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Case-Referent Survey of Young Adults with Mesothelioma: I. Lung Fibre Analyses

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Objectives: Our study aimed to determine the lung tissue concentration of asbestos and other mineral fibres by type and length in persons with mesothelioma aged 50 yr or less at time of diagnosis, compared to controls of similar age and geographical region. In this age group it was thought that most, but not all, work-related exposures would have been since 1970, when the importation of crocidolite, but not amosite, was virtually eliminated.

Methods: Eligible cases were sought from recent reports by chest physicians to the SWORD occupational disease surveillance scheme. Lung tissue samples were obtained at autopsy from 69 male and four female cases, and mineral fibres identified, sized and counted by electron microscopy. Fibre concentrations per μg dry tissue were compared with similar estimates from a control series of autopsies of sudden or accidental deaths. Unadjusted, and adjusted odds ratios calculated by logistic regression, assessed relative risk in relation to fibre type, length and concentration.

Results: Unadjusted and adjusted odds ratios increased steadily with concentration of crocidolite, amosite, tremolite and all amphiboles combined. There was also some increase with chrysotile, but well short of statistical significance. Incremental risk examined in a linear model was as highly significant for all amphiboles together as individually. Short, medium and long amphibole fibres were all associated with increased risk in relation to length. Mullite and iron fibres were significant predictors of mesothelioma when considered without adjustment for confounding by amphiboles, but, after adjustment, were weak and far from statistically significant.

Conclusion: In this young age group, amosite and crocidolite fibres could account for about 80% of cases of mesothelioma, and tremolite for some 7%. The contribution of chrysotile, because of low biopersistence, cannot be reliably assessed at autopsy, but to the extent that tremolite is a valid marker, our results suggest that it was small. The steep linear trend in odds ratio shown by amphiboles combined indicates that their effects may be additive, with increased risk from the lowest detectable fibre level. Non-asbestos mineral fibres probably made no contribution to this disease. Contrary to expectation, however, some 90% of cases were in men who had started work before 1970; this was so whether or not amosite or crocidolite was found in lung tissue.

Keywords: mesothelioma in young adults; occupation; lung fibre analysis

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INTRODUCTION

The steady rise in mortality from mesothelioma in Britain since the 1950s, predicted to continue for some years to come, was investigated in the present study in persons, so far as possible, aged 50 yr or less at time of diagnosis. It was argued that the occupations and lung fibre content of these cases would mainly reflect exposure to asbestos since 1970, when the importation of crocidolite, but not amosite, was virtually eliminated. However, exposure to crocidolite would certainly have continued after that date, mainly as a result of asbestos removal and, as it later proved, almost all cases studied were in persons first employed before 1970.

The investigation had two separate but related components. The first entailed identification of eligible cases, followed by detailed recording of work histories and other relevant data. The second, which is the subject of this report, required collection of lung tissue samples taken at autopsy from as many of these cases as possible, together with similar samples for comparison from accidental or sudden cardiac deaths.

Procedures used in the ascertainment of cases, recording of work histories and occupational analyses which form the background for the present paper are described fully elsewhere (McDonald *et al.*, 2001, this issue). Eligible cases were sought from reports by chest physicians to the SWORD national work-related disease surveillance scheme and were obtained for 115 men and 13 women. Years spent by men in each occupation were compared with expected values from census data. Of 37 industrial occupations analysed, odds ratios were significantly raised in eight: five in the construction industry and the others in shipbuilding and the manufacture of cement and non-metallic mineral products. Only four of the women had been employed in any industrial occupation; the remainder included four in office work, two in nursing, two in sales and one in teaching.

METHODS

Case and control selection

Cases included in the occupational study were followed so far as possible until the end of 1997, by which time most of the 115 men and 13 women with recorded work histories had died. Inquiry indicated that in 98 of these cases there had been an autopsy. The desirability of lung burden analysis with histological confirmation of diagnosis was discussed with the pathologists responsible and as a result, lung tissue and tumour samples were obtained from 69 male and four female cases. Histological review made independently by our two pathologists (CWE and ARG), both considerably experienced in the diagnosis of mesothelioma (Attanoos and Gibbs, 1997), confirmed that in all cases the diagnosis of mesothelioma was at least highly probable. So far as possible, lung tissue samples were obtained as referents from the same pathologists as the cases from accidental or sudden cardiac deaths of similar age, sex and region. As such cases were uncommon, this proved difficult, so the search was extended more widely. This resulted in a much larger number of samples, but relatively few which met the criteria of age and region completely.

Mineral fibre analysis

The methods used for the preparation and electron-microscopic analysis of fibres in lung tissue have been fully described elsewhere (Gibbs and Pooley, 1996). In summary, small pieces of tissue were obtained from different parts of the lung from either wet fixed specimens or paraffin wax blocks, depending on availability. The samples were weighed and then digested in 40% potassium hydroxide solution. A similar piece of lung tissue was weighed wet and dried to constant weight, in order to determine the wet to dry ratio. Tissue embedded in wax blocks was recovered using xylene and ethanol extraction, and then dried to constant weight before preparation. The digested tissue residues were washed, centrifuged, dispersed and then collected by filtration onto cyclopore filters (pore size 0.2 μm; diameter 25 mm). These were carbon coated, the filters dissolved in chloroform, and the carbon filters mounted into gold electronmicroscope support grids for transmission electron microscopy. Random areas of the grid were examined at a magnification of ×22 000. Fibres were identified, counted and sized until 100 or more had been accumulated, or a specified level of detection (0.2 fibres per μg) had been exceeded. All fibrous structures with an aspect ratio of 3:1 or greater were analysed to ascertain their elemental composition using energy dispersion X-ray analysis.

Statistical analysis

The analysis was confined to 69 male cases, whose ages ranged from 36 to 52 yr. Of the 74 controls, 17 were aged five or more years outside this range and were excluded from further study, leaving 57 in all. The degree of matching by age and geographical region is shown in Table 1. To allow for residual confounding, conditional logistic regression was used to estimate the odds ratios, stratifying by the five age groups and regions shown.

The distribution of cases and controls by category of lung fibre concentration — 0 (none detected), 0.1–0.9, 1–9.9, 10–99.9, 100+ fibres per μg — was tabulated for each specific fibre type, for all amphiboles, all asbestos fibres and for seven other types of mineral fibre. Relative risks were estimated relative to the zero fibre concentration group. In order to allow for the effect of one fibre type when considering another, relative risks were also estimated, adjusting for other fibre types by including them simultaneously in the model.

Models were also fitted which allowed for risk to vary continuously with exposure. Linear relative risk models were found to fit much better than the more commonly used log-linear models. For models including more than one fibre type, linear-additive

Table 1. Cases and controls included in analysis, by age group and region

	Age					
	36–39	40–44	45–49	50–56	Total	
Controls	2	13	19	23	57	
Cases	2	16	33	18	69	
	Region					
	Scot and N	NW and NE	Mid (E and N)	Wales and SW	London, SE and E Anglia	Total
Controls	15	16	11	10	5	57
Cases	13	14	17	9	16	69

models fitted much better than the more usual exponential-multiplicative. In these analyses, confidence intervals were calculated using the likelihood profile method of Prentice and Mason (Prentice and Mason, 1986). The proportion of cases attributable to each fibre type was calculated from the linear model slope estimates according to the method described by Bruzzi (Bruzzi *et al.*, 1985).

RESULTS

Asbestos fibres

The distribution in cases and controls (Table 2) shows substantially higher concentrations in cases of all asbestos fibres, all amphiboles, crocidolite, amosite and, although rarer, tremolite; cases also had more chrysotile. Unadjusted odds ratios reflect these differences, with large values for all groups with concentrations of crocidolite, amosite and all amphiboles, increasing with concentration. The odds ratios for tremolite were also elevated, but imprecisely estimated and of limited statistical significance. There were moderately elevated risks with chrysotile fibres, but the association fell well short of statistical significance. The estimated increment in relative risk in the linear model was highest for crocidolite and, among amphiboles, lowest for tremolite, but with overlapping confidence intervals.

Allowing for the effect of other fibre types on risk by entering all four in the model proved difficult, as odds ratios became very unstable. Therefore Table 2 shows the estimates for each specific amphibole allowing for the effect of the other two, and for all amphiboles and chrysotile adjusting for each other. Mutually adjusted patterns remain broadly the same, but with differences between the three amphiboles

Table 2. Distribution of lung fibre concentrations with grouped and continuous odds ratios

Fibre type	Concentration (per μg)	Cases	Controls	Unadjusted OR	Adjusted OR[a]
Crocidolite	0	28	48	1.0	1.0
	0.1–0.9	27	8	5.3 (2.0–14.3)	4.6 (1.3–15.5)
	1.0–9.9	11	1	17.5 (2.0–155)	3.9 (0.3–40.4)
	10.0–	3	0	∞	∞
	Linear model[b]			13.2 (3.3–44.5)	40.0 (2.6–388)
Amosite	0	13	34	1.0	1.0
	0.1–0.9	23	18	5.6 (1.6–18.8)	5.1 (1.4–18.6)
	1.0–9.9	26	5	24.9 (5.7–108)	17.9 (3.5–91.4)
	10.0–	7	0	∞	∞
	Linear model[b]			11.4 (2.8–49.2)	14.3 (2.2–113)
Tremolite	0	55	51	1.0	1.0
	0.1–0.9	13	6	2.2 (0.9–6.6)	2.3 (0.7–8.0)
	1.0–9.9	1	0	–	–
	10.0–	0	0	–	–
	Linear model[b]			6.9 (0.2–30.9)	29.6 (<0–340)
All amphiboles	0	6	28	1.0	1.0
	0.1–0.9	26	24	9.2 (1.9–44.5)	8.8 (1.8–43.5)
	1.0–9.9	28	4	64.7 (9.8–425)	59.9 (9.0–400)
	10.0–	9	1	55.8 (3.9–792)	–
	Linear model[b]			19.4 (4.2–137)	47.6 (6.0–>999)
Chrysotile	0	14	19	1.0	1.0
	0.1–0.9	28	21	1.5 (0.6–3.9)	1.9 (0.5–6.7)
	1.0–9.9	26	16	2.2 (0.8–6.2)	2.2 (0.6–8.4)
	10.0–	1	1	–	–
	Linear model[b]			0.1 (<0–1.2)	2.2 (<0–>999)

[a]Crocidolite, amosite and tremolite are adjusted for each other. Total amphiboles and chrysotile are adjusted for each other.
[b]Average increment in odds ratio per fibre/μg.

Table 3. Estimates of fractions of cases attributable to each fibre type

Fibre type	Attributable fraction		
	Unadjusted	95% CI	Adjusted[a]
Crocidolite	51%	(38–56)	33%
Amosite	70%	(57–77)	46%
Tremolite	12%	(1–18)	7%
All amphiboles	86%	(74–91)	84%
Chrysotile	11%	(0–43)	11%

[a]Adjustments as for Table 2.

well within the bounds of chance. The model with the three amphiboles included separately fitted the data no better than the model combining amphiboles.

Table 3 shows the estimated proportion of cases attributable to each fibre type. The confidence intervals for these estimates reflect uncertainty in the relative risks, but not uncertainty in the selection of the cases. Confidence intervals for adjusted proportions are not shown, as they could not be calculated reliably. Although it is clear that a large majority of cases could be explained by the amphiboles including tremolite, as pure chrysotile fibres do not persist in lung tissue, their contribution is uncertain.

The effect of amphibole fibre length is examined in Table 4 with odds ratios calculated for three ranges: <6, 6–10 and >10 μm. Shorter fibres were more abundant than longer fibres, and as high concentrations of all fibre lengths tended to occur together (correlations from 0.6 to 0.9), discrimination was difficult. Short, medium and long fibres were all associated with mesothelioma risk; those longer than 10μm had the greatest increment in risk per fibre, followed by medium (6–10 μm) and then by short (<6 μm), with coefficients of 417, 116 and 9, mutually adjusted. The difference between the coefficients was on the borderlines of conventional levels of statistical significance (likelihood ratio test =5.9 on 2 df, $P = 0.05$).

Other fibres

Seven types of non-asbestos mineral fibres were identified in all but three of the 69 cases, and in all 57 controls. The detailed distributions are shown in Table 5. Mullite and iron were significant predictors of mesothelioma when considered without adjustment for the confounding effect of amphiboles, with which they were appreciably correlated (Spearman's rank correlation 0.28–0.43). After allowing for this confounding, the associations were weak and far from statistically significant. The remaining five fibre types showed little evidence of association with mesothelioma, with or without adjustment for amphiboles.

DISCUSSION

The results of this study suggest that in the UK, a high proportion of deaths from mesothelioma in young men whose working lives began in the 1960s, and were predominantly in the 1970s or later, resulted from crocidolite or amosite exposure. Although risk per fibre was as high for tremolite as for other amphiboles, it was found less frequently, and so made little contribution to explaining the cases. As tremolite deposits often occur in proximity to chrysotile it can be considered a more biopersistent marker for it. Thus the low proportion of cases attributable to tremolite would also imply a low proportion attributable to chrysotile, though appreciably less than the 20% estimated in a survey of predominantly much older cases across Canada some 20 years ago (McDonald *et al.*, 1989).

As had been observed in two previous studies (McDonald *et al.*, 1989; Rogers *et al.*, 1991), long fibres were associated with greater risk than shorter fibres, but as all sizes were usually found together,

Table 4. Distribution of amphibole fibre concentrations by length, with grouped and continuous odds ratios

Fibre length	Concentration (per μg)	Cases	Controls	Unadjusted OR	Adjusted OR[a]
<6 μm	0	7	28	1.0	1.0
	0.1–0.9	26	24	5.7 (1.5–22.3)	4.1 (1.0–17.1)
	1.0–9.9	29	4	41.9 (7.7–229)	13.4 (2.2–82.9)
	10.0–	7	1	33.6 (2.7–419)	3.6 (0.1–91.4)
	Linear model			13.4 (3.3–67.0)	9.3 (1.1–77.9)
6–10 μm	0	34	51	1.0	1.0
	0.1–0.9	27	6	7.2 (2.4–21.1)	2.9 (0.8–10.4)
	1.0–9.9	6	0	∞	∞
	10.0–	2	0	∞	∞
	Linear model			28.2 (7.1–106)	116 (7.1–>999)
>10 μm	0	40	55	1.0	1.0
	0.1–0.9	24	2	17.1 (3.6–81.9)	4.8 (0.8–27.6)
	1.0–9.9	4	0	∞	∞
	10.0–	1	0	∞	∞
	Linear model			70.9 (14.1–517)	417 (15.8–>999)

[a]One size fraction adjusted for the other two.

Table 5. Distribution of other fibres in cases and controls: details

	Concentration (per μg) 0	0.1–0.9	1.0–9.9	10.0–	Total
Mullite					
Controls	1	8	37	11	57
Cases	1	7	24	34	66
Iron					
Controls	20	29	7	1	57
Cases	8	37	21	0	66
Rutile					
Controls	12	34	11	–	57
Cases	11	39	16	–	66
Muscovite					
Controls	20	30	7	–	57
Cases	26	24	16	–	66
Silica					
Controls	20	29	8	–	57
Cases	30	22	14	–	66
Kaolin					
Controls	53	4	–	–	57
Cases	60	6	–	–	66
Alumina					
Controls	51	2	4	0	57
Cases	58	7	0	1	66

the discrimination was difficult. Risks associated with amphibole concentration were close to linear and perhaps independent of fibre type. Mineral fibres, other than asbestos, showed little or no evidence of a causal association. There is evidence, however, that there probably always has been a low background incidence of mesothelioma in both men and women unrelated to asbestos (McDonald and McDonald, 1993). The adjusted fractions attributable to amphiboles (84%) and chrysotile (11%) do not take this into account, but still leave 5% unexplained (see Table 3).

The clear-cut findings from this study, with their considerable implications for fibre carcinogenesis and the public health, although strongly supported by a large body of epidemiological data (McDonald and McDonald, 1996) and by a recent comprehensive statistical analysis (Hodgson and Darnton, 2000), must be examined nevertheless for the possibility of errors or bias. First there are questions of case and control selection. Probably not more than 70% of mesothelioma cases in the UK are reported to SWORD and lung tissue was obtained for analysis from only some 60% of the eligible cases reported. Although conceivable, we think it improbable that a chest physician's knowledge of a patient's exposure history might affect whether or not a case were reported, but not whether lung tissue at autopsy was later available for analysis. The selection of controls was certainly less than ideal, but without obvious bias in relation to the questions under investigation. More important is the fact that the electronmicroscopic analyses were made, and results recorded, for tissue specimens, identified only by serial numbers unrelated to case/control status.

The most serious and fundamental source of potential bias is the fact that mineral fibres vary in their biopersistence, and in particular that chrysotile is far less durable in lung tissue than crocidolite, amosite or tremolite. As a result, findings for chrysotile at autopsy will reflect recent exposures of little etiological importance rather than those many years ago, whereas those for amphiboles will do the reverse. From animal studies it seems likely that carcinogenicity results mainly from biopersistence (Searl *et al.*, 1999; Miller *et al.*, 1999a,b), and if this is the case the clearance of chrysotile does not invalidate the conclusions from fibre analysis. In humans the evidence that durable agents are more carcinogenic is strong although indirect, consisting of extensive epidemiological evidence that exposure to such biopersistent agents as crocidolite, amosite, tremolite and erionite is followed by a far higher incidence of mesothelioma than occurs after chrysotile exposure (Hodgson and Darnton, 2000). The further indications that chrysotile as mined and milled and used commercially is often contaminated with fibrous tremolite help to complete the picture. Tremolite fibres alone have been shown to carry a high risk of mesothelioma (McDonald *et al.*, 1986) and that their level of concentration in the various chrysotile mines of Quebec is correlated with the incidence of this disease (McDonald and McDonald, 1997).

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