the AB counting was 10-110 AB/g dry. In all the AB positive cases, the SEM-tested asbestos burden was higher than the analytical sensibility. For such cases the SEM asbestos burden range was 60,000-2,000,000 ff/g dry, the case with the lowest SEM count (60,000 ff/g dry) being unexpectedly the one with the greatest LM AB count (110 AB/g dry). For 2 cases testing positive for Asbestos Bodies chrysotile was the only asbestos variant detected in the SEM analysis.

Table 1: main features of each study subject.

	Sex	Age	Residence (Milan)	Birthplace	Work	Tobacco smoke (cigarette/die)
Case 1	F	42	NW	Milan, Italy	Housewife	NS
Case 2	M	78	NW	Teramo, Italy	Retired (career soldier)	10-20
Case 3	M	74	S	Milan, Italy	Retired (automotive industry worker)	> 20
Case 4	M	69	C	Catania, Italy	Retired	FS
Case 5	M	49	NE	Milan, Italy	Computer engineer	NA
Case 6	M	72	NE	Syracuse, Italy	Retired (clerical worker)	> 20
Case 7	F	68	NE	Palermo, Italy	Retired (housewife)	NS
Case 8	M	23	NE	Milan, Italy	Train guard	10-20
Case 9	F	64	NE	Lecco, Italy	Secretary	> 20
Case 10	M	18	S	Romania	Barman	NS
Case 11	M	34	NE	Milan, Italy	Long term unemployed	NS
Case 12	M	51	S	Foggia, Italy	Warehouse-keeper	> 20
Case 13	M	41	C	Milan, Italy	Clockmaker	1-10
Case 14	F	38	NW	Monza, Italy	Make-up artist	10-20
Case 15	M	43	S	Milan, Italy	Unemployed (concierge)	> 20
Case 16	M	59	NW	Mantova, Italy	Retired (businessman in catering industry)	> 20
Case 17	F	48	S	Milan, Italy	Housewife (graphic-designer)	NS
Case 18	F	27	S	Milan, Italy	Student	NS
Case 19	F	49	S	Belluno, Italy	Housewife	> 20
Case 20	M	68	S	Milan, Italy	Retired (clerical worker)	NS
Case 21	F	79	S	Milan, Italy	Retired	1-10
Case 22	M	80	S	Reggio Emilia, Italy	Retired	NA
Case 23	F	22	S	Milan, Italy	Barman	NS
Case 24	M	27	NE	Monza, Italy	Blue collar	1-10

Table 1 cnt: main features of each study subject.

	Sex	Age	Residence (Milan)	Birthplace	Work	Tobacco smoke (cigarette/die)
Case 25	F	30	S	Catania, Italy	Housewife	> 20
Case 26	F	80	S	Genova, Italy	Retired (housewife)	FS
Case 27	M	30	NW	Catanzaro, Italy	Financial analyst	NS
Case 28	F	51	C	Salerno, Italy	Secretary	FS
Case 29	M	71	NW	Foggia, Italy	Retired (clerical worker)	10-20
Case 30	M	48	S	Milan, Italy	Computer programmer	10-20
Case 31	M	33	NE	Milan, Italy	Unemployed (clerical worker)	> 20
Case 32	F	81	S	Turin, Italy	Retired (housewife)	NA
Case 33	F	73	NW	Bari, Italy	Retired (housewife)	NS
Case 34	M	37	NE	Milan, Italy	Barman	> 20
Case 35	M	51	C	Milan, Italy	Medical doctor	> 20
Case 36	M	79	C	Catania, Italy	Retired (professional musician)	NS
Case 37	M	64	NW	Pavia, Milan	Retired (truck driver)	1-10
Case 38	M	64	S	Milan, Italy	Teacher	NS
Case 39	F	83	NE	Milan, Italy	Retired (shop-keeper)	NA
Case 40	F	68	NE	Milan, Italy	Retired (secretary)	NS
Case 41	M	28	NE	Milan, Italy	Long term unemployed	> 20
Case 42	M	27	NE	Milan, Italy	Waiter	10-20
Case 43	F	80	NW	Rome, Italy	Retired (kindergarten teacher)	NS
Case 44	M	22	C	France	Student	NS
Case 45	F	53	NW	Milan, Italy	Press agent	NA
Case 46	F	38	NE	Milan, Italy	Long term unemployed	NA
Case 47	F	22	NW	Kazakhstan	Student	> 20
Case 48	F	49	C	Genova, Italy	Secretary	FS

Table 1 cnt: main features of each study subject.

	Sex	Age	Residence (Milan)	Birthplace	Work	Tobacco smoke (cigarette/die)
Case 49	M	21	NW	Milan, Italy	Cook	NS
Case 50	M	30	S	Milan, Italy	Barman	NS
Case 51	F	23	NW	Romania	Unemployed (shop-assistant)	NA
Case 52	F	55	S	Bari, Italy	Clerical worker	> 20
Case 53	M	21	NW	Milan, Italy	Clerical worker	NS
Case 54	F	35	S	Naples, Italy	Housewife	NS
Case 55	F	44	NE	Naples, Italy	Long term unemployed	1-10

M = Male, F = Female.

NE = North East, NW = North West, C = City Centre, S = South.

NS = Non Smoker, FS = Former Smoker.

NA = Not Available.

Table 2: main results of the LM and SEM analyses.

	AB (LM count)	ASBESTOS FIBERS			TALC FIBERS		TITANIUM DIOXIDE		OTHER FIBERS	
		SEM burden (ff/g dry)	95% CI (ff/g dry)	Fibers type	SEM Burden (ff/g dry)	95% CI (ff/g dry)	SEM Burden (ff/g dry)	95% CI (ff/g dry)	SEM Burden (ff/g dry)	95% CI (ff/g dry)
Case 1	0	400,000	120,000-1,160,000	CA 57% NCA 43%	570,000	180,000-1,320,000	< 1/2 AS		1,500,000	780,000-2,520,000
Case 2	0	420,000	40,000-1,210,000	Chr 60% NCA 40%	< 1/2 AS		< 1/2 AS		4,500,000	2,990,000-6,600,000
Case 3	10/g dry	1,200,000	90,000-2,280,000	CA 47% NCA 53%	< 1/2 AS		250,000	30,000-890,000	870,000	350,000-1,790,000
Case 4	0	90,000	0-520,000	CA 100%	< 1/2 AS		< 1/2 AS		380,000	100,000-960,000
Case 5	60/g dry	110,000	3,000-630,000	Chr 100%	110,000	3,000-630,000	< 1/2 AS		680,000	250,000-1,470,000
Case 6	0	140,000	20,000-690,000	CA 33% NCA 67%	< 1/2 AS		100,000	3,000-530,000	860,000	390,000-1,640,000
Case 7	0	60,000	4,000-690,000	CA 100%	120,000	4,000-690,000	< 1/2 AS		250,000	30,000-890,000
Case 8	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		< 1/2 AS	
Case 9	0	1,900,000	800,000-3,680,000	Chr 12% CA 38% NCA 50%	470,000	60,000-1,680,000	< 1/2 AS		2,800,000	1,450,000-4,890,000
Case 10	0	< 1/2 AS			310,000	60,000-900,000	110,000	3,000-570,000	1,100,000	560,000-2,010,000
Case 11	0	1,110,000	620,000-2,080,000	Chr 91% CA 9%	< 1/2 AS		< 1/2 AS		900,000	410,000-1,700,000
Case 12	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		460,000	130,000-1,180,000
Case 13	0	120,000	3,000-640,000	Chr 100%	< 1/2 AS		< 1/2 AS		1,500,000	800,000-2,570,000
Case 14	0	200,000	20,000-720,000	Chr 50% CA 50%	< 1/2 AS		< 1/2 AS		640,000	210,000-1,490,000
Case 15	0	< 1/2 AS			130,000	4,000-720,000	< 1/2 AS		260,000	30,000-930,000

Table 2 cnt: main results of the LM and SEM analyses.

	AB (LM count)	ASBESTOS FIBERS			TALC FIBERS		TITANIUM DIOXIDE		OTHER FIBERS	
		SEM burden (ff/g dry)	**95% CI (ff/g dry)**	**Fibers type**	**SEM Burden (ff/g dry)**	**95% CI (ff/g dry)**	**SEM Burden (ff/g dry)**	**95% CI (ff/g dry)**	**SEM Burden (ff/g dry)**	**95% CI (ff/g dry)**
Case 16	110/g dry	60,000	3,000-620,000	CA 100%	450,000	120,000-1,140,000	110,000	3,000-620,000	110,000	3,000-620,000
Case 17	0	190,000	30,000-930,000	CA 100%	260,000	30,000-930,000	< 1/2 AS		380,000	80,000-1,120,000
Case 18	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		370,000	80,000-1,080,000
Case 19	0	< 1/2 AS			< 1/2 AS		100,000	3,000-530,000	380,000	80,000-1,110,000
Case 20	60/g dry	170,000	20,000-620,000	Chr 50% NCA 50%	130,000	4,000-710,000	< 1/2 AS		430,000	140,000-1,000,000
Case 21	0	210,000	30,000-1,020,000	NCA 100%	560,000	150,000-1,440,000	< 1/2 AS		850,000	310,000-1,840,000
Case 22	0	520,000	170,000-1,200,000	Chr 50% CA 50%	100,000	3,000-580,000	< 1/2 AS		< 1/2 AS	
Case 23	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		170,000	20,000-610,000
Case 24	0	< 1/2 AS			170,000	20,000-620,000	< 1/2 AS		770,000	350,000-1,460,000
Case 25	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		130,000	4,000-700,000
Case 26	20/g dry	120,000	4,000-690,000	NCA 100%	860,000	350,000-1,780,000	< 1/2 AS		370,000	80,000-1,080,000
Case 27	0	< 1/2 AS			130,000	4,000-700,000	< 1/2 AS		250,000	30,000-920,000
Case 28	0	500,000	140,000-1,280,000	Chr 50% NCA 50%	120,000	4,000-690,000	< 1/2 AS		500,000	140,000-1,280,000
Case 29	0	400,000	80,000-1,160,000	Chr 67% CA 33%	< 1/2 AS		< 1/2 AS		920,000	370,000-1,900,000

Table 2 cnt: main results of the LM and SEM analyses.

	AB (LM count)	ASBESTOS FIBERS			TALC FIBERS		TITANIUM DIOXIDE		OTHER FIBERS	
		SEM burden (ff/g dry)	95% CI (ff/g dry)	Fibers type	SEM Burden (ff/g dry)	95% CI (ff/g dry)	SEM burden (ff/g dry)	95% CI (ff/g dry)	SEM burden (ff/g dry)	95% CI (ff/g dry)
Case 30	0	630,000	200,000-1,470,000	CA 80% NCA 20%	< 1/2 AS		< 1/2 AS		630,000	200,000-1,470,000
Case 31	0	< 1/2 AS			920,000	300,000-2,150,000	< 1/2 AS		1,100,000	410,000-2,400,000
Case 32	0	500,000	140,000-1,280,000	CA 50% NCA 50%	1,400,000	690,000-2,470,000	250,000	30,000-910,000	1,600,000	870,000-2,790,000
Case 33	0	120,000	4,000-660,000	CA 100%	< 1/2 AS		260,000	30,000-930,000	470,000	130,000-1,210,000
Case 34	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		670,000	290,000-1,320,000
Case 35	0	< 1/2 AS			< 1/2 AS		130,000	4,000-740,000	400,000	80,000-1,170,000
Case 36	0	130,000	4,000-740,000	CA 100%	260,000	30,000-960,000	< 1/2 AS		130,000	4,000-740,000
Case 37	0	230,000	30,000-830,000	CA 50% NCA 50%	120,000	3,000-640,000	120,000	3,000-640,000	810,000	320,000-1,660,000
Case 38	0	100,000	3,000-550,000	NCA 100%	500,000	160,000-1,160,000	100,000	3,000-550,000	1,100,000	540,000-1,950,000
Case 39	10/g dry	1,300,000	730,000-2,250,000	CA 7% NCA 93%	760,000	330,000-1,510,000	100,000	3,000-530,000	2,820,000	1,450,000-4,900,000
Case 40	30/g dry	2,000,000	1,150,000-3,270,000	Chr 100%	130,000	4,000-700,000	< 1/2 AS		880,000	350,000-1,820,000
Case 41	0	100,000	3,000-540,000	NCA 100%	100,000	3,000-540,000	100,000	3,000-540,000	680,000	270,000-1,400,000
Case 42	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		770,000	310,000-1,580,000
Case 43	20/g dry	670,000	230,000-1,350,000	NCA 100%	2,400,000	1,290,000-4,150,000	< 1/2 AS		1,100,000	570,000-2,040,000

Table 2 cnt: main results of the LM and SEM analyses.

	AB (LM count)	ASBESTOS FIBERS			TALC FIBERS		TITANIUM DIOXIDE		OTHER FIBERS	
		SEM burden (ff/g dry)	95% CI (ff/g dry)	Fibers type	SEM burden (ff/g dry)	95% CI (ff/g dry)	SEM burden (ff/g dry)	95% CI (ff/g dry)	SEM burden (ff/g dry)	95% CI (ff/g dry)
Case 44	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		690,000	220,000-1,620,000
Case 45	0	< 1/2 AS			< 1/2 AS		90,000	3,000-480,000	610,000	240,000-1,250,000
Case 46	0	100,000	3,000-560,000	NCA 100%	200,000	20,000-730,000	< 1/2 AS		500,000	160,000-1,180,000
Case 47	0	< 1/2 AS			140,000	4,000-760,000	< 1/2 AS		680,000	220,000-1,600,000
Case 48	0	< 1/2 AS			90,000	3,000-500,000	< 1/2 AS		1,300,000	750,000-2,200,000
Case 49	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		120,000	4,000-680,000
Case 50	0	< 1/2 AS			< 1/2 AS		< 1/2 AS		490,000	160,000-1,150,000
Case 51	0	260,000	30,000-960,000	CA 100%	< 1/2 AS		< 1/2 AS		400,000	80,000-1,160,000
Case 52	0	290,000	30,000-1,040,000	NCA 100%	430,000	90,000-1,260,000	290,000	10,000-1,590,000	1,400,000	690,000-2,630,000
Case 53	0	120,000	4,000-670,000	NCA 100%	120,000	4,000-670,000	< 1/2 AS		240,000	30,000-870,000
Case 54	0	150,000	5,000-850,000	CA 100%	1,100,000	430,000-2,200,000	< 1/2 AS		1,500,000	730,000-2,800,000
Case 55	0	40,000	3,000-470,000	CA 100%	330,000	90,000-860,000	< 1/2 AS		330,000	90,000-860,000

LM = Light Microscope, SEM = Scanning Electron Microscope.

95% CI = 95% Confidence Interval. AS = Analytical Sensibility.

Chr = Chrysotile, CA = Commercial Amphibole, NCA = Non-Commercial Amphibole..

Asbestos fibers were SEM-detected in 35 cases (63.6%), while 20 cases had an asbestos burden lower than the ½ analytical sensibility. 60% of cases with an asbestos burden lower than the ½ analytical sensibility was represented by subjects younger than 30 years. Three cases showed a pure chrysotile burden, 24 cases a pure amphibole burden and 8 cases a mixed chrysotile + amphibole burden. Chrysotile fibers were thus detected in 20% of all the experimental cases, whereas amphibole fibers in 58.2%. In cases showing a mixed chrysotile and amphibole burden, the chrysotile amount ranged from 12% to 91% of the total burden and was joined by a Non-Commercial Amphiboles burden in 50% of subjects and by a Commercial Amphiboles burden in 62.5%. Among the cases showing a detectable amphibole burden (either pure or chrysotile-mixed), 13 cases had a pure Commercial Amphiboles burden, 11 cases had a pure Non-Commercial Amphiboles burden and 8 cases had a combined Commercial + Non-Commercial Amphiboles burden. Commercial Amphibole fibers were detected in 40% of all the experimental cases, whereas Non-Commercial Amphibole fibers in 34.5%. The Non-Commercial Amphiboles burden was mainly represented by tremolite fibers (92.5%).

The median values and interquartile ranges for the chrysotile burden and the amphiboles burden in the whole population are reported in Table 3. The maximum estimated asbestos burden was 2,000,000 ff/g dry (100% chrysotile fibers), while the maximum estimated amphiboles burden was 1,672,000 ff/g dry.

Table 3: global asbestos burden *versus* chrysotile burden *versus* amphiboles burden.

	Asbestos fibers	**Chrysotile fibers**	**Amphibole fibers**
Median value (ff/g dry)	110,000	51,600	91,600
Interquartile range (ff/g dry)	62,250-275,000	46,600-65,000	60,000-180,000

Based on the Helsinki Criteria for the occupational exposure to asbestos, 3 cases showed an amphibole burden higher than the established cut off for fibers longer than 1 µm (1,000,000 ff/g dry) and other 10 cases showed an amphibole burden greater than 100,000 ff/g dry for fibers longer than 5 µm.

Figure 1 illustrates the asbestos burden for all subjects separately in males and females. The median value in females was 150,000 ff/g dry (IQR 63,000-400,000) while it was 95,750 (IQR 61,500-170,000) in males ($p = 0.31$).

Figure 1: Box-plot of asbestos burden in the whole population, by gender (F = females, M = males).

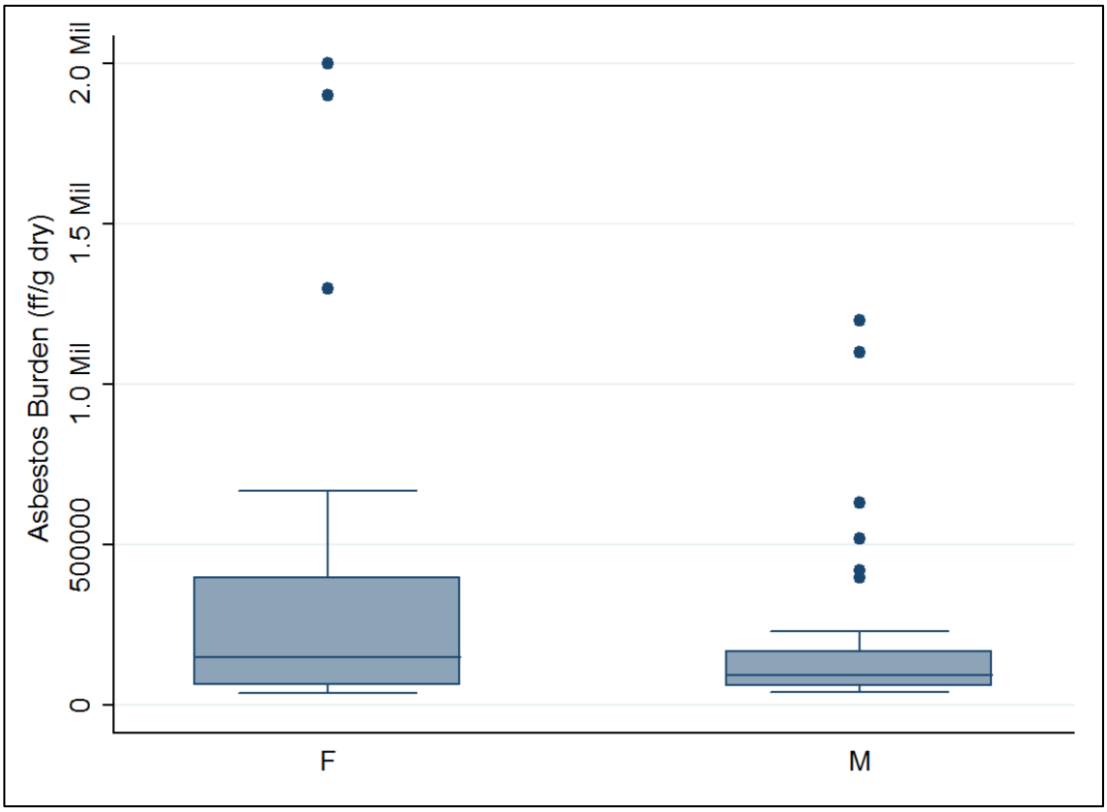


Figure 2 shows the association between asbestos burden and age at death in all subjects: the percent change in asbestos burden per 1-year increase in age at death was 2.81 (95% CI 1.54-4.09, $p < 0.001$) indicating a positive trend with increasing age.

When we considered the amphiboles burden variation according to the sex variable (Figure 3), the differences were not statistically significant ($p = 0,22$). The median values were 120,000 (IQR 61,600-260,000) in females and 77,500 (IQR 58,300-130,000) in males.

The positive trend with age at death was confirmed (Figure 4): percent change in amphiboles burden per 1-year increase in age at death = 2.33 (95% CI 1.22-3.45, $p < 0.001$).

If we consider only subjects (52 cases) having an amphibole SEM-counting lower than the 1,000,000 ff/g dry cut off for occupational exposure (figure 5 and 6) the results did not consistently change. As predictable, the annual percent increase for the lung amphiboles burden is much lower (1.16%, see Figure 6) after excluding the few cases having an extremely high SEM amphibole counting.

Figure 2: relationship between asbestos burden and age at death in all subjects.

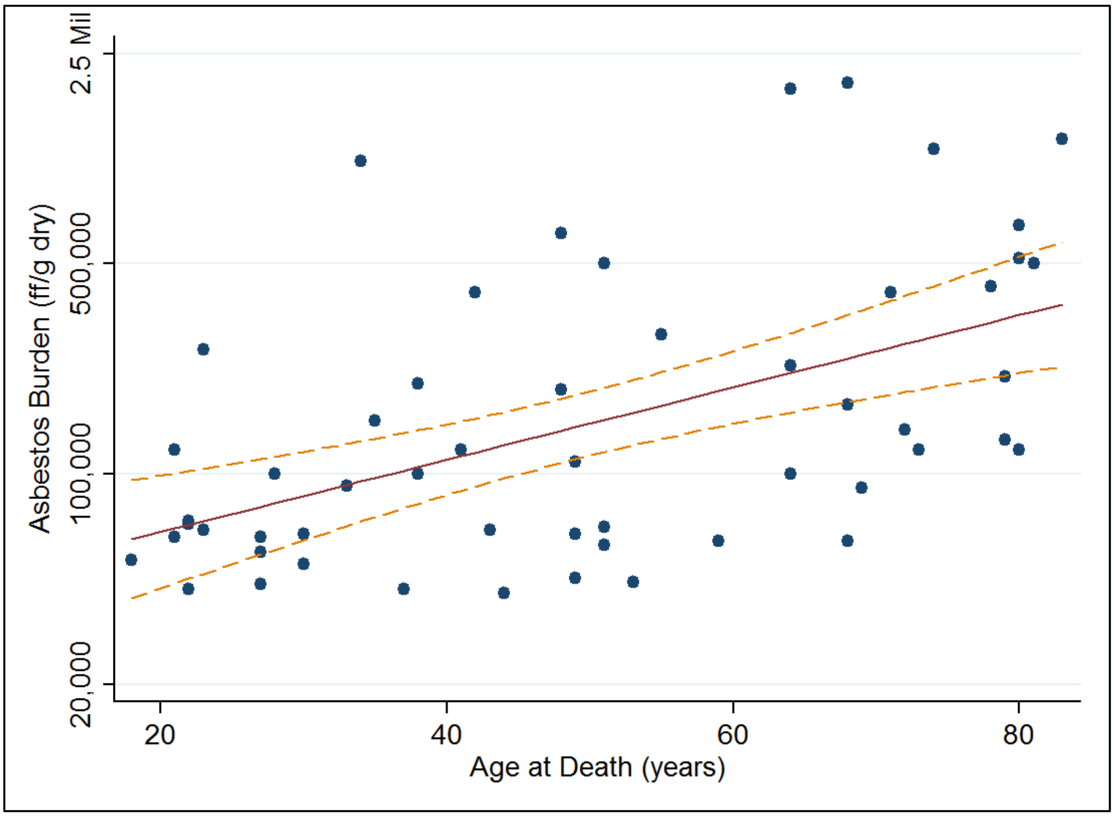


Figure 3: Box-plot of amphiboles burden in the whole population, by gender (F = females; M = males).

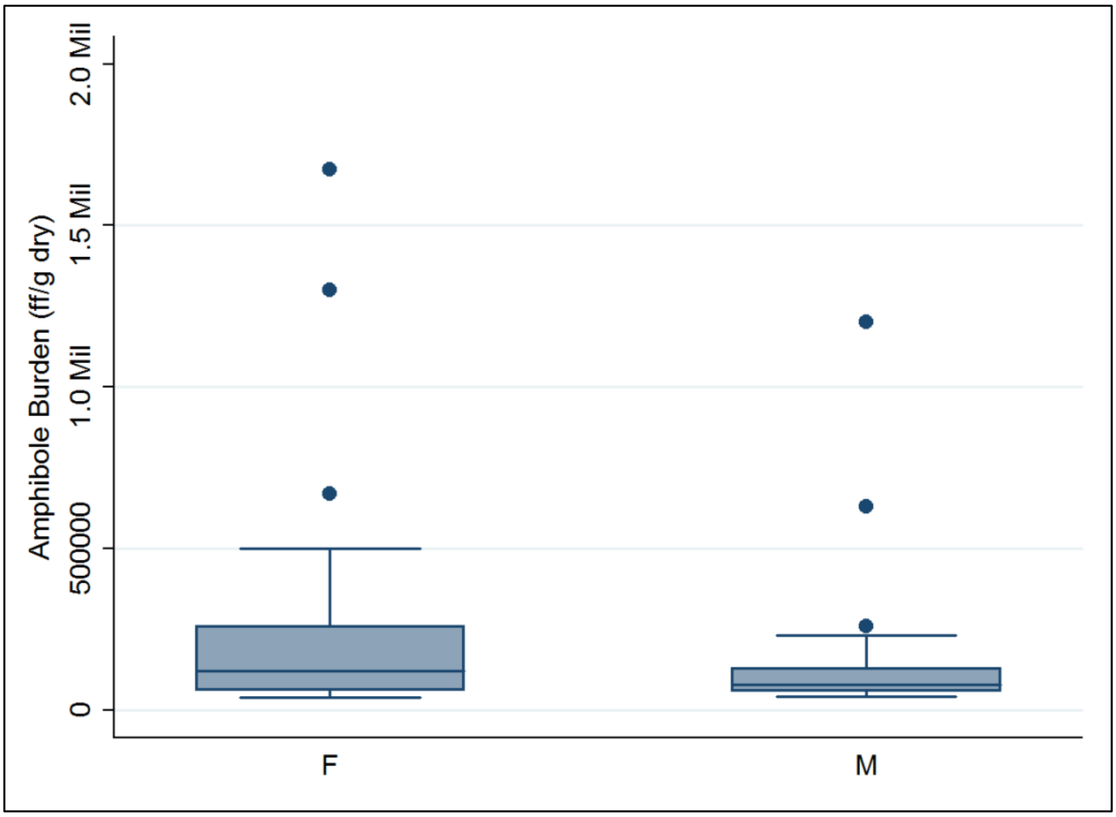


Figure 4: relationship between amphiboles burden and age at death in the whole population.

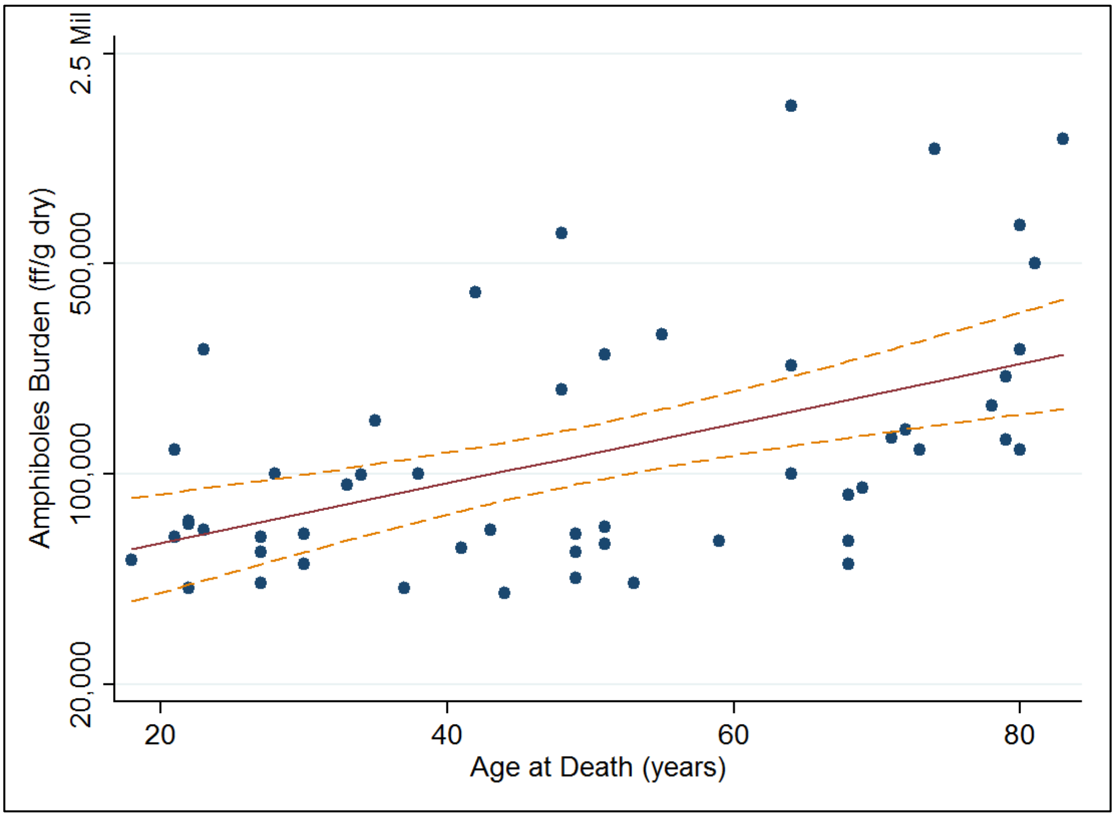


Figure 5: Box-plot of amphiboles burden in the 52 cases with < 1,000,000 amphiboles ff/g dry stratified by gender (F = females; M = males).

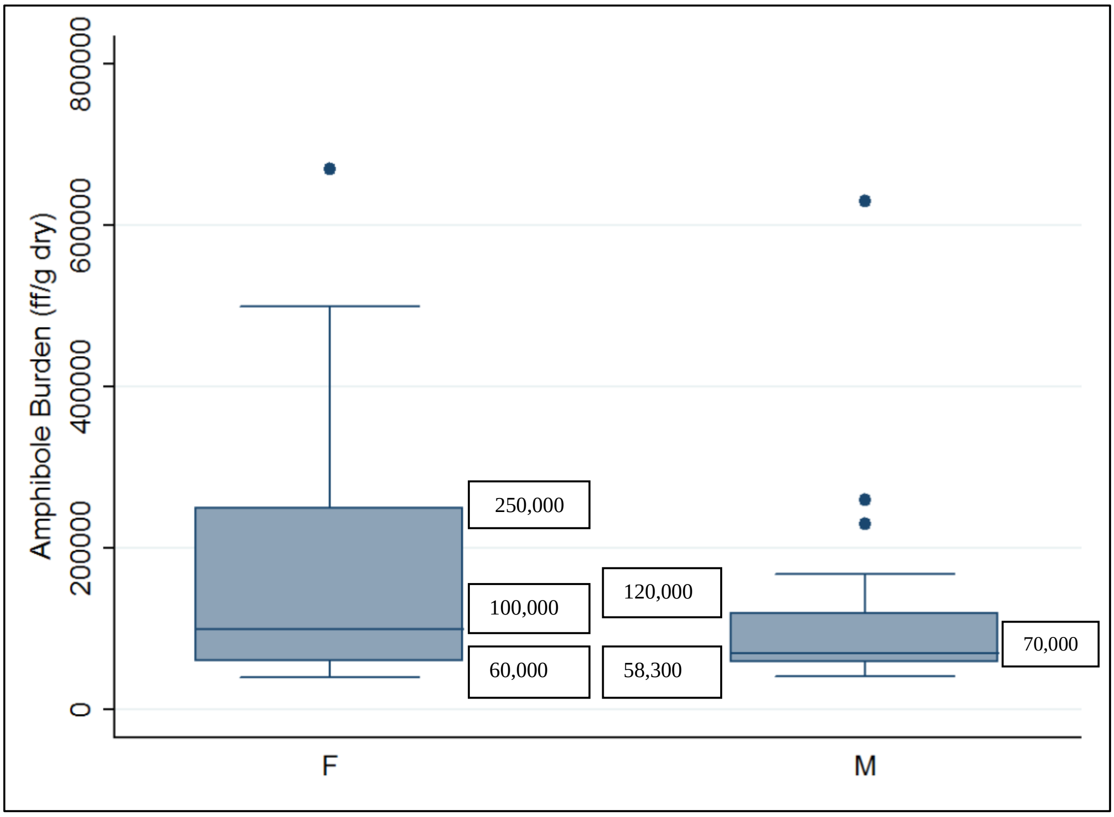


Figure 6: relationship between amphiboles burden and age at death in cases < 1,000,000 amphiboles ff/g dry.

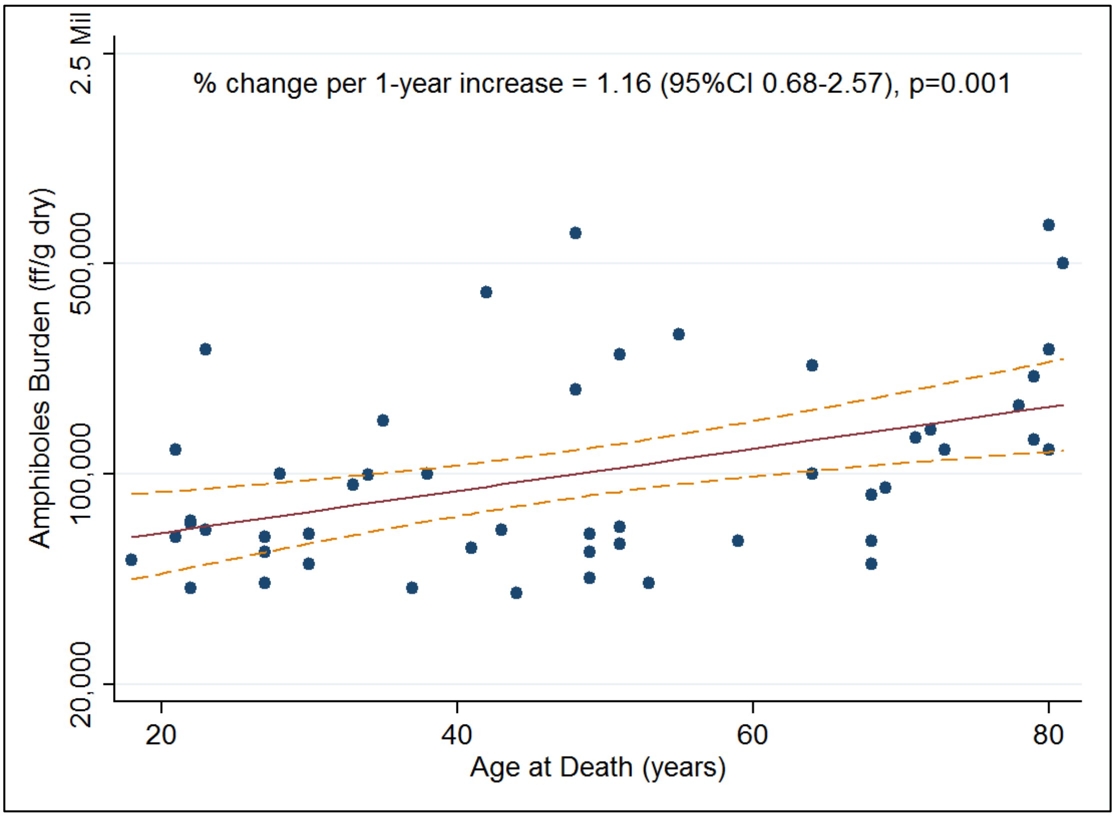


Table 4 details the main characteristics of the 13 cases having an asbestos burden above the Helsinki cut offs for the occupational exposure to asbestos (10 cases having > 100,000 amphibole fibers longer than 5 μm/g dry and 3 cases having > 1,000,000 amphibole fibers longer than 1 μm/g dry). The mean age was 57.5 ± 19.8 years, significantly higher (p 0.013) than the subgroup having an amphiboles burden lower than the Helsinki Criteria for occupational exposure. The distribution by gender, residential district and smoking habits did not differ. Three cases showed a positive Asbestos Bodies counting in LM ranging from 10 AB/g dry to 20 AB/g dry.

Because of the poor available notations about the work histories of the experimental cases, it was not possible to carefully analyze the subgroup above the Helsinki Criteria according to singular job experiences and singular occupational exposures to asbestos: it is nonetheless remarkable the presence inside such subgroup of a retired automotive industry worker and of a retired truck driver. Further and more detailed investigations about the lifetime work experiences of the cases AB positive and the cases above the Helsinki Criteria have been started during the final phase of the present PhD study but have not yet been completed. This approach has been made possible thanks to collaboration with OCCAM (OCcupational CAncer Monitoring) that

Table 4: main characteristics of the 13 cases having and amphibole burden higher than the Helsinki Criteria for occupational exposure.

	Age Sex	Amphiboles ff/g dry	AB/g dry	Talc ff/g dry	Other inorganic ff/g dry	Residence Birthplace	Smoking habit	Work
Case 1	42 F	fibers longer than 5 µm: 400,000	0	570,000	1,500,000	NW Milan	Non Smoker	Housewife
Case 6	72 M	fibers longer than 5 µm: 140,000	0	< ½ x AS	860,000	NE Syracuse, Italy	> 20	Retired (clerical worker)
Case 17	48 F	fibers longer than 5 µm: 190,000	0	260,000	380,000	S Milan	Non Smoker	Housewife (graphic-designer)
Case 21	79 F	fibers longer than 5 µm: 105,000	0	560,000	850,000	S Milan	1-10	Retired
Case 32	81 F	fibers longer than 5 µm: 200,000	0	1,400,000	1,600,000	S Turin, Italy	Not Available	Retired (housewife)
Case 37	64 M	fibers longer than 5 µm: 115,000	0	120,000	810,000	NW Pavia, Italy	1-10	Retired (truck driver)
Case 43	80 F	fibers longer than 5 µm: 286,000	20	2,400,000	1,100,000	NW Rome, Italy	Non Smoker	Retired (kindergarten teacher)
Case 52	55 F	fibers longer than 5 µm: 145,000	0	430,000	1,400,000	S Bari, Milan	> 20	Clerical worker
Case 53	21 M	fibers longer than 5 µm: 120,000	0	120,000	240,000	NW Milan	Non Smoker	Clerical worker
Case 54	35 F	fibers longer than 5 µm: 150,000	0	1,100,000	1,500,000	S Naples, Italy	Non Smoker	Long term unemployed (housewife)
Case 3	74 M	fibers longer than 1 µm: 1,200,000	10	< ½ x AS	870,000	S Milan	> 20	Retired (automotive industry worker)
Case 9	64 F	fibers longer than 1 µm: 1,672,000	0	470,000	2,800,000	NE Lecco, Italy	> 20	Secretary
Case 39	83 F	fibers longer than 1 µm: 1,300,000	10	760,000	2,820,000	NE Milan	Not Available	Retired (shop-keeper)

AB = morphologically typical Asbestos Bodies. M = male, F = female. AS = Analytical Sensibility. NW = North West, NE = North East, S = South.

investigates occupational cancer risk by industrial sectors.

When we analyzed the asbestos lung burden across the four residence districts in Milan (North East, North West, South and City Centre), the birthplace (Milan *versus* outside) and the smoking habits (ever smokers *versus* non-smokers), no statistical differences were found.

A special analytical focus was about the dimensions of the detected asbestos fibers. Table 5 summarizes the analytical results about asbestos fiber dimensions.

From the whole experimental population a global amount of 111 asbestos fibers were measured by SEM: 40 Non-Commercial Amphibole fibers, 32 Commercial Amphibole fibers and 39 chrysotile fibers. Chrysotile fibers were significantly shorter and thinner than amphiboles fibers, while Commercial Amphibole fibers were significantly thinner than the Non-Commercial Amphibole fibers.

The mean aspect ratio (length/width) value decreased from the chrysotile fibers to the Commercial Amphibole fibers and yet to the Non-Commercial Amphibole fibers, about 70% of the detected chrysotile fibers showing a diameter < 0,1 µm versus the 25% of the Commercial Amphibole fibers and the 0% of the Non-Commercial Amphibole fibers. An aspect ratio lower than 10 was recorded in 0% of chrysotile fibers, in 12.5% of Commercial Amphibole fibers and in 40% of Non-Commercial Amphibole fibers. Only 13.5% of all asbestos fibers were longer than 10 µm, while about 60% was shorter than 5 µm and 47.7% were both ultrashort and ultrathin fibers (length < 5 µm and diameter < 0.25 µm). Less than 10% of all detected asbestos fibers were Stanton fibers (fibers longer than 8 µm and thinner than 0.25 µm), Lippmann fibers variant 1 (fibers longer than 5 µm and thinner than 0.1 µm) or Lippmann fibers variant 2 (fibers longer than 10 µm and thinner than 0.15 µm). A further analysis was performed about the prevalence of the long asbestos fibers: 34% of the whole experimental population had no asbestos fibers longer than 5 µm, while 56% had no asbestos fibers longer than 10 µm. About 80% of the whole population had no Stanton fibers, while nearly 90% showed no Lippmann fibers variant 1 or Lippmann fibers variant 2.

Even though the distinction between true asbestiform fibers and non-asbestiform cleavage fragments was not a declared focus of the present work, data from table 5 show the relative amount of Commercial Amphiboles and Non-Commercial Amphiboles respectively fitting the American Society for Testing and Materials criteria for class 2 fibers (ASTM class 2 fibers hypothesis: fibers longer than 10 µm or thinner than 1 µm being true asbestiform fibers) [144]. The very high percentage of fibers with a width < 1 µm across all the subgroups allows here the reader to equalize the official ASTM class 2 criteria to the revision hypothesis later advanced -but finally rejected- by the Workplace ASTM subcommittee (fibers longer than 10 µm and thinner than 1 µm being true asbestiform fibers). The previous notation about the percentage of fibers having an aspect ratio lower than 10 also answers the claim about this aspect ratio value to be a reliable cut off between true asbestiform fibers (AR usually > 10) and cleavage fragments (AR usually < 10) [145].

Table 5: dimensional analysis of the detected asbestos fibers (CA = Commercial Amphiboles; NCA = Non-Commercial Amphiboles).

	All asbestos fibers	**Chrysotile fibers**	**CA fibers**	**NCA fibers**
Length x diameter µm: geometric mean values (95% CI)	4.19 (2.49-5.89) x 0.19 (0.14-0.24)	2.74 (2.19-3.43) x 0.09 (0.08-0.10)	4.86 (3.28-7.20) x 0.18 (0.14-0.23)	5.65 (4.39-7.28) x 0.47 (0.40-0.57)
Mean aspect ratio (length/width)	20.6	30.7	26.9	11.25
% of fibers ≥ 10 µm in length	13.5%	5.1%	21.8%	15%
% of fibers ≥ 5 µm in length	37.8%	20.5%	34.3%	57.5%
% of fibers < 1 µm in length	94.6%	100%	96.8%	87.5%
% of fibers ≤ 0.1 µm in width	31.5%	69.3%	25%	0%
% of Stanton fibers	7.2%	7.7%	12.5%	2.5%
% of Lippmann fibers (hypothesis 1)	4.5%	10.2%	3.1%	0%
% of Lippmann fibers (hypothesis 2)	3.6%	5.1%	6.2%	0%

The SEM analysis also extended to the counting of talc fibers, titanium-containing fibers and inorganic fibers other than asbestos.

Talc fibers were positively detected in 56.4% of cases, titanium-containing fibers in 25.5% and other inorganic fibers other than asbestos in 96.4%. The median values from the whole experimental population were respectively 110,000 ff/g dry (interquartile range 50,750-285,000 ff/g dry), 51,500 ff/g dry (interquartile range 48,000-81,500 ff/g dry) and 640,000 ff/g dry (interquartile range 375,000-910,000 ff/g dry).

No statistical influence of the variables sex, residential district, birthplace and smoking habit was observed on the median values of talc, titanium and other inorganic fibers burdens.

Figures 7 and 8 respectively illustrate the relationships between the talc fibers burden and the miscellaneous inorganic fibers burden with age at death. The percent change per 1-year increase in age at death was 1.74 (95% CI 0.29-3.22, p = 0,020) for the talc burden and 0.96 (95% CI 0.24-2.16, p = 0,114) for the other inorganic fibers burden.

Figure 7: relationship between talc fibers burden and age at death in the whole population.

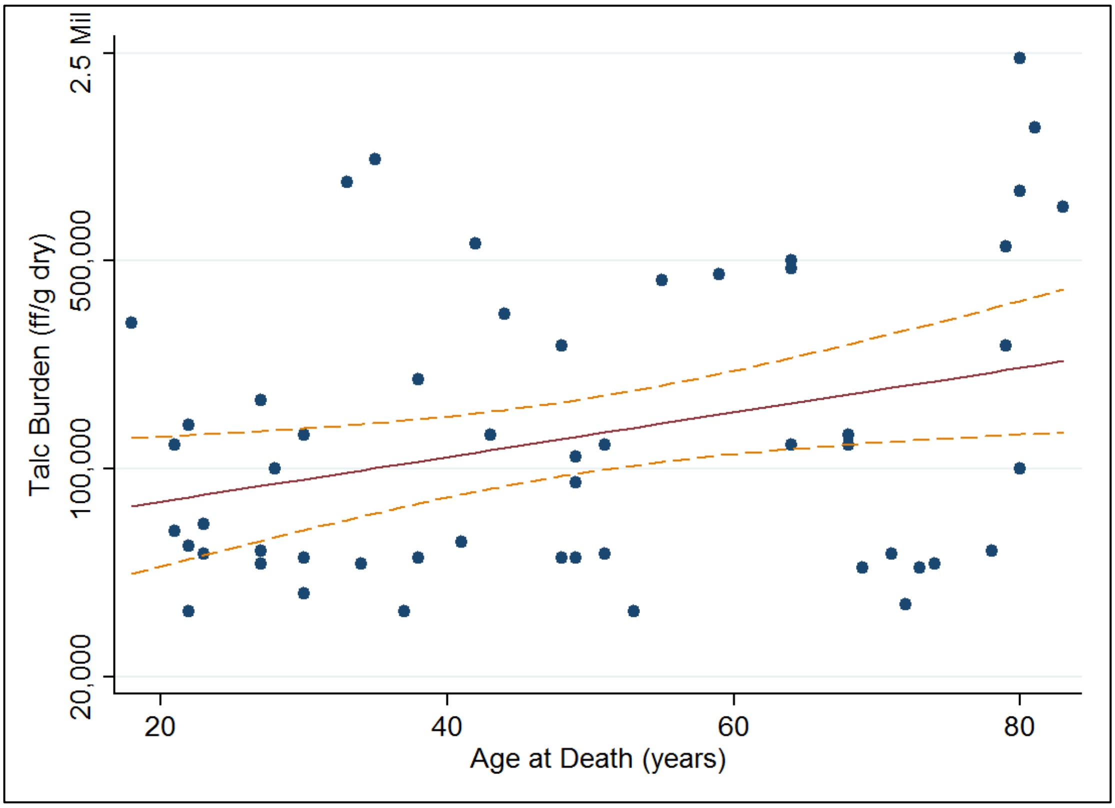


Figure 8: relationship between other inorganic fibers burden and age at death in the whole population.

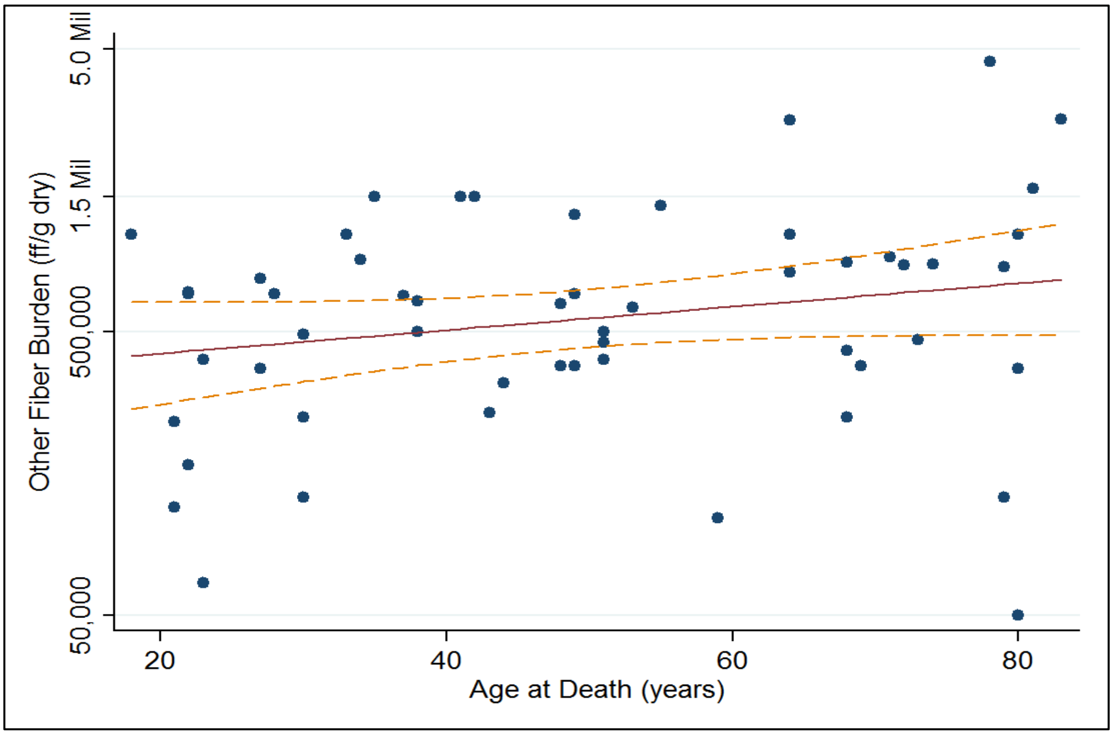
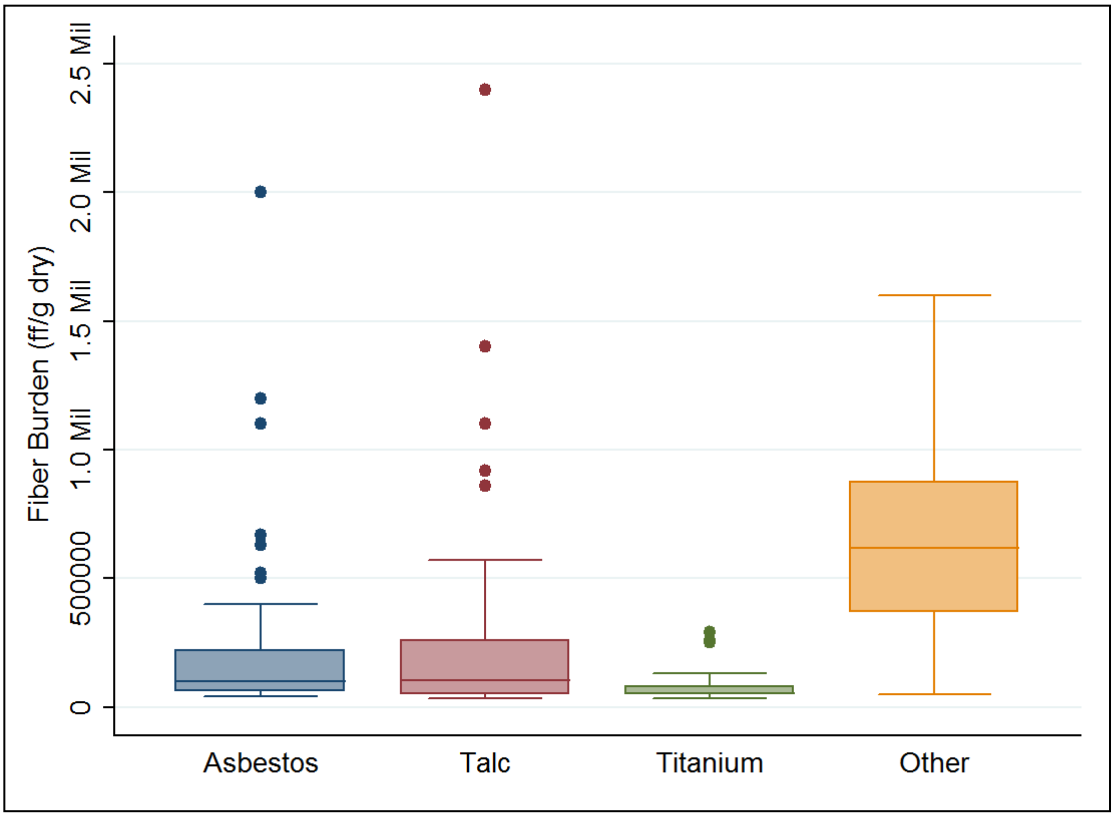


Figure 9: asbestos burden *versus* talc burden *versus* titanium burden *versus* other inorganic fibers burden in the whole population.



DISCUSSION AND CONCLUSIONS

We investigated the lung asbestos burden in a sample of necropsies collected between 2009-2011 at the Institute of Forensic Medicine of Milan. The study population included 55 subjects residents in Milan at the time of death, stratified by gender (30 males and 25 females) and age (15 cases ≤ 30 years, 21 cases 31-60 years and 19 cases > 60 years). The study population can be considered a representative sample of the Milan resident population, yet avoiding the anagraphic biases usually connected with consecutive and unselected necroscopic series (series younger and much more male-oriented than the whole background population).

Milan is a post-industrial and tertiary-oriented town, far enough from relevant natural sources of asbestos dust and historically free from major asbestos primary manufactures. However, Milan is also a high-traffic metropolitan town widely scattered by either professional and residential buildings still containing asbestos-rich components (asbestos estimated volume in Milan territory at September 2008: 784,808 m^3) [146], that is Milan is not a town expected free from asbestos pollution. The Milan surroundings are mainly industrial on the north side and mainly rural on the south side.

Based on environmental measurements performed in 1991, the background asbestos-pollution in the city was mainly a chrysotile-pollution with prevalence of ultrashort and ultrathin fibers: such background pollution tended to be constant across the four seasons of the year and was not influenced by the vehicular traffic rates and by the land use in the different urban districts [147]. Milan asbestos pollution (mean value 12,1 ± 4,6 ff/L) was significantly lower than the background pollution of a small town hosting primary asbestos manufactures (Casale Monferrato: 48,4 ± 33,5 ff/L) but was higher than the environmental pollution from other Italian medium-sized towns (Brescia: 5,6 ± 1,5 ff/L, Ancona: 6,00 ± 1,2 ff/L, Firenze: 1,8 ± 1,0 ff/L, Bologna 3,3 ± 3,6 ff/L) [147] and also from Rome [148]. Based on official data from the Lombardy Region and from the Lombardy ARPA Laboratories [146], repeated measurements between 1999 and 2008 showed mean asbestos atmospheric level always lower than 0,07 ff/L for fibers longer than 5 µm. The asbestos environmental burden was constituted by 65% chrysotile fibers and 35% amphibole fibers. In 2008 the mean asbestos atmospheric level for ultrathin fibers was lower than 2,5 ff/L.

In short, Milan is nowadays a 1,300,000 inhabitants town showing a decreasing background level of secondary asbestos-pollution with infrequent spikes of primary asbestos-pollution and a strongly prevalent non-occupational asbestos exposure of the resident people. As already suggested by other authors in different industrialized countries [254], the younger class people today living in Milan (people born or grown up after the 1992 asbestos ban) can be considered to be ever exposed only to a light secondary asbestos pollution, while people belonging to the 30-60 years and the > 60 years classes must be considered to have remotely suffered more intense asbestos exposures either in occupational or non-occupational circumstances.

More than 60% of our population presented SEM-detectable asbestos fibers inside the lung parenchyma, with amphiboles fibers being more frequently detected than chrysotile fibers (58.2% of cases showing detectable amphibole fibers *versus* 20% showing detectable chrysotile fibers). Commercial and Non-Commercial Amphiboles were equally represented, the former coming from the indoor and outdoor releasing from asbestos-containing materials and the latter mainly originating as a natural contaminant of either the industrial chrysotile or the cosmetic talc [149-153]. The greater biopersistence of the amphiboles rather than chrysotile may explain the higher detection frequency for amphibole fibers even in the sample from a low-amphibole and a high-chrysotile polluted town. Such a ratio between amphiboles and chrysotile positive cases should also be prudentially analyzed in the suggested perspective of an intrinsic SEM bias towards the underestimation of chrysotile fibers [43,154].

The asbestos lung burden represents the final result of a lifetime dynamic accumulation as suggested by our findings that showed a linear positive trend of the lung asbestos burden with increasing age. In addition, 80% of study subjects younger than 30 years had an asbestos lung burden lower than the SEM analytical sensitivity. As stated before, the ≤ 30 years old people and the > 30 years old people analyzed in our study probably faced different pattern of daily asbestos exposures, with older subjects well crossing the asbestos-exploitation era: the reported “age-lung fibers burden” relationship should therefore be analyzed also remembering that > 30 years people do not represent an automatic reliable forecast for younger subjects.

About 5% (3 cases) exceeds the Helsinki amphiboles 1,000,000 ff/g dry cut off for occupational exposure. For one case an occupational exposure to asbestos is plausible: he worked in an automotive industry well-known as contaminated by asbestos.

Combining the two amphiboles statements from the 1997 Helsinki Consensus Report about probabilistic occupational asbestos exposures (1,000,000 ff/g dry cut off for all amphibole fibers and 100,000 ff/g dry cut off for amphibole fibers longer than 5 µm), 23.6% of our population would set above the suggested threshold values. A thorough explanation of these findings is however limited by the lack of a detailed lifetime work history for every case enrolled in the experimental population. All the attempts performed so far to investigate the occupational exposure of our cases (live pre-autoptic interviews + phone interviews with the subject's relatives) did not get satisfactory data. An ongoing search is in progress to obtain data on past work histories thanks to the collaboration with OCCAM (OCcupational CAncer Monitoring) that investigates occupational cancer risk by industrial sectors. For each case a linkage with social security files available at the National Social Security Institute from 1974 has been planned in order to obtain data on past employment. For each year of employment the employing firm, its economic branch and the white collar *versus* blue collar status are available.

However, the evidence of more than 20% of our cases having an amphibole count higher than the Helsinki cut off for occupational exposure to asbestos suggests the need to develop reference values for the residents in the city of Milan. The Helsinki Document itself actually stimulates any asbestos-dealing laboratory to the production of its own reference values from the pertinent background population and also states that the intrinsic reliability of a reference population comes from the stable evidence of a median asbestos burden for occupationally exposed people substantially higher than the corresponding reference population median burden [25].

The 6 analytical teams referring to the European Respiratory Society Asbestos Task Force published in 1998 their own reference values for the background populations, 5 teams not declaring any reference value for the amphibole fibers longer than 5 µm and just 1 team declaring the reference value 180,000 ff/g dry for the amphibole fibers longer than 5 µm [32]. Matching the analytical evidences of the present work with the 180,000 ff/g dry cut off for amphibole fibers longer than 5 µm, just 12.7% of our population (6 cases) would be labelled as having an abnormal lung retention of amphibole dust.

To further complicate matters, the Helsinki Report seems to equalize SEM and TEM techniques, whereas some authors partially explain the well-known inter-laboratory variability in asbestos burden estimations with the different performance of the currently available microscopic counting techniques [27,89,155]. Even though a general consensus about the 1,000,000 ff/g dry cut off for all the amphibole fibers do exist, such vigorously debated problems reinforce the need for the proper definition of reliable reference values by every single laboratory performing asbestos counting analyses and recommend the use of external reference values just for speculative purposes.

In our population the variables sex, residential district, birthplace and smoking habit did not significantly influence the median value of the asbestos burden. Previous studies already showed no influence of gender [238,241,243,257] and smoking habit [254] on the asbestos lung burden from the general population.

A comparison of the asbestos lung burden in the general populations from different countries or cities [33,99,127,181,237-259] is made difficult by the great variability related to the environmental characteristics of the study areas, the period ob observation, the selection criteria and the size of the examined population, the employed analytical techniques and the counting rules [156]. Such variability further underlines the need to build local and specific reference values.

About 50% of the asbestos fibers recovered from the lung parenchyma of our population was composed by ultrashort and ultrathin fibers, while fibers longer than 10 µm represented a fraction slightly above 10%. Such evidence matches well with the already discussed Milan environmental asbestos pollution [147], but also suggests an extraordinary biopersistence of the long amphibole fibers [157] (80% of the detected fibers longer than 5 µm being in fact amphibole fibers). There is no definite way to infer from the SEM images of a lung fiber its past splitting behaviour, that is a SEM image cannot reliably distinguish “native” fibers from fibrous fragments generated inside the lung parenchyma. Chrysotile is considered the asbestos variety most susceptible to intrapulmonary splitting, its mean dimensions being thus regularly expected as considerably lower than those of amphiboles. Among the asbestos fibers recovered from our experimental cases, chrysotile fibers were significantly shorter and thinner than amphibole fibers and Non-Commercial Amphibole fibers are thicker than the commercial ones.

In substantial agreement with previous independent observations (Table 6), the chrysotile fibers showed a very short mean length and a thin diameter, the Non-Commercial Amphibole fibers differed from the Commercial Asbestos fibers because of a lower aspect ratio and all the asbestos varieties increased their mean aspect ratios along with the increasing of their mean lengths.

In the lungs of the examined population there was just a minimal contamination from (asbestos) fibers traditionally supposed to hold a fibrogenic [158,159] or a carcinogenic metric [160,161]. In our experimental cases the typical asbestos burden was mainly made up by short and thin fibers yet to be definitely understood in their oncogenic potential [150,162-168].

The Asbestos Bodies prevalence in our experimental series was relatively low in agreement with the small fraction of asbestos fibers longer than 10 µm detected in the SEM analyses [56,76]. The Asbestos Bodies concentration in all the AB positive cases was well below the Helsinki Criteria for inferring an asbestos occupational exposure and also well below the 100 AB/g wet cut off for detecting AB in routine histological analysis of the lung [169,170]. The detected range of AB burden in positive cases was in full agreement with the previous literature statements about a mean general population AB burden lower than 20-50 AB/g wet and lower than 200-500 AB/g dry [56,76,171-173,227]. In the present study, only the morphologically typical Asbestos Bodies were counted and no investigation about the chemical composition of the Bodies core was performed. Even though a typical microscopic AB-morphology correlates well with the asbestos composition of the fibrous core [56,65,66,175,176], the absence of chemical analyses on the core of the detected bodies prudentially recommends to underline that the used label “morphologically typical Asbestos Body” does not mean “body with an assessed asbestos core”.

No Asbestos Bodies were found among the cases younger than 30 years. Being the Asbestos Bodies formation a relatively rapid process [58,184], such evidence can be better explained by a theory focused on the decrease of long airborne asbestos fibers in the last 20-30 years rather than by a theory suggesting the lack of sufficient time for creating Asbestos Bodies in young-dying people. However, the positive relationship between the Asbestos Bodies prevalence and the increasing age is an inconstant finding across the available scientific literature [53,177,185,186].

A comparison between the findings in our study population and the results obtained by the same SEM laboratory in two distinct populations of Italian asbestos-exposed workers [142,143] was also performed. This comparison was not a pure juxtaposition between occupational- and environmental-exposed subjects (as recommended by the Helsinki Report to validate a reference value for background asbestos population), because the two occupational-exposed groups were in reality made of workers affected by asbestos-related diseases.

The two occupational-exposed population respectively included 11 mesothelioma cases among asbestos- and nonasbestos-textile workers [143] and 12 cases with asbestos-related diseases (5 mesotheliomas, 4 lung cancers, 1 asbestosis + pleural plaques and 2 pure pleural plaques) among asbestos-cement workers [142].

In the former group the highest values of asbestos fibers were detected in the asbestos-textile subgroup (4 cases) and in 3 cases employed in jute recycling with concentrations ranging between 9,1 and 397 millions ff/g dry, well above the range observed in our study population. The total fiber concentrations in the other 4 non asbestos-textile workers (silk and cotton production workers) ranged from 0,33 to 1,2 millions ff/g dry and overlapped with the concentrations observed in our population.

Among the asbestos-cement workers 11 out of the 12 examined cases had an asbestos lung burden higher than 2,5 millions ff/g dry. Only 1 subject affected by lung cancer had a total fiber concentration of 1,32 millions ff/g dry, a value lower than our maximum measured concentration of 2,0 millions ff/g dry.

As regard the type of asbestos fibers, in both the occupational-exposed groups a 100% amphibole fibers detection frequency was observed (*versus* 58.2% in our population). Chrysotile fibers were detected in 20% of our population, in 25% of the asbestos-cement workers and in 72.8% of the asbestos-textile workers series.

In substantial agreement with other international experiences about the general population [89,187], the fibers lung burden from our experimental cases also comprised talc fibers, titanium-containing fibers and other inorganic fibers and the non-asbestos fibers burden greatly outnumbered the asbestos fibers burden. Talc is a well-known human fibrogenic pathogen [78,188-191] and in our experimental series the median value of the talc fibers burden appears to be very similar to the median value of the asbestos fibers burden. The SEM analysis did not allow a definite distinction among the inorganic fibers other than asbestos and a TEM analysis would be therefore needed to properly investigate such component of the fibrous lung burden and eventually to focus on the Man Made Vitreous Fibers and the Refractory Ceramic Fibers subgroups [13,192,193].

The present work aimed at describing the asbestos lung burden from a necroscopic sample of the people today living in Milan, thus giving a first contribution to the creation of reliable reference values for the asbestos lung burden of the Milan population.

To establish reliable reference values for such analyses is at the same time mandatory for a good clinical and forensic practice but also very difficult to realize [89,194,195]. According to the European Respiratory Society Asbestos Task Force [32], the creation of reliable reference values for the asbestos lung burden estimations is a multistep task with contemporary focus on necroscopic series free from asbestos-related diseases and on surgical series from patients undergone lung surgery either for asbestos-related pathology or not. In this perspective, the present work is just a first contribution to the creation of reliable reference values for the asbestos lung burden estimations and there is the pivotal need for further research on Milan lung surgical series.

A better and thorough evaluation of the results from the present work will be done after improving the exposure history collection for all the 55 cases enrolled in the experimental population: as stated before, an ongoing research is in progress.

The results of the present study are first of all influenced by the employed population selection criteria, sampling procedures, analytical techniques and counting rules. As a preliminary warrant about the reliability of the assessing results [196], all the pre-analytical and analytical steps have been performed by trained specialists according to published and shared methodologies and all the SEM-analyses (the only ones probably expected to produce the effective reference values for asbestos lung counting) have been performed at the certified Laboratory of the Environmental Protection Agency of Lombardy (ARPA).

The collected results come after about 20 years from the 1992 Italian asbestos ban and put together at least 2 subgroups of people probably facing in their lifetime a very different pattern of asbestos exposure (1^{st} age class people *versus* 2^{nd} and 3^{rd} age class people). After just 20 years from the total asbestos ban, the results from such a general investigation on the whole resident population have to be considered as intermediate evidences along a period of ever-decreasing environmental exposure to asbestos in Milan. The results from the present study need therefore to be updated in the future [196] to promptly detect any differences in the asbestos lung burden of the Milan residents after demographic increasing of the people having faced in their lifetime only very mild secondary asbestos pollution. This update will be another crucial factor for a good clinical and forensic practice about asbestos-related diseases.

The typical fibrous lung content of Milan residents also comprises talc fibers, titanium-containing fibers and a great amount of inorganic fibers other than asbestos, talc and titanium. The global amount of fibers other than asbestos greatly outnumbers the asbestos lung content and the estimated median talc burden is very similar to the median asbestos burden. The miscellaneous group of the inorganic fibers other than asbestos, talc and titanium extends to the Man Made Vitreous Fibers and the Refractory Ceramic Fibers: such fibers need a further dedicated experimental focus by TEM-analysis, as similarly do ultrathin and ultrashort asbestos fibers and also cleavage fragments from non-asbestiform amphiboles [144,145,149].

Available data suggest a progressive lifetime accumulation of all the major inorganic fiber types inside the lung parenchyma of the Milan inhabitants.

Table 6: asbestos fibers dimension from 10 different studies about the general population.

	Chrysotile fibers	**CA fibers**	**NCA fibers**
Churg et al 1980 [241]	90% < 5 µm in length 1.9% > 10 µm in length Mean AR for fibers < 5 µm in length: 61 Mean AR for fibers 5-10 µm in length: 200 Mean AR for fibers > 10 µm in length: 340	25% < 5 µm in length 20.6% > 10 µm in length Mean AR for fibers < 5 µm in length: 32 Mean AR for fibers 5-10 µm in length: 68 Mean AR for fibers > 10 µm in length: 160	60% < 5 µm in length 7.4% > 10 µm in length Mean AR for fibers < 5 µm in length: 18 Mean AR for fibers 5-10 µm in length: 25 Mean AR for fibers > 10 µm in length: 30
Churg et al 1986 [247]	94% < 5 µm in length 1% > 10 µm in length Mean length 1.1 µm Mean AR 24		For tremolite fibers 92% < 5 µm in length 0% > 10 µm in length Mean length 1.6 µm Mean AR 6.5
Paoletti et al 1991 [183]	For all asbestos fibers Length range 1-8 µm Mean length 3 µm		
Langer et al 1994 [234]	99.4 % < 5 µm in length 90.2% < 1 µm in length		
Magnani et al 1998 [181]		For all amphiboles fibers Mean dimensions 11.7 x 0.40 µm	
Dodson et al 1999 [237]	86.4% < 5 µm in length Mean dimensions 1.81 x 0.06 µm Mean AR 29.3	For amosite fibers 75% < 5 µm in length For crocidolite fibers 100% < 5 µm in length	For tremolite fibers 89% < 5 µm in length Mean dimensions 2.40 x 0.26 µm Mean AR 9.11

Table 6 cnt: asbestos fibers dimension from 10 different studies about the general population.

	Chrysotile fibers	**CA fibers**	**NCA fibers**
Dodson et al 2000 [257]	For all asbestos fibers Mean dimensions 2 x 0.15 µm Mean AR 13.9		
Tossavainen et al 2000 [258]	Mean dimensions 2.2 x 0.08 µm		
McDonald et al 2001 [259]		3.5% of all amphibole fibers > 10 µm in length	
Present work	For all asbestos fibers 62.2% < 5 µm in length 13.5% > 10 µm in length Mean dimensions 4.19 x 0.19 µm Mean AR 20.6		
	79.5% < 5 µm in length 5.1% > 10 µm in length Mean dimensions 2.74 x 0.09 µm Mean AR 30.7 Mean AR for fibers < 5 µm in length 24.4 Mean AR for fibers > 5 µm in length 74.9	65.7% < 5 µm in length 21.8% > 10 µm in length Mean dimensions 4.86 x 0.17 µm Mean AR 26.9 Mean AR for fibers < 5 µm in length 16.3 Mean AR for fibers > 5 µm in length 70.2	42.5% < 5 µm in length 15% > 10 µm in length Mean dimensions 5.65 x 0.47 µm Mean AR 11.25 Mean AR for fibers < 5 µm in length 5.8 Mean AR for fibers > 5 µm in length 31.2

CA = Commercial Amphiboles, NCA = Non-Commercial Amphiboles. AR = Aspect Ratio.

SUMMARY

The present study analyzed the asbestos lung burden from a necroscopic series of the Milan general population. The study was performed on 55 cases free from asbestos-related disease undergone a judicial autopsy at the Forensic Institute of Forensic Medicine of Milan in the period running from 2009 to 2011.

For each study case multiple lung samples were digested and vacuum-filtered on 0.2 µm pore size polycarbonate membranes and then were analyzed by both traditional Light Microscopy (for counting of morphologically typical Asbestos Bodies) and EDXA-Scanning Electron Microscopy (for counting of all asbestos fibers). The SEM-analysis also extended to the count of inorganic fibers other than asbestos.

The Asbestos Bodies prevalence in the series was 14.5% with the positive cases having an AB count ranging from 10 to 110 AB/g dry. No Asbestos Bodies were found in the subjects younger than 30 years.

Asbestos fibers were SEM-detected in 63.6% of the study cases, with a higher detection frequency for amphiboles than for chrysotile (58.2% *versus* 20%). An asbestos content lower than the SEM analytical sensibility was found in 80% of the subjects younger than 30 years. Commercial Amphiboles were detected as frequently as NonCommercial Amphiboles. NonCommercial amphiboles were mainly represented by tremolite fibers.

The estimated median value was 110,000 ff/g dry (IQ range 62,250-275,000 ff/g dry) for all the asbestos fibers, 91,600 ff/g dry (IQ range 60,000-180,000 ff/g dry) for the amphibole fibers and 51,600 ff/g dry (IQ range 46,600-65,000 ff/g dry) for the chrysotile fibers.

The maximum estimated burden for all asbestos fibers was 2,000,000 ff/g dry. Thirteen cases showed an amphibole burden higher than the Helsinki cut offs for occupational exposure: three cases showed a total amphibole burden higher than 1,000,000 ff/g, while other 10 cases showed an amphibole burden for fibers longer than 5 µm higher than the 100,000 ff/g dry.

A comparison was performed between our results and the results coming from two distinct occupational-exposed populations examined by the same SEM laboratory. The maximum measured asbestos burden in our population was lower than the minimum measured asbestos burden among asbestos-textile workers, jute

recycling workers and asbestos-cement workers. Just one asbestos-cement worker and 4 silk/cotton-textile workers showed asbestos lung concentrations overlapping our experimental results.

A positive linear relationship was observed between asbestos lung burden and age at death. Sex, residential district, birthplace and smoking habit did not significantly influence the median asbestos lung burden.

The mean dimension for the detected asbestos fibers was 4.19 x 0.19 μm with a 20.6 mean aspect ratio. Chrysotile fibers (mean dimension 2.74 x 0.09 μm) were significantly shorter and thinner than amphibole fibers, the NonCommercial Amphibole fibers (mean dimension 5.65 x 0.47 μm) being also significantly thicker than the Commercial Amphibole fibers (mean dimension 4.86 x 0.17 μm). Asbestos fibers traditionally supposed to be fibrogenic and carcinogenic in humans were very infrequently detected.

The median talc burden was very similar to the median asbestos lung burden and the global non-asbestos fibers lung burden well outnumbered the asbestos fibers burden. Also for inorganic fibers other than asbestos a positive linear relationship with age at death was observed.

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APPENDIX 1

Results from main studies examining the AB prevalence in the general population

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Cauna et al 1965 USA [197]	100 necroscopic cases. 53 M and 47 F	Analysis on lung smears	**41%**. 47% in M and 34% in F	
Elmes et al 1965 UK [198]	200 M from routine necropsies as control population. 100 cases in the age range 50-59 years + 100 cases in the age range 60-69 years	LM analysis on typical histologic lung sections	**20.5%**. 14% in the 50-59 years old series, 27% in the 60-69 years old series	Inclusion criterion for the control population: no diagnosis of lung carcinoma or mesothelioma
Thomson 1965 1^{st} series) South Africa 2^{nd} series) USA [199]	1^{st} series) 500 cases from consecutive necropsies, age > 15 years. 2^{nd} series) 500 cases from consecutive necropsies, age > 15 years	LM analysis on basal lung smears	1^{st} series) **26.4%**. 30.4% in M and 20% in F. 31.6% in the 55-64 years old subgroup (max). 2^{nd} series) **27.2%**. 31.6% in M and 20.4% in F. 31.9% in the > 75 years old subgroup (max).	

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Anjilvel et al 1966 Canada [200]	100 cases from random hospital necropsies. 56 M and 44 F Age > 26 years	LM analysis on scraping and squeezing lung smears	**48%**. 57% in M and 36% in F. 30% in the 26-45 years old subgroup and 50% in the > 45 years old subgroup	4 cases with lung cancer
Meurman 1966 Finland [201]	264 cases from consecutive necropsies. 148 M and 116 F. Age > 15 years	LM analysis on iron-stained lung sections	**57.6%**. 60.1% in M and 54.3% in F. 70% in urban residents and 49% in rural residents	
Hourihane et al 1966 UK [202]	115 cases from hospital routine necropsies	LM analysis on 30 µm-thick unstained lung sections	**24.3%**	
Roitzsch 1967 Germany [203]	250 cases	Analysis on lung smears	**43.2%**	10 cases with pleural plaques
Hefin Roberts 1967 UK [204]	100 cases from consecutive hospital necropsies. 62 M and 38 F. Age range 24-85 years	LM analysis on basal lung smears	**23%**. 37% in M and 0% in F. 47% of AB-positive cases < 5 AB	13 cases with pleural plaques, 3 cases with lung cancer, 1 case with asbestosis and 4 cases with wide lung fibrosis

Authors Year Place	Population	Methods	AB prevalence	Comment
Ghezzi et al 1967 Italy [182]	100 cases from random hospital necropsies. 64 M and 36 F. Age range 25-83 years	LM analysis on apical and basal lung smears	**51%**. 54% in M and 44% in F. 14% in the < 50 years old subgroup and 60-66% in the > 50 years old subgroup	
Polliack et al 1968 Israel [205]	100 cases	Analysis on lung smears	**26%**. 29.1% in M and 22.2% in F	
Ashcroft 1968 UK [206]	311 cases from unselected necropsies. 196 M and 115 F. Age > 15 years	LM analysis on basal lung smears	**20.3%**. 25.5% in M and 11.3% in F. 25% in urban residents and 3.5% in rural residents. No AB in M< 25 years and in F< 35 years	11 cases with lung cancer, 1 case with mesothelioma
Bignon et al 1969 France [207]	1st series) 45 cases 2nd series) 103 cases	LM analysis on sodium hypochlorite digested lung samples	1st series) **98%** 2nd series) **99%**	
Dicke et al 1969 USA [208]	100 cases from consecutive necropsies. 66 M and 34 F. Age range 16-88 years	LM analysis on apical and basal lung smears	**18%**. 23% in M and 12% in F. No AB-positive cases in the < 30 years old subgroup	

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Peacock et al 1969 Italy [180]	109 cases from consecutive hospital necropsies. 63 M and 46 F. Age range 23-90 years	LM analysis on scraping lung smears	**0.9%**	4 cases with lung cancer
Xipell et al 1969 Australia [209]	200 cases. 138 M and 62 F	Analysis on lung digested samples	**43.5%**. 44.2% in M and 41.9% in F	1 case with pleural plaques
Um 1971 UK [210]	From consecutive hospital necropsies, age > 20 years: 1st series, from 1936) 127 cases, 82 M and 45 F 2nd series, from 1946) 100 cases, 61 M and 39 F 3rd series, from 1956) 100 cases, 51 M and 48 F 4th series, from 1966) 100 cases, 55 M and 45 F	LM analysis on 5µm- and 30µm-thick lung sections	1st series) **0%** 2nd series) **3%** 3rd series) **14%** 4th series) **20%**	
Nizze 1971 Germany [211]	234 cases. 121 M and 113 F	Analysis on digested lung samples	**9%**. 11% in M and 8% in F	

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Plamenac et al 1971 Bosnia [212]	100 cases. 55 M and 45 F	Analysis on lung smears	**38%**. 52.7% in M and 20% in F	
Rosen et al 1972 USA [213]	86 cases from lung surgery and necropsies	LM analysis on sodium hypochlorite digested and filtered lung samples	**93%**	1 case with mesothelioma and some cases with lung cancer
Smith et al 1972 USA [214]	100 cases from consecutive necropsies. 66 M and 34 F. Age > 16 years	LM analysis on sodium hypochlorite digested and filtered lung samples	**100%**	6 cases with lung cancer
Hagerstrand et al 1973 Sweden [215]	97 cases from consecutive necropsies. 59 M and 38 F. Age range 30-93 years	Analysis on lung smears and on 30 μm-thick lung sections	**48.4%**. 54.2% in M and 39.4% in F	29 cases with pleural plaques, 4 cases with lung cancer and 1 case with mesothelioma
Bianchi et al 1973 Italy [216]	50 cases from unselected hospital necropsies. 24 M and 26 F. Age range 48-89 years	LM analysis on centrifuged lung squeezing fluid	**70%**. 79% in M and 61% in F	2 cases with lung cancer and 1 case with mesothelioma
Fondimare et al 1974 France [217]	52 unselected cases	LM analysis on digested and filtered lung samples	**100%**. 92% of cases < 100 AB/4 g wet lung	

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Bianchi et al 1974 Italy [218]	50 cases from unselected hospital necropsies. 34 M and 16 F. Age range 28-83 years	LM analysis on sodium hypochlorite digested lung samples	**96%**	Some cases with lung cancer
Doniach et al 1975 UK [219]	394 cases from consecutive hospital necropsies. 216 M and 178 F. Age > 16 years.	LM analysis on 30µm-thick unstained lung section	**37%**. 42% in M and 30% in F. 60.9% in heavy manual workers and 12.1% in clerical workers. 45-53% in residents in industrial areas	58 cases with lung carcinoma, 25 cases with pleural plaques. Exclusion criterion: diagnosis of asbestosis or mesothelioma
Breedin et al 1976 USA [220]	100 cases from consecutive necropsies. 70 M and 30 F. Age > 16 years	LM analysis on sodium hypochlorite digested and filtered lung samples	**93%**. 91% in urban residents and 95% in rural residents	Exclusion criteria: lung neoplasm, known occupational exposure to asbestos
Bhagavan et al 1976 USA [177]	From consecutive necropsies: 1st series, from 1940-1949) 61 cases 2nd series, from 1950-1959) 47 cases 3rd series, from 1970-1972) 145 cases	LM analysis on sodium hypochlorite digested and filtered lung samples	1st series) **40.9%** 2nd series) **61.7%** 3rd series) **91.1%**	
Gordon et al 1976 USA [221]	28 cases from a hospital 1928-1932 necroscopic archive. 9 M and 19 F. Age range 17-76 years	LM analysis on sodium hypochlorite digested and filtered lung samples	**39.3%**	1 case with lung cancer

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Bianchi et al 1976 Italy [222]	50 cases from unselected necropsies. 24 M and 26 F. Age range 23-90 years	LM analysis on chemically digested lung samples	**88%**	
Churg et al 1977 USA [53]	252 cases from 234 necropsies and 18 lung surgeries. 152 M and 100 F. Age > 40 years	LM analysis on sodium hypochlorite digested and filtered lung samples	**96%** Women and white collar men with unimodal AB count < 50/g wet. Blue collar men with bimodal AB count with peaks < 50 g/wet and 100-499 g/wet	54 cases with lung cancer
Francis et al 1977 Scandinavia [223]	198 cases from unselected necropsies		**7%**	66 cases with pleural plaques
Roggli et al 1980 USA [224]	52 cases as control population. Mean age 58.5 years	LM analysis on sodium hypochlorite digested and filtered lung samples	**92%**	3 cases with lung cancer and 1 case with peritoneal mesothelioma
Rubino et al 1980 Italy [225]	218 M from unselected hospital necropsies	LM analysis of lung smears	31.8% in cases with no pleural plaques. 49.2% in cases with < 100 cm^2 pleural plaques. 87.5% in cases with > 100 cm^2 pleural plaques	67 cases with pleural plaques
Bianchi et al 1981 Italy [226]	100 cases from consecutive hospital necropsies	LM analysis on chemically digested lung samples	**94%**	Pleural plaques in 72% of M and 33% of F

Authors Year Place	**Population**	**Methods**	**AB prevalence**	**Comment**
Steele et al 1982 UK + New Zealand [186]	From lung surgery and unselected necropsies: 1st series, from UK) 319 cases. 286 M and 33 F. Age range 16-92 years 2nd series from NZ) 248 cases. 196 M and 52 F. Age range 15-92 years. 3rd series, from UK) 106 cases.75 M and 31 F	LM analysis in 30µm-thick unstained lung section for the 1st series. LM analysis on KOH digested and Perls stained lung samples for the 2nd series and the 3rd series	1st series) 13% in M and 0% in F. 2nd series) **75%**. 78% in M and 63% in F. 3rd series) **80%**. 83% in M and 74% in F	1st series) 196 cases with lung cancer 2nd series) 167 cases with lung cancer 3rd series) 2 cases with mesothelioma and 50 cases with lung cancer
Andrion et al 1982 Italy [227]	996 cases from unselected necropsies		**12.4%**	
Andrion et al 1982 Italy [228]	Two series as control populations: 1st series) 26 cases from hospital necropsies. 23 M and 3 F. Age range 42-80 years. 2nd series) 39 cases from surgeries. 34 M and 5 F. Age range 35-72 years	LM analysis on 30 µm-thick unstained lung sections	1st series) 26% in M and 0% in F. 2nd series) 23.5% in M and 20% in F	Exclusion criterion for control populations: diagnosis of lung cancer, occupational exposure to asbestos
Betta 1982 Italy [229]	100 samples from consecutive hospital necropsies. 71 M and 29 F. Mean age 56.8 years	LM analysis on scraping lung smears	**52%** 57.7% in M and 35.7% in F. 76% in urban residents and 45.9% in non-urban residents	

Authors Year Place	Population	Methods	AB prevalence	Comment
Johansson et al 1987 Sweden [230]	89 necroscopic cases as control population. Mean age 67 years	LM analysis on 25 µm-thick unstained lung sections	**13.5%**	Exclusion criterion for the control population: known exposure to dust causing pneumoconiosis
Haque et al 1988 USA [231]	46 cases from paediatric necropsies. Age range 1-27 months	LM analysis on sodium hypochlorite digested lung samples	**21.7%**	
Wu et al 1988 Japan [232]	92 necropsy cases	Analysis on digested lung samples	**94.6%**	60 cases with lung cancer
Shishido et al 1989 Japan [178]	From necropsies and lung surgery: 1st series) 1937-1941 2nd series) 1947-1951 3rd series) 1958-1963 4th series) 1970-1973 5th series)1980-1981	LM analysis of sodium hypochlorite digested lung samples	1st series) **10%** 2nd series) **18%** 3rd series) **70%** 4th series) **74.4%** 5th series) **81%**	
Hiraoka et al 1990 Japan [233]	369 cases from unselected hospital necropsies as control population. 249 M and 120 F. Age > 35 years	LM analysis on chemically digested and filtered lung samples	80.2% of cases 0-19 AB/g wet. 11.1% of cases 20-199 AB/g wet. 7.1% of cases 200-1,999 AB/g wet. 1.6% of cases ≥ 2,000 AB/g wet	Exclusion criterion for the control population: diagnosis of lung cancer
Langer et al 1994 USA [234]	3000 cases from hospital necropsies. 1971 M and 1029 F	LM analysis on lung samples	**48.6%** 51.4% in M and 42.4% in F	

Authors Year Place	Population	Methods	AB prevalence	Comment
Arenas-Huertero et al 1994 Mexico [179]	180 cases from hospital necropsies in the 1975-1988 period. 104 M and 76 F	LM analysis on sodium hypochlorite digested lung samples	**50%** in 1975 **93%** in 1977 **96%** in 1981 **70%** in 1982 **86%** in 1983 **86%** in 1988	
Monso et al 1995 Spain [235]	1st series) 18 cases from an urban area. Mean age 62.2 years. 2nd series) 16 cases from a rural area. Mean age 62.2 years		1st series) **50%**. AB mean count 52.35/g dry. 2nd series) **12.5%.** AB mean count 5.37/g dry	3rd series, 8 cases with lung carcinoma) AB prevalence 25%, mean AB count 20.59/g dry
King et al 1996 USA [236]	16 cases selected as control population. Age range 36-83	LM analysis on sodium hypochlorite digested lung samples	**12.5%**	Inclusion criterion for the control population: no history of occupational asbestos exposure
Magnani et al 1998 Italy [181]	31 cases from unselected hospital necropsies	LM analysis on sodium hypochlorite digested lung samples	**80.7%**	Inclusion criterion: no known occupational exposure to asbestos
Dodson et al 1999 USA [237]	33 necropsy cases without known occupational exposure to asbestos. 23 M and 10 F	LM analysis on sodium hypochlorite digested and filtered lung samples	**21.2%**	Inclusion criterion: LM FB count ≤ 20/g wet

Authors Year Place	Population	Methods	AB prevalence	Comment
Liu et al 2001 China [238]	107 cases from randomized hospital necropsies. 44 M and 63 F	PCLM analysis on chemically digested lung samples	35.7% in M and 39.5% in F	Inclusion criteria for the control population: no diagnosis of lung cancer, death due to acute myocardial infarction or to accidental death

AB = Asbestos Bodies, FB = Ferruginous Bodies.
F = Females, M = Males
LM = Light Microscope, PCLM = Phase Contrast Light Microscope.
KOH = potassium hydroxide.

APPENDIX 2

Results from main studies examining the asbestos fibers burden in the general population

Authors Year Place	**Population**	**Methods**	**Asbestos fibers count**	**Comment**
Ashcroft 1973 UK [239]	48 cases from routine necropsies as control population divided into 2 subgroups. 1st subgroup) 18 cases with ≥ 1 AB in the preliminary LM analysis. 2nd subgroup) 30 cases with no AB in the preliminary LM analysis	Analysis on chemically digested lung samples	1st subgroup) range 0-7,760,000 ff/g dry. 11% of cases having no detectable fibers 2nd subgroup) range 0-298,000 ff/g dry. 57% of cases having no detectable fibers	Inclusion criterion for the control population: no diagnosis of malignancies
Whitwell et al 1977 UK [240]	100 unselected necropsy cases as control population. 72 M and 28 F. Age > 20 years	PCLM analysis on chemically digested lung samples. Counting of both coated and uncoated asbestos fibers > 6 µm in length	57% of the population < 10,000 ff/g dry, 71% of the population < 20,000 ff/g dry, 15% of the population > 50,000 ff/g dry	Inclusion criteria for the control population: no diagnosis of lung cancer, mesothelioma or other “industrial disease”. 55 cases with pleural plaques
Churg et al 1980 USA [241]	21 necropsy cases. 11 M and 10 F. Age > 40 years, mean age 64 years	EM analysis on sodium hypochlorite digested lung samples	Chrysotile range 12,000-680,000 ff/g wet, mean chrysotile value 130,000 ff/g wet. Amphibole range 1,300-75,000 ff/g wet, mean amphibole value 25,000 ff/g wet. Chrysotile and tremolite detected in 100% of cases, commercial amphiboles detected in 52% of cases	Inclusion criterion: preliminary AB count < 100/g wet. 4 cases with lung cancer, 1 case with pleural plaques

Authors Year Place	**Population**	**Methods**	**Asbestos fibers count**	**Comment**
Gylseth et al 1981 Norway [242]	12 cases from consecutive necropsies as control population. 8 M and 4 F. Age range 49-90 years	SEM analysis on ashed lung samples	Range 100,000-2,300,000 ff/g dry. 75% of cases < 1,000,000 ff/g dry	Controls died of cardiovascular disease
Churg 1982 USA [99]	25 cases as a control population. 24 M and 1 F. Mean age 65 years	EM analysis on sodium hypochlorite digested and filtered lung samples	Mean value for all asbestos fibers 99,000 ff/g wet. Mean values for chrysotile fibers 68,000 ff/g wet. Mean values for non-commercial amphiboles 30,000 ff/g wet. Mean values for amosite fibers 1,000 ff/g wet	Inclusion criteria for the control population: no known occupational exposure to asbestos, absence of pleural plaques, AB count < 100/g wet lung
Stovin et al 1982 UK [243]	112 cases from consecutive necropsies. 87 M and 25 F. Age range 45-74 years	PCLM analysis on KOH digested lung samples. PCLM counting of all fibers > 8 µm in length	31% of cases having no detectable fibers. Median value ≈ 5,000 ff/g dry	10 cases with pleural plaques, 42 cases with lung cancer and 4 cases with mesothelioma
Mollo et al 1983 Italy [244]	82 cases	LM analysis on sodium hypochlorite digested lung samples. LM count of fibers > 10 µm in length	Maximum uncoated fibers count 12,500 ff/g dry. Maximum coated fibers count 150 AB/g dry	82 cases without known asbestos exposure
Mowè et al 1984 Norway [245]	36 necropsy cases as control population. Mean age 67.9 years	SEM analysis on ashed lung samples. SEM counting of all inorganic fibers	Range 0-4,800,000 ff/g dry. Median value 300,000 ff/g dry.	Inclusion criterion for the control population: death due to cardiovascular pathology

Authors Year Place	Population	Methods	Asbestos fibers count	Comment
Mowè et al 1985 Norway [246]	28 M from hospital necropsies as control population. Mean age 68.1 years	SEM analysis on ashed lung samples	Range 0-4,800,000 ff/g dry. 25% of cases > 1,000,000 ff/g dry	Exclusion criterion for the control population: diagnosis of lung cancer or chronic pulmonary disease
Churg et al 1986 Canada [247]	20 cases from unselected necropsies as control population	EM analysis on sodium hypochlorite digested lung samples. EM counting of all fibers > 0.5 μm in length	Chrysotile range 0-1,300,000 ff/g dry; chrysotile median value 200,000 ff/g dry. Tremolite range 0-1,200,000 ff/g dry; tremolite median value 200,000 ff/g dry	Inclusion criteria for the control population: no known occupational exposure to dust of any kind
Tuommi et al 1989 Finland [248]	15 cases from unselected necropsies as control population	SEM analysis on ashed lung samples. Mineral fibers counting	Range < 10,000-3,200,000 ff/g dry. 20% of cases > 1,000,000 ff/g dry	
Albin et al 1990 Sweden [33]	96 cases as control population	TEM analysis on chemically digested lung samples	Asbestos fibers median value 29,000,000/g dry. Amphibole fibers median value 150,000 ff/g dry	
Tuommi et al 1991 Finland [249]	13 M from unselected necropsies as control population. Mean age 60 years	SEM and TEM analysis on lung samples	77% of cases < 1,000,000 ff/g dry. 23% of cases ≥ 1,000,000. 100% of cases < 10,000,000 ff/g dry	
Tuommi et al Finland 1991 [250]	9 M from sudden death necropsies as control population. Age range 37-67 years	SEM analysis on ashed lung samples	Range < 100,000-600,000 ff/g dry	

Authors Year Place	**Population**	**Methods**	**Asbestos fibers count**	**Comment**
Langer et al 1994 USA [251]	126 cases from hospital necropsies	TEM analysis on chemically digested lung samples. TEM chrysotile counting only	Range for positive cases 1,800,000-15,700,000 ff/g dry.	Positive cases with TEM counting ≥ 28 chrysotile fibrils
Tossavainen et al 1994 Finland [252]	10 M from unselected hospital necropsies as control population	SEM analysis on ashed lung samples	Range < 100,000-1,600,000 ff/g dry. Mean value 500,000 ff/g dry	
Karjalainen et al 1994 Finland [253]	300 M from urban subjects necropsies. Age range 35-69 years	SEM analysis on ashed lung samples. SEM counting for fibers ≥ 1 μm in lenght	For the subgroup without pleural plaques: median value 160,000 ff/g dry; range 0-2,900,000 ff/g dry; 8% of such cases ≥ 1,000,000 ff/g dry. For the subgroup with moderate pleural plaques: median value 400,000 ff/g dry; range 0-4,700,000 ff/g dry. For the subgroup with widespread pleural plaques: median value 570,000 ff/g dry; range 0-160,000,000 ff/g dry	3 cases with lung carcinoma, 168 cases with pleural plaques (80 cases with moderate pleural plaques and 88 cases with widespread pleural plaques)
Karjalainen et al 1994 Finland [254]	300 M from sudden death necropsies. Age range 35-69 years	SEM analysis on ashed and filtered lung samples. SEM counting of all fibers ≥ 1μm	Range < 300,000-163,000,000 ff/g dry. 18% of cases > 1,000,000 ff/g dry	3 cases with lung cancer

Authors Year Place	Population	Methods	Asbestos fibers count	Comment
Karjalainen et al 1994 Finland [255]	297 M from sudden death necropsies as control population. Age range 35-69 years, mean age 52 years	SEM analysis on ashed lung samples. SEM counting of all fibers > 1μm in length	82.8% of cases < 1,000,000 ff/g dry. 2.3% of cases > 5,000,000 ff/g dry	Exclusion criterion for the control population: diagnosis of lung carcinoma
Dufresne et al 1996 Canada [256]	49 cases from routine necropsies as control population divided into 2 subgroups. 1st subgroup) 23 cases born before 1940. 2nd subgroup) 26 cases born after 1940	TEM analysis on sodium hypochlorite digested and filtered lung samples	1st subgroup) Mean value for fibers < 5 μm in length 700,000 ff/g dry. Mean value for fibers 5-10 μm in length 134,000 ff/g dry. Mean value for fibers > 10 μm in length 74,000 ff/g dry 2nd subgroup) Mean value for fibers < 5 μm in length 162,000 ff/g dry. Mean value for fibers 5-10 μm in length 65,000 ff/g dry. Mean value for fibers > 10 μm in length 42,000 ff/g dry	
Magnani et al 1998 Italy [181]	31 cases from unselected hospital necropsies	TEM analysis on sodium hypochlorite digested and filtered lung samples. TEM counting of all fibers	No asbestos fibers detected in 54.8% of cases. Mean value for asbestos fibers 24,000 ff/g dry. Mean value for chrysotile fibers 5,000 ff/g dry. Mean value for crocidolite fibers 10,000 ff/g dry. Mean value for tremolite fibers 6,000 ff/g dry	Inclusion criterion: no known occupational exposure to asbestos

Authors Year Place	Population	Methods	Asbestos fibers count	Comment
Dodson et al 1999 USA [237] Dodson et al 2000 USA [257]	33 necropsy cases. 23 M and 10 F. Age range 12-73 years	TEM analysis on sodium hypochlorite digested and filtered lung samples	Range 0-290,000 ff/g dry. Mean value for the 0-290,000 ff/g dry interval: 84,000/g dry. Mean value for the 32,000-290,000 ff/g dry interval: 120,000/g dry. Chrysotile = 35% of all detected asbestos fibers. Chrysotile detected in 42.4% of cases	Inclusion criteria: no known occupational exposure to asbestos, lung histology negative for asbestos-related diseases, preliminary LM FB count ≤ 20/g wet
Tossavainen et al 2000 Russia [258]	23 cases from necropsies as control population. 17 M and 6 F. Age range 1 month-81 years, mean age 49 years	SEM analysis on ashed and filtered lung samples. SEM counting of all fibers > 1μm in length	Chrysotile range 100,000-14,600,000 ff/g dry; chrysotile mean value 2,630,000 ff/g dry. Tremolite + anthophyllite range < 100,000-700,000 ff/g dry; mean tremolite + anthophyllite value 180,000 ff/g dry	Inclusion criterion for the control population: no known occupational asbestos exposure
Liu et al 2001 China [238]	107 cases from unselected hospital necropsies as control population. 44 M and 63 F	PCLM analysis on chemically digested lung samples	Median values: 0 ff/g dry in the 10-40 years old subgroup, 32,000 ff/g dry in the 40-70 years old subgroup, 52,000 ff/g dry in the > 70 years old subgroup. Median value for M 32.500/g dry. Median value for F 37,000/g dry. 18.8% of the control population with values > 100,000 ff/g dry	Inclusion criteria for the control population: no diagnosis of lung cancer, death due to acute myocardial infarction or to exogenous accident

Authors Year Place	**Population**	**Methods**	**Asbestos fibers count**	**Comment**
McDonald et al 2001 UK [259]	57 cases from necropsies as control population. Age range 36-52 years	TEM analysis on KOH digested lung samples	For chrysotile: 33.4% of cases < DL, 36.8% of cases 100,000-900,000 ff/g dry, 28% of cases 1,000,000-10,000,000 ff/g dry, 1.8% of cases > 10,000,000 ff/g dry. For amphiboles: 49.1% of cases < DL, 42.1% of cases 100,000-900,000 ff/g dry, 7.0% of cases 1,000,000-10,000,000 ff/g dry, 1.8% of cases > 10,000,000 ff/g dry	Inclusion criterion for the control population: sudden or accidental death
Roggli et al 2010 USA [127]	20 cases from necropsies	SEM analysis	Range: 4,000-169,000 ff/g dry. Median value 31,000 ff/g dry	Inclusion criteria: normal lungs at autopsy, preliminary AB count within the general population range

AB = Asbestos Body, FB = Ferruginous Body.
F = Females, M = Males.
DL = Detection Limit.
LM = Light Microscope, PCLM = Phase Contrast Light Microscope, EM = Electron Microscope, SEM = Scanning Electron Microscope, TEM = Transmission Electron Microscope.
KOH = potassium hydroxide.

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