Eur Respir J,
1988, 1, 818-822

Electron microscopic analysis of asbestos body cores from the Belgian urban population

E. Moulin, N. Yourassowsky, P. Dumortier, P. De Vuyst, J.C. Yernault

Electron microscopic analysis of asbestos body cores from the Belgian urban population. E. Moulin, N. Yourassowsky, P. Dumortier, P. De Vuyst, J.C. Yernault.

ABSTRACT: Typical ferruginous bodies considered as asbestos bodies (AB) were collected from the lungs of 19 asbestos-exposed and 25 non-exposed urban subjects. Of the 319 body cores analysed by energy dispersive spectrometry (EDS), 315 were asbestos. The non-asbestos cores were talc and crystalline silica. 89.2% of the asbestos cores were commercial amphiboles (amosite/crocidolite), 7% were chrysotile and 3.8% were non-commercial amphiboles (anthophyllite/tremolite). The commercial amphibole bodies were found in exposed and non-exposed subjects and chrysotile bodies mostly in exposed subjects. The non-commercial amphibole bodies were detected in non-exposed patients with low lung AB levels; this background contamination would be more difficult to detect in lungs containing large amounts of bodies due to occupational exposure. Chrysotile bodies and tremolite/anthophyllite bodies were not observed together. We suggest that in Belgium the source of non-commercial amphiboles exposure is not contamination by chrysotile.

Eur Respir J., 1988, 1, 818–822.

Research Unit on Toxicity of Mineral Particles, Chest Department, Erasme Hospital, Université Libre de Bruxelles, Belgium.

Correspondence: E. Moulin, Chest Department, Erasme Hospital, 808 route de Lennik, 1070 Brussels, Belgium.

Keywords: Asbestos bodies; human lung tissue; X-ray analysis.

Received: January 1988; Accepted after revision May 25, 1988.

Asbestos fibres coated with ferroprotein are referred to as asbestos bodies [1]. In light microscopy, these structures have thin straight transparent central cores with a regularly segmented or continuous yellow to brown coating [2, 3]. These typical asbestos bodies (AB) can usually be distinguished in light microscopy from the ferruginous bodies built on non-asbestos cores such as coal, talc or other material, which have brown to black cores or broad yellow cores, usually with an irregular coating [3].

Even though AB represent a small proportion of the total fibre burden of the lungs (1%) [4], counting them by light microscopy in appropriate lung tissue digestates allows a rough evaluation of occupational, as well as environmental, exposure to long asbestos fibres [5–8]. In this connection, a previous report from our laboratory [9] confirms their presence in virtually all the lungs from exposed and unexposed adults in the Belgian urban population.

The aim of the present study was to confirm that the central core of what we considered as a typical AB is in fact asbestos. Another purpose was to examine whether any difference in the central core type exists between subjects with occupational exposure, and between these subjects and people submitted only to environmental contamination. Of the six naturally occurring asbestos types, chrysotile, crocidolite and amosite are, from an industrial point of view, the most important source of both environmental and occupational exposure. The remaining three types, tremolite, actinolite and anthophyllite, are of little economic importance but can be contaminants of other minerals such as talc and chrysotile [10]. Although chrysotile can become coated in laboratory animals [11] and can be found in this form in chrysotile miners with asbestos [12], it has been pointed out that AB extracted from human lungs usually have amphibole and only rarely chrysotile cores [6, 7, 13, 14]. Therefore, we examined by electron microscopy the central core of 376 AB collected from the lungs of 44 subjects for whom total lung AB content was known.

Materials and methods

Subject selection

AB were collected from autopsy and surgical lung specimens. The specimens were selected on the basis of the presence or absence of asbestos exposure according to an occupational questionnaire. Occupational histories were obtained by direct questioning for surgical patients and by medical records and interviews with relatives of autopsy subjects (table 1).

A group of 19 subjects (all males; age range 45–82 yrs; mean 61.7 yrs) were selected, who had known occupational exposure to asbestos other than in chrysotile mining, milling and brake-lining factories. AB

Table 1. – Subject data

Case number	Autop/ Surg	Smoke pk yr	Age yr	Sex	Occupation		AB·g^{-1} dry lung Mean
Exposed group							
1	S	40	60	M	Roadwork foreman.		21,561
2	S	26	46	M	Industrial furnace worker.		11,376
3	S	42	66	M	Industrial furnace worker.		33,819
4	S	30	62	M	Heating mechanic.		39,628
5	S	65	61	M	Refractory material worker.		10,128
6	S	30	58	M	Iron foundry worker.		6,520
7	S	60	69	M	Heating mechanic.		9,738
8	S	30	45	M	Heating mechanic.		9,135
9	S	40	60	M	Iron foundry worker.		5,018
10	S	43	66	M	Heating mechanic.		5,700
11	S	20	65	M	Garage mechanic.		5,997
12	A	smoker	66	M	Decorator.		9,381
13	A	smoker	82	M	Plumber.		8,150
14	A	38	56	M	Dental technician.		49,162
15	S	53	70	M	Thermal assay worker.		1,044
16	S	37	66	M	Welder.		1,018
17	S	45	51	M	Thermal assay worker.		1,161
18	S	36	62	M	Coachwork mechanic.		1,260
19	S	34	61	M	Refractory material worker.		3,350
Non-exposed group							
20	S	40	61	M	Truck driver.	BC	28,070
21	S	44	72	M	Plasterer.	BC	31,648
22	S	40	73	M	Administrative empl.	WC	10,786
23	S	50	69	M	Bailiff.	WC	7,551
24	S	34	65	M	Storekeeper.	BC	4,364
25	S	30	74	M	Earthenware worker.	BC	6,110
26	S	36	47	M	Roadworker.	BC	2,896
27	S	25	70	M	Storekeeper.	BC	1,180
28	S	68	54	M	Painter.	BC	2,409
29	S	60	56	M	Decorator.	BC	870
30	S	30	72	M	Construction worker.	BC	1,618
31	S	40	59	M	Administrative empl.	WC	2,334
32	S	44	59	M	Lawyer.	WC	1,390
33	S	70	71	M	Administrative empl.	WC	2,250
34	S	40	63	M	Insurance empl.	WC	956
35	S	32	72	M	Caterer.	WC	812
36	A	65	65	M	Truck driver.	BC	1,777
37	A	65	56	M	Truck driver.	BC	1,304
38	A	none	75	M	Typist.	BC	1,378
39	A	none	50	M	Taxi driver.	BC	3,985
40	A	smoker	62	F	Safety belt factory w.	BC	1,910
41	A	none	74	M	Hairdresser.	WC	407
42	A	smoker	75	F	Nurse.	WC	3,167
43	A	none	88	F	Housewife.	WC	2,169
44	A	smoker	85	M	Bailiff.	WC	1,850

BC: blue collar worker; WC: white collar worker; S: surgical specimen; A: autopsy; AB: asbestos body

concentrations, expressed per gram of dry lung tissue, had been determined by light microscopy in other studies from our laboratory [9]. The AB counts ranged from 1,018–49,162 (mean 12,270) AB·g^{-1}. Five subjects had a mean lung content lower than 4,335 AB·$^{-1}$, a value that was calculated in our laboratory as an upper limit of non-specific exposure (unpublished data).

A group of 25 unexposed subjects (3 females, 22 males; age range 47–88 yrs; mean 66.8 yrs) were also selected. Of these 14 were blue collar (BC) workers and 11 white collar (WC) workers. The AB counts ranged from 407–31,648 (mean 4,959) AB·g^{-1}. Nineteen subjects had a mean lung content lower than 4,335AB·g^{-1}.

Preparation of AB for electron microscopic analyses

We used lung filter preparations mounted on a glass slide for light microscopy counting. The technical pro-

cedure used to collect AB for light microscopy counts has been extensively described previously [9].

For the subjects with more than 4,335 AB·g^{-1}, small squares (2 × 2 mm) of filter were cut out, carbon coated and mounted on electron microscope grids. The filter was then dissolved in acetone, leaving the carbon film with embedded particles on the grid. By this method, a sufficient number of AB could be randomly detected on the grids.

For the subjects with low concentrations (<4,335 AB·g^{-1}), because of the relative paucity of AB on the filter, the positions of the "typical" bodies had first to be localized and pointed on the filter with a high precision object marker before cutting [15].

Electron microscopic analyses

The analyses were performed using a Philips EM400T fitted with an Edax PV9900 energy dispersive spectrometry (EDS) system. For each subject, 4–20 body cores were examined. Differentiation of central asbestos fibres from other fibrous particles and identification of asbestos type were made by integrating all information available from morphological observations, electron diffraction and microchemical analysis.

AB were detected by inspection of the transmission image at a magnification of ×4,600 and morphological characters were evaluated at a magnification of × 28,000.

If possible, areas of the cores without a coating were selected for the chemical analysis to minimize the contribution of the ferroprotein to the content of the spectra.

UICC standard asbestos reference samples (anthophyllite, amosite, chrysotile, crocidolite) and the University of Liège tremolite sample number 6801 were used to provide reference EDS spectra and selected area electron diffraction (SAED) patterns. Each chemical analysis was performed using an 80 kV accelerating voltage and an acquisition time of 4–100 s. A thick condenser aperture and a beryllium holder were selected to minimize the background content of the spectra [16], usually allowing the detection of sodium and manganese peak values, so that amosite and crocidolite could be distinguished in most cases (>90%).

In addition, SAED was used to distinguish chrysotile from anthophyllite and to visually assess the crystallinity of non-asbestos cores when encountered.

Statistical analysis

The Chi-squared test was used to compare the proportions of commercial amphibole, non-commercial amphibole and chrysotile cores analysed between the two series of subjects.

Results

Among 376 bodies found by inspection of the transmission image, microchemical analysis was possible for 319 (84.8%). For the remaining 57 bodies, a too complete ferroprotein coating did not permit chemical analysis (table 2).

Table 2. – Comparison of the proportions of asbestos core types between the two groups of subjects

	Exposed group (n=19)	Non-exposed group (n=25)
Commercial amphiboles	141 (n=19) (89.2%)	140 (n=24) (89.2%)
Chrysotile (*)	17 (n=8) (10.8%)	5 (n=3) (3.2%)
Non-commercial (**)	0	12 (n=7) (7.6%)

χ^2 test between the 2 groups; *: χ^2=5.22(p<0.025); **: χ^2=6.32 (p<0.01)

3–18 bodies (median 8) were identified for each subject in the exposed series and 3–11 bodies (median 6) in the non-exposed series.

Of the 319 body cores identified, 315 (98.8%) were asbestos fibres. The non-asbestos cores, detected in four subjects, were consistent with crystalline silica fibres in three cases and an elongated talc platelet in one case.

Commercial amphiboles were the major core material identified (89.2%) with 63% of bodies composed of amosite, 19.4% of crocidolite and 6.8% amosite/crocidolite, respectively (when sodium and manganese

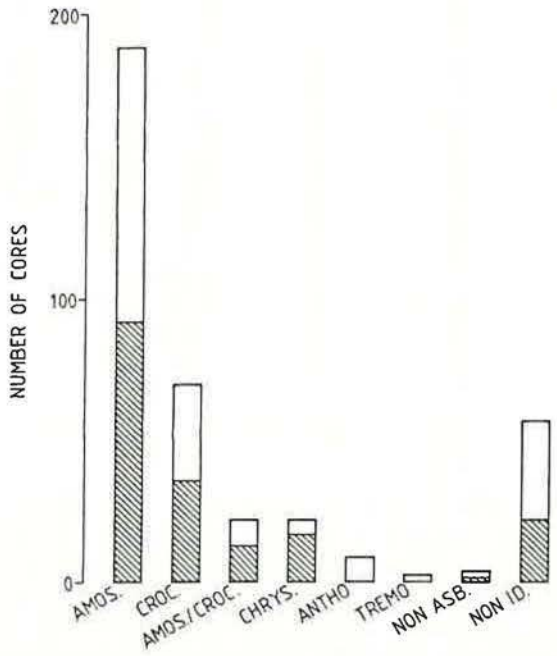

Fig. 1. – Type and number of body cores detected in exposed subjects and non-exposed subjects. Hatched areas represent the number of body cores in exposed subjects. White areas represent the number of body cores in non-exposed subjects.

peak values were not easily discerned). Chrysotile represented 7% of the remaining cores and the non-commercial amphiboles, anthophyllite and tremolite 2.8% and 1%, respectively (fig. 1).

The commercial amphibole cores were found in all but one case (a non-exposed subject). In addition, chrysotile bodies were detected in eight exposed and three non-exposed subjects. The non-commercial amphibole bodies were found in seven non-exposed subjects, including the three women.

In table 2 the statistical comparison of the proportions of these three asbestos core types between the two series of subjects is given. On the one hand, no significant difference was observed (χ^2=0.78) for the proportions of commercial amphibole cores between the two groups. On the other hand, a significant difference was found for the proportions of chrysotile cores (χ^2=5.22; $p<0.025$) and for the proportions of non-commercial amphibole cores (χ^2=6.32; $p<0.01$).

Discussion

Our study has confirmed that the central fibre of the ferruginous bodies in our population that we considered by light microscopy as typical asbestos bodies are true asbestos in 98.8% of the cores. The non-asbestos cores are rare in these typical bodies.The vast majority of the cores encountered are the two commercial amphiboles, amosite and crocidolite with a predominance of the former. Therefore, AB counts by light microscopy may be considered as a marker to evaluate the commercial amphibole burden in our series of exposed and non-exposed subjects. Indeed, high values (up to 30,000 $AB \cdot g^{-1}$) found in non-exposed subjects obviously reflect ignored, indirect or forgotten exposures.

Our results indicate a trend to detection of only a small number of chrysotile bodies. As indicated in reports from other countries [5–7, 13, 14], the relatively small number of chrysotile bodies, despite chrysotile being the commercial asbestos most often used, can be related to the high clearance rate of chrysotile or to its presence in the form of fibres too small to form AB.

Small numbers of anthophyllite and tremolite bodies were detected in some unexposed subjects (33% of this series) with a lung mean AB content less than 4,335 $AB \cdot g^{-1}$.

If one hypothesizes that the non-commercial amphiboles represent part of the non-occupational background contamination, roughly the same in every urban dweller, it is more likely that these types of bodies will be detected in people with low concentrations in which they will constitute a relatively higher proportion of the AB varieties present. If this hypothesis is true, this background contamination would be more difficult to demonstrate in lungs containing very large amounts of AB built on commercial varieties due to heavy occupational exposure.

No association of chrysotile bodies and anthophyllite-tremolite bodies was observed. One may suggest that the source of the non-commercial amphibole exposure does not originate from contamination by chrysotile. However, asbestos bodies form readily on long fibres present in the lung [5–6, 7, 12]. In this connection, in people with occupational exposure to chrysotile, in which a relatively high number of long chrysotile fibres can be found [12], asbestos bodies are frequently formed on this asbestos type. The lungs of the general population contain essentially short fibres of chrysotile [17]. Thus, one can also make the hypothesis of possible environmental pollution by long fibres of amphibole and small fibres of chrysotile. This could lead to an inhalation of long tremolite or anthophyllite fibres together with small chrysotile fibres, so that only AB based on amphibole could be detected. These hypothesesremain, however, to be confirmed by further studies.

Acknowledgements: We sincerely thank A. Van Muylem for his help in the statistical interpretation of the data. We also thank P.Vanderhoeft for reviewing the manuscript and Miss M. Onrubia for typing it.

References

1. Pooley FD. – Asbestos bodies, their formation, composition and character. *Environ Res*, 1972, 5, 363–379.
2. Churg AM, Warnock ML. – Asbestos bodies and other ferruginous bodies, their formation and clinical significance. *Am J Pathol*, 1981, 102, 447–456.
3. Churg AM, Warnock ML, Green N. – Analysis of the cores of ferruginous (asbestos) bodies from the general population. II. True asbestos bodies and pseudoasbestos bodies. *Lab Invest*, 1979, 40, 31–38.
4. Davis JMG, Gylseth B, Morgan A. – Assessment of mineral fibers from human lung tissue. *Thorax*, 1986, 41, 167–175.
5. Churg AM, Warnock ML. – Asbestos fibers in the general population. *Am Rev Respir Dis*, 1980, 122, 69–78.
6. Churg AM, Warnock ML. – Analysis of the cores of asbestos bodies from members of the general population. Patients with probable low-degree exposure to asbestos. *Am Rev Respir Dis*, 1970, 120, 781–786.
7. Roggli VL, Pratt PC, Brody AR. – Asbestos content of lung tissue in asbestos associated diseases: a study of 110 cases. *Br J Ind Med*, 1986, 43, 18–28.
8. Dodson R, Greenberg D, Williams M, Corn C, O'Sullivan M, Hurst G. – Asbestos content in lungs of occupationally and non-occupationally exposed individuals. *J Am Med Assoc*, 1984, 252, 68–71.
9. De Vuyst P, Dumortier P, Moulin E, Yourassowsky N, Rocmans P, de Francquen P, Yernault JC. – Asbestos bodies in bronchoalveolar lavage reflect lung asbestos bodies concentration. *Eur Respir J*, 1988, 1, 362–367.
10. Wagner PC. – Mineral fibers and mesothelioma. *Thorax*, 1986, 41, 161–166.
11. Davis JMG. – Further observations on the ultrastructure and chemistry of formation of asbestos bodies. *Exp Med Pathol*, 1970, 13 (Suppl.), 346–356.
12. Holden J, Churg A. – Asbestos bodies and the diagnosis of asbestosis in chrysotile miners. *Environ Res*, 1986, 39, 232–236.
13. Dodson RF, Williams MG, Hurt GA. – Method for removing the ferruginous coating from asbestos bodies. *J Toxicol Environ Health*, 1983, 11, 959–966.
14. Gylseth B, Bauman RH. – Topographic and size distri-

bution of asbestos bodies in exposed human lungs. *Scand Work Environ Health*, 1981, 7, 190–195.
15. Dumortier P, De Vuyst P. – Object marking, a bridge between light and analytical electron microscopy for particles characterization. *J Elect Micros Tech*, (in press).
16. Goldstein JI, Williams DB. – Spurious X-rays produced in the scanning transmission electron microscope. *SEM*, 1978, I, 427–434.
17. Churg A. – Fiber counting and analysis in the diagnosis of asbestos-related disease. *Hum Pathol*, 1982, 13, 381–392.

RÉSUMÉ: Des corps ferrugineux "typiques" considérés comme corps asbestosiques (CA) ont été extraits de poumons chez des sujets urbains exposés à l'amiante (19 cas) et non-exposés (25 cas). 315 des 319 fibres centrales analysées sont de nature asbestosique. Les fibres restantes sont constituées de talc ou de silice critalline. Les amphiboles commerciales (amosite/crocidolite) représentent 89,2% des fibres d'amiante, le chrysotile 7% et les amphiboles non-commerciales (anthophyllite/ trémolite) 3,8%. Les CA sur amphiboles commerciales sont détectés aussi bien chez les sujets exposés que non-exposés et les CA sur chrysotile se retrouvent surtout chez les exposés. Les CA sur amphiboles non-commerciales s'observent chez des sujets non-exposés à faible empoussiérage pulmonaire; cette contamination d'origine environementale probable semble difficile à mettre en évidence dans les poumons des sujets fortement empoussiérés en raison d'une exposition professionnelle. La coexistence de CA sur chrysotile et CA sur trémolite/anthophyllite n'est pas observée. En Belgique, la source d'exposition aux amphiboles non-commerciales pourrait être sans aucune relation avec une contamination par du chrysotile.