Ann. Occup. Hyg., Vol. 56, No. 6, pp. 660–670, 2012
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on behalf of the British Occupational Hygiene Society [2012]
doi:10.1093/annhyg/mer126

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Asbestos Fibre Burden in the Lungs of Patients with Mesothelioma Who Lived Near Asbestos-Cement Factories

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Received 8 December 2010; in final form 16 November 2011; published online 12 January 2012

Background: Epidemics of malignant mesothelioma are occurring among inhabitants of Casale Monferrato and Bari never employed in the local asbestos-cement (AC) factories. The mesothelioma risk increased with proximity of residence to both plants.

Objectives: To provide information on the intensity of environmental asbestos exposure, in the general population living around these factories, through the evaluation of the lung fibre burden in mesothelioma patients.

Methods: We analysed by a scanning electron microscope equipped with X-ray microanalysis wet (formalin-fixed) lung tissue samples from eight mesothelioma patients who lived in Casale Monferrato or Bari and underwent surgery. Their occupational and residential history was obtained during face-to-face interviews. Semi-quantitative and quantitative indices of cumulative environmental exposure to asbestos were computed, based on residential distance from the AC plants and duration of stay.

Results: The lung fibre burden ranged from 110 000 to 4 300 000 fibres per gram of dry lung (f/g) and was >1 000 000 f/g in three subjects. In four cases, only amphibole fibres were detected. Environmental exposures had ceased at least 10 years before samples were taken. No patient had other definite or probable asbestos exposures. A linear relationship was observed between the lung fibre burden and all three indices of environmental cumulative exposure to asbestos.

Conclusions: Environmental exposure to a mixture of asbestos fibres may lead to a high lung fibre burden of amphiboles years after exposure cessation. The epidemiological evidence of an increased mesothelioma risk for the general population of Casale Monferrato and Bari, associated with asbestos contamination of the living environment, is corroborated.

Keywords: environmental exposure; lung asbestos fibre burden; pleural malignant mesothelioma

INTRODUCTION

It has long been recognized that mesothelioma incidence is proportional to cumulative exposure to asbestos, as reflected by the high occurrence of this neoplasm in industries where the mineral was used. On the other hand, the dose–response relationship has no established threshold and exposures at home or in the neighbourhood of sources of asbestos environmental contamination entail a substantial risk of mesothelioma (Bignon *et al.*, 1989; Bourdès *et al.*, 2000). Only very limited data, however, exist on the mineral fibre content of the lungs in cases with

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environmental exposures (Magee *et al.*, 1986; Howel *et al.*, 1999).

According to the most recent statistics from the National Mesothelioma Registry (Marinaccio *et al.*, 2010), 4.7% of all cases in Italy from 1993 to 2004 were due to environmental exposures and almost 50% of them occurred in the neighbourhood of asbestos-cement (AC) plants. In Casale Monferrato (Piedmont) and Bari (Apulia), two large AC factories were active, from 1907 to 1986 and from 1934 to 1989, respectively. Epidemiological studies showed that a large number of cases occurred among subjects never employed in AC production and the mesothelioma risk increased with residential proximity to the local AC plant (Maule *et al.*, 2007; Musti *et al.*, 2009).

Asbestos fibre concentration in ambient air has been inadequately measured in both areas, as the few available data were collected when production had been reduced or after it stopped (Marconi *et al.*, 1989; Chiappino *et al.*, 1991). Furthermore, only one study provides information on the lung fibre burden in deceased AC workers and members of the general population (Magnani *et al.*, 1998).

The lung fibre burden can be estimated with well-established techniques (De Vuyst *et al.*, 1998) and is considered an index of the cumulative dose of asbestos. Cut-off values suggesting occupational exposure have been proposed (Henderson *et al.*, 1997). A positive relationship between the lung fibre burden and indices of external occupational exposure was found in several studies on workers exposed to asbestos and in population-based case–control studies (Gylseth *et al.*, 1981b; Mowé *et al.*, 1985; Wagner *et al.*, 1988; Tuomi *et al.*, 1991; Takahashi *et al.*, 1994).

The Mesothelioma Registries of the Brescia Province and Veneto Region currently retrieve on an *ad hoc* basis lung tissue samples from individuals affected by mesothelioma undergoing chest surgery or examined 'post-mortem'. We report on the lung fibre burden of patients who lived in Casale Monferrato or Bari, where they could have incurred environmental exposures, on the relationship with indices of cumulative external exposure to asbestos that we developed *ad hoc* and on the comparison with previous findings on workers employed in various industrial settings. The study was approved by the Institutional review board of the Brescia Local Health Authority.

SUBJECTS AND METHODS

Case finding

The Brescia and Veneto mesothelioma registries contributed the cases for this study, who were included in their records after they had been treated in hospitals located in Brescia or in Veneto, well outside the areas and regions of exposure, either because they had changed their residence or because they had sought advice and care in these hospitals. The Brescia and Veneto mesothelioma registries are part of the network of the Italian National Mesothelioma Registry, established in agreement with the 1991 Italian regulation on the protection of workers exposed to asbestos. They conform, therefore, to the rules established by the national guidelines to register incident cases and to investigate and assess asbestos exposures (Nesti *et al.*, 2003).

In addition to the basic exposure assessment procedure, lung tissue samples from individuals for whom surgery (pleuropneumonectomy) or post-mortem examination has been carried out are collected, stored in formaldehyde solution, and sent to a single laboratory for lung fibre count and characterization.

All the malignant mesothelioma cases included in this study arose in the pleurae and were confirmed by histological examination of neoplastic tissues, completed with immunohistochemical staining.

Personal history

Lifelong occupational and residential histories had been registered during face-to-face interviews, conducted by trained interviewers using the questionnaire of the National Mesothelioma Registry (Nesti *et al.*, 2003). During interview, a detailed description of all jobs and places of residence is obtained, including the neighbourhoods of each dwelling. The questionnaire allows information on other circumstances of exposure to asbestos, such as the presence of asbestos-containing materials at home and the occupations of persons with whom the patients lived, to be collected as well.

Exposure assessment

The likelihood of occupational exposure to asbestos had been assessed by raters with specific knowledge of the past local asbestos industries. Exposure was classified as 'definite', 'probable', and 'possible' (Nesti *et al.*, 2003).

The present study includes only cases with no definite or probable occupational exposure to asbestos, who lived at least 1 year in Casale Monferrato or Bari.

The AC plants in Casale Monferrato and Bari

The 'Eternit' plant in Casale Monferrato was the first and largest AC factory in Italy, active from 1907 to 1986. It employed up to 1500 workers (Maule *et al.*, 2007). Corrugated sheets, pipes, and

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pressure pipes were produced, using mixtures of commercial types of asbestos, including chrysotile, crocidolite (~10% of all asbestos), and amosite (in small amounts and for one specific product only). The plant was in the outskirts of the town, that reached 40 000 inhabitants in the 1980s, and lay at a distance of 500–2000 m downwind, with a quarter considerably closer. The warehouse where all products were stocked before shipping was located on the opposite side of the town, close to the railway station. Airborne asbestos fibre concentration in Casale Monferrato was measured only a few months before production stopped or after the factory was shut down. In 1984, when the number of employed workers had dropped to ~300 from a previous maximum of ~1500, concentrations ranging from 1 to 11 asbestos fibres >5 μm per litre (f/l) were found by scanning electron microscope analysis of samples collected at various distances, up- and downwind of Eternit, with 4- to 8-h sampling times (Marconi *et al.*, 1989). The highest value was observed close to the plant, and the lowest in the city area farthest away from it. Measurements in transmission electron microscope on samples of ambient air obtained in 1990–1991 in residential areas of the town, with sampling times as long as 3 weeks, were still in the range of 2.2–7.4 f/l for fibres >5 μm (Chiappino *et al.*, 1991).

The 'Fibronit' factory in Bari was active from 1934 to 1989, produced sheets and pipes and employed up to 500 workers. It was located in a popular district (the Japigia quarter of Bari) that experienced a huge growth in inhabitants after World War II, with apartment buildings being risen in close proximity of the plant since the 1950s. A mixture of commercial asbestos fibres was used, with chrysotile representing ~80%, crocidolite 15%, and amosite 5%. Fibre measurements are not available, but in the mid-1970s, the concentration in ambient air of particles (<5 μm in size) averaged 1.6 l^{-1} close to the plant (Musti *et al.*, 2009).

The two factories had some common features: important amounts of crocidolite were used, production leftovers were abandoned in uncontrolled dumping sites within the towns' borders or given away to everyone who could be interested in re-using them, and a large excess in mortality from malignant mesothelioma was observed in members of the general population never employed at either plant, with the excess decreasing according to residential distance from the factories (Maule *et al.*, 2007; Musti *et al.*, 2009). Furthermore, no major remediation action had been undertaken up to 2008, when in Casale Monferrato, a programme to remove contamination from the former industrial premises and from the waste dumping sites was started. In Bari, after a first provisional intervention in 2009, a remediation project is due to the start in 2011.

Development of ad hoc exposure indices

Semi-quantitative indices of asbestos exposure intensity have been developed for the present study, assuming that intensity of exposure was proportional to contamination of the environment surrounding the factory and that contamination decreased with distance. They are based, thus, on the distance between places of residence and AC plants. Distance was categorized in bands (0–499, 500–999, 1000–1499, 1500–1999, and ≥2000 m). The first index (Index 1) assumes that contamination in each band was the base 10 logarithm of that in the previous closer band (i.e. 1000, 100, 10, 1, and 0.1, respectively), whereas the second index (Index 2) assumes that it was its square root (i.e. 256, 16, 4, 2, and 1.41, respectively).

In addition, quantitative exposure assessment was carried out (Index 3). To this purpose, asbestos fibre concentration in the ambient air was assumed to decrease around the Eternit factory in Casale Monferrato from 100 f/l to 10, 3, 1, and 0.1 in the distance bands, respectively, of 0–499, 500–999, 1000–1499, 1500–1999, and ≥2000 m. The starting value, applied to the closest band, is one order of magnitude larger than that actually measured by Marconi *et al.* (1989) and was chosen considering that when the measurement campaign was conducted, production at Eternit had dropped to a fraction of its maximum value. The same pattern of concentrations was estimated to have occurred at considerably closer distances from the Fibronit factory in Bari and the Eternit warehouse in Casale Monferrato, i.e. at 0–99, 100–299, 300–599, 600–999, and ≥1000 m.

To obtain the individual cumulative exposure index, cumulative exposure estimates (in f/l × y) were first calculated for all residence periods of an individual: the concentration (in f/l) applying to the appropriate combination of source and distance band was multiplied by the corresponding duration of residence (in years). Such estimates were then summed up across all places of residence for every case, disregarding the 10 years preceding diagnosis.

Lung fibre burden measurement

Wet (formalin-fixed) lung tissue samples were freeze dried to avoid shrinkage of the tissue during the dehydration process (Manke *et al.*, 1987). About

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50 mg of freeze-dried lung were completely ashed at low temperature with an oxygen plasma asher (Emitech K1050X) to remove organic matter. The forward power of the plasma asher was kept to a maximum of 100 W to limit possible breakage of asbestos bundles and fibres during the ashing process (Gylseth *et al.*, 1981a). The ash was suspended in a solution made of 20 ml distilled water, 20 ml isopropyl alcohol, and 20 ml hexane; vigorously shaken; and the solution was filtered on a polycarbonate filter (0.2-μm pore size). The use of ultrasound was avoided to prevent fibre breakage. The filter was completely ashed at low temperature (at the same power settings as above) and the ash was suspended in 48 ml distilled water plus 2 ml ethyl alcohol. Two polycarbonate (0.2-μm pore size) filters were then prepared, the first with 10 ml and the second with 20 ml of solution. The filter with the best load of particles was used for the analysis (De Vuyst *et al.*, 1998; Wang *et al.*, 2000; Somigliana *et al.*, 2008). Analysis was carried out using a scanning electron microscope equipped with X-ray fluorescence microanalyzer at ×12 000 magnification (Leica Stereoscan 420 + Oxford PentaFETx3 and ZEISS EVO 40 + Oxford XMAX). Fibres were counted and measured when corresponding to the following criteria: (i) length > 1 μm, diameter < 3 μm, and aspect ratio $\geq 3{:}1$ and (ii) identification as commercial asbestos (amosite, crocidolite, and chrysotile) or as tremolite structures. With our microanalysis systems, fibres with diameter >0.13 μm were clearly identifiable, while fibres with diameter <0.05 μm were not identifiable and were not counted. Fibres with diameter between 0.05 and 0.13 μm had an X-ray signal-to-noise ratio too low to be distinguished over the background for the peaks of Na and Mg. In this class of fibre diameter, we could not, therefore, discriminate between the two main types of commercial amphibole asbestos (amosite and crocidolite). For this reason, we always grouped as commercial amphibole asbestos all counts of crocidolite or amosite. We separately counted elongated fibre-like tremolite structures and included this count in our estimation of the lung fibre burden because we had no possibility to distinguish cleavage fragments from tremolite asbestos based on microscopy alone. On the other hand, environmental sources of tremolite in Italy are known to be confined to specific areas in the Alps and Apennines, none of which is close to Casale Monferrato or Bari (Pasetto *et al.*, 2004). We believe, therefore, that the most important source of tremolite structures in our case series was tremolite contamination of the chrysotile asbestos used in the two AC plants.

Further details of the analytical technique are given in a supplementary data, available at *Annals of Occupational Hygiene* online.

In Fig. 1, two examples of commercial asbestos amphibole fibres, found in one of the cases in the present series, are shown together with their X-rays' spectra. Panel A shows a crocidolite fibre with 0.3 μm diameter, clearly identifiable by the X-ray spectrum in Panel B. Panel C shows an amphibole fibre of 0.12 μm width. Its X-ray spectrum (Panel D) does not allow to distinguish between crocidolite and amosite.

Fibre concentration is reported per gram of dry lung tissue (f/g), with 95% confidence interval (CI) and the detection limit (DL). The DL was defined as the upper boundary of the 95% CI when no fibre was found. When fibres are found, the DL is an indicator of the analytical sensitivity (ISO/FDIS 14966:2002, 2002).

The analyst had no knowledge of the exposure histories of cases.

Other (occupational) exposure groups

From the files of the Brescia and Veneto registries, mesothelioma cases with already examined lung specimens and with occupational exposure were identified. Occupational exposures had occurred during employment in asbestos works (in the following text: asbestos industries) or in industries where asbestos-containing materials had been used (non-asbestos industries), as reported in previous papers (Barbieri *et al.*, 2008, 2010a,b; Merler *et al.*, 2009). The original data will be used here to compare with findings among the present study subjects.

Reference group

As an internal reference group, the same centre analysed the fibre burden in lung tissue samples obtained at autopsy in 13 individuals with heart disease who died suddenly as inpatients at the Spedali Civili of Brescia, i.e. the largest (and the only teaching) hospital in Brescia province. These subjects represent a reasonable comparison group as they had not been diagnosed with any asbestos-related disease and are not expected to have had any specific pattern of occupational or non-occupational exposure to asbestos. Their exposure histories were collected through interviews with next of kin.

Statistical analysis

Linear regression models were fitted with each of the semi-quantitative and quantitative cumulative exposure indices as explanatory variable and the

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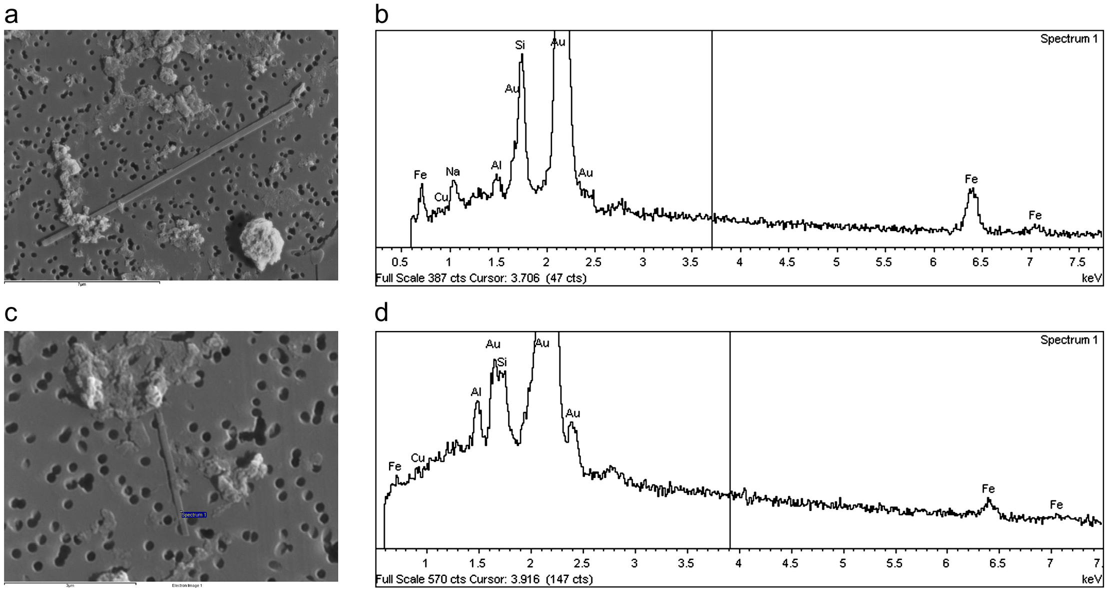

Fig. 1. (A) Microphotograph in scanning electron microscopy at ×12 000 magnification of a commercial asbestos amphibole fibre (diameter ≈ 0.3 μm). (B) X-ray spectrum of the fibre in (A). Presence of a detectable Na peak, identifying the fibre as crocidolite asbestos. (C) Microphotograph in scanning electron microscopy at ×12 000 magnification of a commercial asbestos amphibole (diameter ≈0.11 μm). (D) X-ray spectrum of the thin fibre in (C). The Na and Mg peaks are not detectable over the background, preventing the possibility to distinguish amosite from crocidolite.

lung fibre burden as outcome variable. Analysis of variance was used to examine differences in the distribution of lung fibre burden by exposure group. All analyses were carried out with the statistical software package Stata 9.2 (Stata 9.2, 2007). Statistical significance level for testing was set at 95%, and 95% CIs were computed.

RESULTS

Eight mesothelioma cases (five men and three women) fulfilled all inclusion criteria. Their lung tissue samples came from pleuropneumonectomies carried out at the Chest Surgery Units of the Spedali Civili of Brescia (six cases), Ospedale dell'Angelo of Venice, Mestre (one case), and Azienda Ospedaliera of Padua (one case).

Their clinical features are summarized in Table 1, along with residential histories, exposure indices, and lung fibre burden.

Seven cases had an epithelial and one a biphasic form of mesothelioma. The mean age at diagnosis was 54.0 years (±10.4 SD). The personal history showed that they were never employed in asbestos factories. Other circumstances of definite or probable exposure at work could be ruled out as well.

The five patients who had lived in Bari had seven residence periods, at distances ranging from 200 to 2000 m from the Fibronit plant, whereas the three patients who inhabited Casale Monferrato had eight residence periods in town, at distances ranging from 300 to 1800 m from the Eternit factory or warehouse. It seems relevant to report that the mother of Case number 4, who shared period and place of residence in Bari with her son, was diagnosed in 2004 with pleural mesothelioma; she had no other exposure circumstance.

The lung tissue samples used to measure the burden of asbestos fibres had been taken 10–46 years (mean: 22.2, 95% CI: 10.7–33.8) after the last residential exposure, and the analyses were conducted 12–46 years (mean: 24.1, 95% CI: 13.4–34.9) after exposure ceased.

A detectable amount of measurable asbestos fibres was present in all cases, and the concentration ranged from 110 000 to 4 300 000 f/g. It was >1 000 000 f/g, i.e. the cut-off value suggested to ordinarily distinguish individuals with occupational exposure to asbestos, in three cases and the 95% CI included 1 000 000 in further three cases. Amphibole fibres were the predominant (in three cases) or exclusive (in four cases) fibres found.

The geometric mean of fibre diameters varied from 0.12 to 0.24 μm and that of lengths from 2.1 to 3.5 μm.

A linear relationship was observed between the lung fibre burden and all indices of environmental

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Table 1. Pleural mesothelioma patients and results on asbestos lung fibre burden. Asbestos fibres, concentration per gram of dry lung tissue.

ID	Gender, age,[a] type	Year of diagnosis and analysis	Periods of residence and duration (years)		Distance from the plant (m)	Index 1, Index 2, Index 3[b]	Other asbestos exposures	Lung fibre burden				
								Fibre count (95% CI)	DL	Fibre type (%)	Dia, μm	Len, μm
Bari—Fibronit (1934–1989)												
1	F, 51, epithelial	2005 and 2007	1960–1977 and 1984–1991	17 and 7	500, 1000	177.0, 30.0, 51.7	None identified	2 300 000 (1 330 000–3 670 000)	400 000	A (100)	0.12	3.2
2	M, 36, epithelial	2005 and 2008	1969–1976	7	2000	0.1, 1.0, 0.7	None identified	110 000 (0–630 000)	330 000	T (one fibre)	nd	nd
3	M, 53, biphasic	2009 and 2009	1967–1971	4	200	400.0, 102.0, 40.0	None identified	1 700 000 (890 000–2 860 000)	302 000	A (85), C (15)	0.24	3.5
4	F, 56, epithelial	2009 and 2009	1979–1990	11	1500	1.1, 2.2, 1.1	1979–1992: use of blanket on the ironing table, possibly made of asbestos-containing textiles	160 000 (30 000–760 000)	315 000	A (67), C (33)	nd	nd
5	M, 65, epithelial	2007 and 2009	1970–1994 and 1995–1997	24 and 2	1000, 2000	24.0, 10.0, 2.7	1983–1997: pastry cook, possible presence of asbestos-containing oven insulation	390 000 (80 000–1 140 000)	308 000	A (100)	0.21	3.5
Casale Monferrato—Eternit (1907–1986)												
6	M, 56, epithelial	2006 and 2008	1950–1955, 1955–1980, 1980–1990[c], 1991–1996	5, 25, 10, 5	800, 1200, 300, 900	1135.0, 283.6, 155.6	None identified	4 300 000 (2 750 000–6 180 000)	480 000	A (100)	0.15	3.3
7	M, 46, epithelial	2003 and 2009	1962–1967, 1967–1970[c], 1970–1989, 1989–1991	5, 3, 19, 3	1100, 300, 1800, 1800	307.1, 83.1, 44.2	1957–1991: father, possible exposure as a car repairer (asbestos-containing brake and clutch linings)	610 000 (200 000–1 410 000)	360 000	A (100)	0.16	3.2
8	F, 69, epithelial	2010 and 2010	1941–1956	15	1000	15.0, 6.0, 45.0	1964–1967: husband, possible exposure as an air forces jet pilot (fireproof overalls)	860 000 (430 000–1 530 000)	180 000	A (77), T (23)	0.22	2.1

Dia, geometric mean of diameter; Len, geometric mean of length; nd, not determined; A, commercial asbestos amphiboles (amosite/crocidolite); C, chrysotile; T, non-commercial asbestos (tremolite).
[a]At diagnosis.
[b]Semi-quantitative indices (Index 1, Index 2), quantitative index (f/l × y) (Index 3). See also text for explanation.
[c]Distance from the Eternit warehouse, not from the main plant.

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cumulative exposure (Table 2). The best fit was observed for the model using as a predictor the quantitative index, i.e. Index 3 in Table 2, with the asbestos fibre burden increasing by 26 700 f/g per cumulative exposure unit (in f/l × y).

We found (Table 3) significant differences in the lung fibre burden distribution of our study cases when compared on the one hand with mesothelioma cases characterized by past occupational exposures and on the other hand with the internal reference group of 13 individuals with heart disease who died suddenly. The average fibre burden among environmental cases was ~50% of the mean for mesothelioma cases with occupational exposure to asbestos in non-asbestos industries, and the range of fibre burden overlapped that of mesothelioma cases with occupational exposure in asbestos industries. The reference group had average fibre concentration equal to 166 000 ± 72 000 SD f/g (maximum value 360 000). The difference with the mean value for the cases here studied was statistically significant. No asbestos fibre was detected in seven members of this group, and in two members, only a single chrysotile fibre was identified; in the remaining subjects, at most two commercial amphibole asbestos fibres were found. Exposure to asbestos in this group could be assessed less satisfactorily than in the others as only proxy interviews could be carried out. In 3 of 13 subjects, information was not sufficient to evaluate the probability of exposure; in 3 of 10 of the remaining subjects, occupational exposure to asbestos was considered possible in various non-asbestos productions; no definite or probable exposure was identified.

DISCUSSION

Mesothelioma occurrence in the general population in Casale Monferrato and Bari has been the object of various epidemiological studies.

According to the mesothelioma registry of Piedmont, from 1980 to 2008 among inhabitants of Casale Monferrato, 454 incident cases were observed and 373 of them were never employed at Eternit. Interviews, conducted according to the national guidelines, were available for 304 cases and, according to the exposure assessment, for 102 (33.6%) of them, residence at various distances from the AC factory or its warehouse was the only identified exposure circumstance (D. Mirabelli, personal communication). In a population-based case–control study on pleural malignant mesothelioma, among members of the general population never exposed to asbestos at work, the risk of developing this malignancy was found to decrease according to the distance from the factory of the longest-held residence. The odds ratio (OR) was 10.5 (95% CI: 3.8–50.1) for living close to the factory boundary and was still over unity at 10 km (Maule *et al.*, 2007). A necropsy study was conducted analysing lung tissue samples from a series of consecutive post-mortem examinations carried out at the Santo Spirito Hospital in Casale Monferrato. The concentration of asbestos bodies—by light microscopy—and asbestos fibres—in transmission electron microscopy—was determined, and the presence and grade of asbestosis was histologically assessed. Among individuals never employed at Eternit, concentrations of asbestos bodies were significantly higher among those who lived in Casale Monferrato compared with inhabitants of the other, surrounding towns, and two cases of histological Grade 2 asbestosis were identified (Magnani *et al.*, 1998).

Among Bari inhabitants, 78 new mesothelioma cases occurred between 2000 and 2009, according to the Apulia mesothelioma registry, and 23 (29.5%) had been classified as having residence in proximity to the Fibronit plant as their only asbestos exposure (D. Cavone, personal communication). In a population-based case–control study conducted with an approach closely similar to that used in Casale Monferrato, the mesothelioma OR for people living at <500 m from the factory was 5.29 (95% CI: 1.18–23.74), levelling off at distances >1000 m (Musti *et al.*, 2009).

The overall evidence offered by these epidemiological studies, thus, is that environmental asbestos exposure was associated with mesothelioma, and the risk was related with residential distance from the AC factories.

The cases included in our study had lower ages at diagnosis compared with >9000 cases described in the Third Report of the National Mesothelioma Registry, who were on average 68.3 years old at diagnosis (Marinaccio *et al.*, 2010). This observation might suggest that exposures occurred at young ages, which would be expected for those born in these towns, although selection of younger patients for pleuropneumonectomy is another possible explanation.

How do the values we observed compare with those reported previously? Comparisons of analyses of lung fibre burden by different laboratories are difficult due to methodological differences in sample treatment, type of electron microscope, magnification, and counting rules (Dodson and Atkinson, 2006). In this discussion, we will therefore limit ourselves to analyses, reported in Table 3, on mesothelioma patients engaged in AC or asbestos textiles

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Table 2. Relationship between asbestos lung fibre burden and indices of environmental cumulative exposure. Linear regression models (see text for explanation of indices).

Model	*P* value	R^2	Intercept	Coefficient
1. Lung fibre (asbestos) burden versus semi-quantitative Index 1, logarithmic decay of contamination by increasing distance band	<0.01	0.81	443.0 (−268.9 to 1155.1)	3.3 (1.7–4.9)
2. Lung fibre (asbestos) burden versus semi-quantitative Index 2, square root decay of contamination by increasing distance band	<0.01	0.77	463.1 (−336.5 to 1262.6)	13.0 (5.8–20.2)
3. Lung fibre (asbestos) burden versus quantitative exposure assessment (in f/l × y) Index 3	<0.001	0.88	166 900 (−436 900 to 770 800)	26 700 (17 200–36 100)

production, as construction workers or employed in steel mills, carried out between 2000 and 2009 at the same laboratory by the same reader (Barbieri *et al.*, 2008, 2010a,b; Merler *et al.*, 2009).

Although the range of concentrations found in the lungs of workers with exposure to asbestos was wider, and average values were higher than in the present study, the distributions of values in occupationally and non-occupationally exposed persons partially overlapped. The reference group, which had only limited opportunities of exposure to asbestos, had a significantly lower lung fibre burden.

In three cases, we found concentrations >1 000 000 f/g of amphibole fibres with length >1 μm, a value proposed as a cut-off to identify subjects with an occupational exposure to asbestos, even when no evidence of such exposure is present in their histories (Henderson *et al.*, 1997).

It is also interesting that amphibole fibres were regularly found, whereas chrysotile was present in only two cases and even then it was not the predominant type of fibre. It is known that chrysotile is less persistent (Churg and Wright, 1994), so that the time elapsed between the end of the most recent exposure and the moment when lung tissue samples were taken could explain this finding, as in our case series, the tissue samples had been obtained on average 22.5 years after cessation of exposure. At the same time, as in both Eternit and Fibronit production, crocidolite was used, especially for pressure pipes, the predominant presence of 'amphiboles' we observed (no distinction between crocidolite and amosite was possible) may be a marker of environmental contamination from the two plants, as already pointed out in the necropsy study in Casale Monferrato (Magnani *et al.*, 1998). From this point of view, a role of generic environmental pollution in determining the lung fibre burden in the present case series is unlikely, as it would be constituted essentially by chrysotile fibres (Lim *et al.*, 2004).

Our main finding is the demonstration of a linear relationship between the lung fibre burden and indices of cumulative dose in individuals with environmental exposures in well-studied settings and without other definite or probable routes of exposure to asbestos. The model using the quantitative exposure assessment as predictive variable not only had the best model fit but also had the advantage of expressing the relationship as increase in lung fibre concentration (in f/g) per unit increase in cumulative exposure (in f/l × y).

The semi-quantitative indices are relatively crude, as they take into account only the residential distance from the sources, thus implying no difference in power among them. Nonetheless, they predicted satisfactorily the lung fibre burden and could be applied to other, reasonably similar industrial sources of environmental asbestos pollution to obtain an approximate exposure assessment when data on past asbestos concentrations are missing, as is often the case.

Considering the results on the relationship between lung fibre burden and the quantitative exposure assessment, the concentration of measurable asbestos fibres was estimated to increase by 26 700 f/g for a unit (1 f/l × y) increase in cumulative exposure. Given the clearance of asbestos fibres and the average 22.5 years lag between exposure cessation and tissue samples collection, during exposure or immediately after it, the lung fibre burden was most likely considerably higher.

All cases included in the present analysis had left Casale Monferrato or Bari at the time they were diagnosed with mesothelioma. The occurrence of malignant mesotheliomas in individuals who had moved from these towns is not surprising, given the induction–latency period of this neoplasm. The number of cases among residents observed by the Piedmont and, respectively, Apulia mesothelioma registries, thus, has been reduced by the mobility of the two

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Table 3. Lung asbestos fibre burden in workers exposed in various industries. Mean concentration, fibres per gram of dry lung tissue.

Industry (or setting) group	Cases	Diagnoses	Mean fibre burden (SD)	Minimum − maximum values	Type of fibres (% range)	Reference	*P* value for differences of means[a]
1. Sudden death as inpatient (reference group)	13	Heart diseases	166 000 (72 000)	−360 000	—	This study	<0.01 versus Group 2; <0.01 versus Group 3; <0.001 versus Group 4
2. Environmental exposure (this study)	8	MM	1 300 000 (1 400 000)	110 000 − 4 300 000	A (67–100), C (15–33), T (nd–23)	This study	NS versus Group 3; <0.001 versus Group 4
3. Asbestos exposure in non-asbestos industries[b]	24	MM	2 800 000 (3 500 000)	260 000 − 12 000 000	A (7–100), C (nd–93), T (nd–50)	Barbieri *et al*., 2010a,b; Merler *et al*., 2009	<0.001 versus Group 4
4. Asbestos industries[c]	9	MM	136 400 000 (101 400 000)	3 500 000 − 286 000 000	A (60–100), C (5–45), T (nd)	Barbieri *et al*., 2008, 2010a	—

MM, malignant mesothelioma; A, commercial asbestos amphiboles (amosite/crocidolite); C, chrysotile; T, non-commercial asbestos (tremolite); nd, not detected.
[a]*P* value <0.0001 for the analysis of variance by exposure group.
[b]Textiles (non-asbestos), steel mills, and construction industry.
[c]Asbestos cement and asbestos textiles.

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populations and any assessment of the overall burden of cancers attributable to environmental exposures to asbestos based on incidence data in these areas should be assumed to be incomplete.

CONCLUSIONS

In the absence of systematic measurements of asbestos fibre concentrations in the ambient air of Casale Monferrato and Bari when the two factories were fully active, our results provide information on past exposure to asbestos associated with contamination of the urban environment. In addition, this case series represents a relatively large data set compared with published data on fibre burden in mesothelioma cases with environmental exposures and has the advantage of including an accurate reconstruction of exposure circumstances.

While environmental exposure, as a consequence of the type of fibres used by the two factories, was to a mixture of asbestos fibres, predominantly amphiboles were detected in the lungs of patients, due, we presume, to the slower lung clearance of amphiboles.

The asbestos fibre burden in lung tissues was strictly related with indices of cumulative environmental exposure to asbestos. It was higher on average than in the reference group and partially overlapping with the values we observed in groups with occupational exposure. This finding suggests that environmental exposures have been substantial in the populations living around these two plants and is in agreement with the epidemiological evidence of a marked increase in mesothelioma risk.

SUPPLEMENTARY DATA

Supplementary data can be found at http://annhyg.oxfordjournals.org/.

Acknowledgements—We are indebted with all colleagues who assisted us in data collection and in particular with Dr A. Tironi, Department of Pathology and University of Brescia; Dr B. Murer, Department of Pathology, Ospedale dell'Angelo Mestre; Dr A. Galligioni, Department of Pathology, University of Padua; Dr. F. Gioffrè, Occupational Health Unit, Department of Prevention, Padua; and Dr. E. Romeo, Department of Epidemiology, Local Health Authority Roma E. Two anonymous reviewers helped us improve considerably this article and we want to express them our gratitude for the attention they dedicated to our work.

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