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Tremolite and Mesothelioma

VICTOR L. ROGGLI*, ROBIN T. VOLLMER, KELLY J. BUTNOR and THOMAS A. SPORN

Departments of Pathology, Duke University and Durham VA Medical Centers, Durham, NC 27710, USA

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Background: Exposure to chrysotile dust has been associated with the development of mesothelioma and recent studies have implicated contaminating tremolite fibers as the likely etiological factor. Tremolite also contaminates talc, the most common non-asbestos mineral fiber in our control cases.

Methods: We examined 312 cases of mesothelioma for which fiber burden analyses of lung parenchyma had been performed by means of scanning electron microscopy to determine the content of tremolite, non-commercial amphiboles, talc and chrysotile. The vast majority of these patients were exposed to dust from products containing asbestos.

Results: Tremolite was identified in 166 of 312 cases (53%) and was increased above background levels in 81 cases (26%). Fibrous talc was identified in 193 cases (62%) and correlated strongly with the tremolite content (P < 0.0001). Chrysotile was identified in only 32 cases (10%), but still correlated strongly with the tremolite content (P < 0.0001). Talc levels explained less of the tremolite deviance for cases with an increased tremolite level than for cases with a normal range tremolite level (22 versus 42%). In 14 cases (4.5%) non-commercial amphibole fibers (tremolite, actinolite and/or anthophyllite) were the only fiber types found above background.

Conclusions: We conclude that tremolite in lung tissue samples from mesothelioma victims derives from both talc and chrysotile and that tremolite accounts for a considerable fraction of the excess fiber burden in end-users of asbestos products.

Keywords: asbestos; mesothelioma; tremolite; talc; chrysotile; amphiboles

INTRODUCTION

Tremolite is a hydrated calcium magnesium silicate [$Ca_2Mg_5Si_8O_{22}(OH)_2$] belonging to the amphibole group of minerals. It is a known contaminant of chrysotile asbestos and also contaminates fibrous talc and vermiculite (McDonald *et al.*, 1989; Langer *et al.*, 1991). In addition, tremolite is the most common amphibole fiber found in lung samples from our control populations (Srebro and Roggli, 1994).

Due to the fragility of chrysotile asbestos and its tendency to be removed from the lungs at much greater rates than amphiboles, it has been suspected that contaminating tremolite might be responsible for mesotheliomas occurring in chrysotile miners and millers (McDonald *et al.*, 1989). Recent studies have leant further support to this hypothesis in that mesotheliomas in Thetford chrysotile miners and millers were much more likely to occur in the five central mines, where there is greater contamination by tremolite as compared with the more peripheral mines (McDonald *et al.*, 1997). Furthermore, it has been suggested that processed (i.e. milled) chrysotile contains little or no tremolite contaminant (Churg, 1988; Craighead, 1995).

Our laboratory has performed fiber analyses on a large number of mesothelioma patients, most of whom were exposed to asbestos-containing products as end-users. We have previously shown that amosite asbestos is the most common fiber type found in the lungs of these workers (Roggli *et al.*, 1993). It is the purpose of this study to evaluate the relationship between levels of tremolite, non-commercial amphiboles, talc and chrysotile and to compare these findings with the occupational exposure information in a series of 312 mesothelioma cases for which a lung fiber burden analysis had been performed.

*Author to whom correspondence should be addressed. Tel: +1-919-286-0411 ext. 6615; fax: +1-919-286-6818; e-mail: roggli.v@durham.va.gov

MATERIALS AND METHODS

Case selection

The study group consisted of all mesothelioma cases in the database of one of the authors (V.L.R.) for which a fiber burden study with identification of fiber types had been performed. The diagnosis of mesothelioma was based on previously published criteria (Roggli *et al.*, 1992a). These included the gross distribution of tumor, histological appearance and immunohistochemical findings. In some cases histochemistry and electron microscopy were also performed. In addition, occupational information was reviewed for cases with a tremolite content that exceeded our background range (see below). In all cases the diagnosis of mesothelioma was made independently of asbestos exposure history or determination of tissue mineral fiber content.

Mineral fiber analysis

Fiber analysis was performed on lung tissue samples obtained either at time of surgery (lobectomy or pneumonectomy) or at autopsy. Most lung samples were formalin fixed, and a few were paraffin embedded. For the latter cases a correction factor (0.7) was applied to the final calculation so that the results were comparable to the formalin fixed tissue results (Roggli *et al.*, 1986). The tissue was digested in sodium hypochlorite solution and the residue collected on a 0.4 μm pore size polycarbonate filter as previously described (Roggli, 1992). The filter was mounted on a carbon disc with colloidal graphite and sputter coated with gold or platinum for examination by scanning electron microscopy.

Scanning electron microscopy was performed with a JEOL JSM 6400 scanning electron microscope operated at an accelerating voltage of 20 kV, screen magnification of 1000× and a scan rate of 10 s/frame. Both coated (asbestos body) and uncoated fibers ≥5 μm in length were counted, with a fiber defined as a mineral particle with an aspect (length to width) ratio of at least 3:1 and roughly parallel sides. A total of 100 fields (filter area of ~2.37 mm^2) or 200 fibers, whichever occurred first, were counted for each sample. Blank filters were also examined and all reagents were pre-filtered to avoid contamination with fibers (Roggli, 1992).

The elemental composition of individual mineral fibers was determined by means of energy dispersive X-ray analysis (EDXA). Asbestos fibers were classified as amosite, crocidolite, tremolite, anthophyllite, actinolite, chrysotile or talc based on their morphology and X-ray spectra as previously described (Fig. 1; Roggli, 1989; Roggli *et al.*, 1992b). For each case 5–50 fibers (average 20 fibers) were classified by EDXA. The detection limit for each fiber type varied somewhat from case to case, depending on the concentration of fibers in the lung tissue and the actual number of fibers analyzed by EDXA for that case.

Statistical analysis

For the purposes of examining the relationship between levels of tremolite or non-commercial amphibole fibers (combined tremolite, actinolite and anthophyllite) and talc or chrysotile only those cases in which at least one of these four fiber types was identified were included. These criteria were met by 248 cases, three of which were individuals exposed to tremolite/actinolite environmentally in Turkey. Since this environmental exposure is not related to either chrysotile or talc, these three cases were excluded

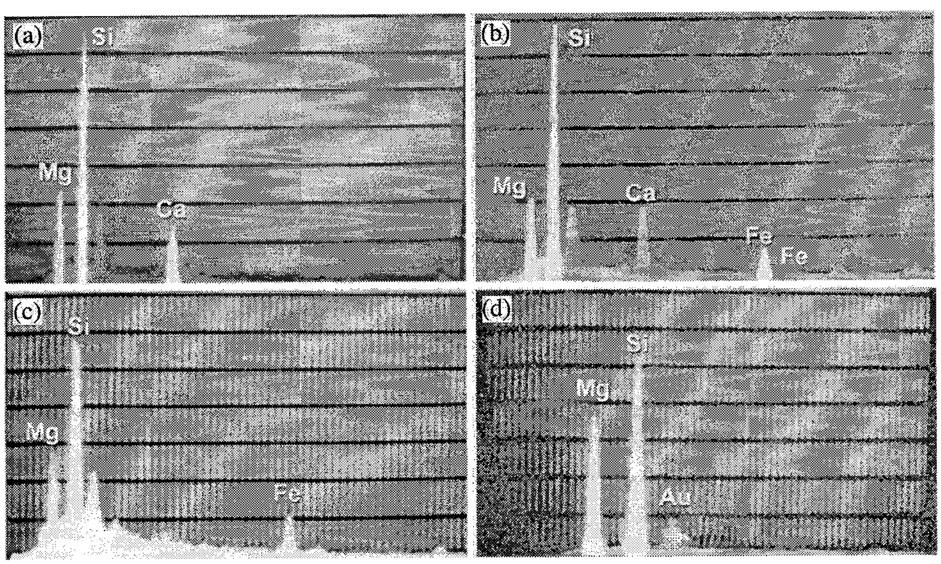

Fig. 1. Energy dispersive X-ray spectra for tremolite (a), actinolite (b), anthophyllite (c) and chrysotile (d). Characteristic elemental composition for each fiber type is shown. The gold peak is due to sputter coating of the sample to reduce charging artifacts.

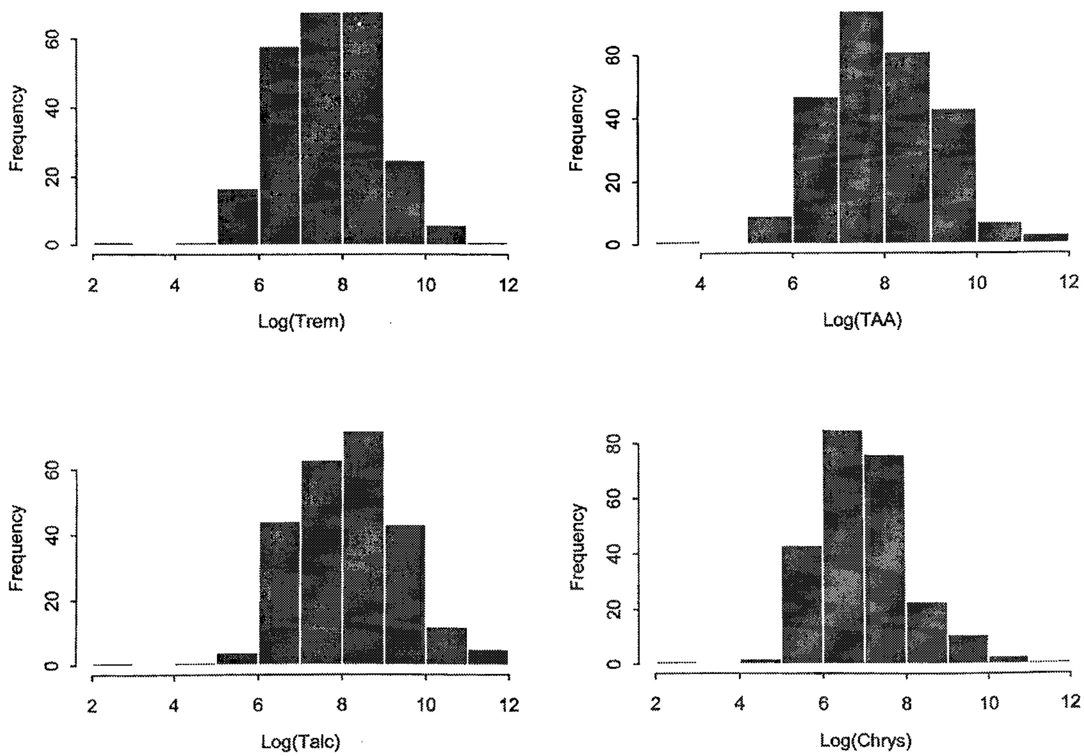

Fig. 2. Frequency distribution for four of the fiber types analyzed in this study: tremolite, TAA (tremolite, actinolite and anthophyllite), talc and chrysotile. The values are log normally distributed.

from the analysis, leaving 245 cases. As fiber burden levels have been shown to be log normally distributed (Fig. 2), a log transformation of the fiber counts was performed. Zero values were recorded as half the detection limit for the purposes of this analysis. A general linear model was used to examine the relation between chrysotile and talc as independent variables and tremolite or non-commercial amphiboles as the dependent variables (McCullagh and Nelder, 1989). Residual deviance (residual sum of squares) was determined using the S-Plus statistical package (MathSoft Inc., Seattle, WA). Univariate analysis was also used to examine the relationship between total non-commercial amphiboles, tremolite, talc and chrysotile. Statistical significance was considered for P values <0.05.

RESULTS

Tremolite was detected in 166 of 312 cases (53%), with a median value of 2840 fibers/g wet lung tissue for fibers ≥5 μm in length. The range of values was 120–292000 fibers/g. Non-commercial amphibole fibers of any type (tremolite, actinolite or anthophyllite) were detected in 210 cases (67%), with a median value of 3160 and a range of 26–454000 fibers/g. Talc was identified in 193 cases (62%), with a median value of 3910 and a range of 280–138000 fibers/g. In contrast, chrysotile was identified in only 32 cases (10%), with a median value of 1800 and a range of 540–124000 fibers/g. These values are compared with our background range in Table 1.

Tremolite exceeded our background range in 81 cases (26%). Exposure information was available for 70 of these cases, and the exposure categories for these cases are summarized in Table 2. The occupational categories in this table are generally considered to be end-users of asbestos-containing products or bystanders of end-users. Of interest is the observation that six of these cases occurred as household contacts of asbestos workers, whereas three of the cases were building occupants. The one brake mechanic with excess tremolite also had excess levels of amosite, suggesting an alternative means of exposure.

There were 14 cases (4.5%) in which non-commercial amphibole fibers were the only fiber type found in excess in the lung tissue samples. These cases are of particular interest and are summarized in Table 3. Three of these cases occurred in individuals from Turkey with environmental exposures to tremolite/actinolite (Zeren *et al.*, 2000). Three additional cases were household contacts of asbestos workers and three were building occupants. One case worked in a pattern shop in a silica plant, where the primary exposure was to talc.

The correlation among tremolite, non-commercial amphibole fibers, talc and chrysotile is summarized in Table 4. Multivariate analysis of the 245 cases for which at least one of these mineral species was

Table 1. Non-commercial amphibole, talc and chrysotile concentrations in 248 mesothelioma cases and 19 controls[a]

	Tremolite	TAA	Talc	Chrysotile
Mesothelioma				
No. of cases	166	210	193	32
Median	2840	3160	3910	1800
Range	120–292 000	26–454 000	280–138 000	540–124 000
Controls[b]				
Median	<600	<600	<600	<600
Range	<170–2540	<170–2540	210–10 200	<80–1000

TAA, tremolite + actinolite + anthophyllite.
[a]Values represent the median and range for fibers ≥5 μm in length per gram of wet lung tissue as determined by analytical scanning electron microscopy.
[b]Controls include 19 cases with no evidence of asbestos-related disease, no history of asbestos exposure and normal lungs at autopsy.

Table 2. Exposure information for 81 mesothelioma cases with elevated tremolite content

Exposure category	No.[a]
Shipyard worker	12
Household contact[b]	10
Pipefitter/welder	9
Electrician[c]	5
Plumber/construction	5
Steamfitter/boiler worker	4
Power plant worker	4
US Navy/merchant marine	4
Railroad worker	3
Building occupant	3
Insulator	2
Oil refinery worker	2
Other[d]	9
No information	11

[a]Total exceeds 81 because a few cases had more than one exposure.
[b]Contacts include shipyard worker (four), insulator (two), chemical plant worker, pipefitter/welder, tool grinder and glass company worker and power plant worker (one each).
[c]Shipyard, paper mill and chemical plant.
[d]Includes silica plant/pattern shop, machinist in a glass manufacturing plant, brake mechanic, steel worker, asbestos textile plant worker, dry cleaning plant assembler, painter/plasterer, sheet metal worker and truck driver (insulation).

detected showed that talc accounted for 42% of the residual deviance of tremolite concentrations, while an additional 16% was accounted for by chrysotile ($P < 0.0001$). Similarly, talc accounted for 36% of the deviance of non-commercial amphibole concentrations, while an additional 12% was accounted for by chrysotile ($P < 0.0001$). In addition, a strong correlation was observed using pair-wise regressions between tremolite and non-commercial amphiboles, tremolite and talc, tremolite and chrysotile, non-commercial amphiboles and talc and non-commercial amphiboles and chrysotile ($P < 0.0001$ for all comparisons).

When the multivariate analysis was restricted to the 81 cases with an elevated tremolite burden, talc explained only 22% of the deviance of tremolite concentrations ($P < 0.0001$), while an additional 6% was accounted for by chrysotile ($P < 0.03$). When the analysis was restricted to only the 32 cases in which chrysotile was actually detected, talc explained 54% of the deviance of tremolite concentrations ($P < 0.0001$), while an additional 4% was accounted for by chrysotile ($P = 0.12$) (see Table 4).

Figure 3 shows the comparison between the predicted log concentration of tremolite based on the log concentrations of talc and chrysotile and the observed log concentration of tremolite. The association is highly significant, although there is still considerable scatter of the data around the line of perfect agreement.

DISCUSSION

We have found in a large series of patients with malignant mesothelioma in which fiber burden analyses had been performed on lung tissue samples that tremolite fibers are commonly observed. These tremolite levels exceeded background in >25% of these cases. The levels of tremolite were strongly associated with concentrations of fibrous talc, which accounted for just over 40% of the deviance in tremolite concentrations. However, when cases with tremolite levels in excess of background were examined, fibrous talc accounted for just over 20% of the deviance. Therefore, in these cases some other source must be invoked to explain the tremolite deviance.

Although chrysotile concentrations were also strongly correlated with tremolite fiber burden, chrysotile accounted for a smaller percentage of the tremolite deviance. In this regard it should be noted that chrysotile does not accumulate in lung tissue samples to the extent that the amphiboles do, as it is broken down into smaller fibrils that are more readily cleared from the lungs. These smaller fibrils would have been missed by our technique, since we only counted fibers

Table 3. Mesothelioma cases for which non-commercial amphiboles were the only fiber type present in excess levels

Case no.	Age sex	Diagnosis	Exposure	Duration	Tremolite	TAA	Talc	Chrysotile
1	ND M	SPl	Environmental exposure, Turkey	ND	292000	454000	<24700	<24700
2	67 M	EPl	Environmental exposure, Turkey	Lifetime	<15900	286000	<15900	<15900
3	ND M	EPl	Environmental exposure, Turkey	ND	23500	30400	<3010	<3010
4	54 M	EPl	Household contact[a]	18 yr	7460	17700	13100	<2760
5	56 M	BPl	Painter/plasterer	38 yr	8110	16200	60800	<4060
6	57 F	EPl	Household contact[b]	1–2 yr	8100	16200	<8100	<8100
7	61 M	BPl	Shipyard worker	8 days	11700	11700	<5850	<5850
8	57 M	SPl	Supervised construction	1 yr	9550	11100	6530	<500
9	59 F	SPl	Household contact[c]	2–3 yr	5030	6330	3250	<1130
10	73 F	BPl	Silica plant/pattern shop	34 yr	5540	6070	46 100	<3030
11	59 M	BPl	Radar man, US Navy	4–5 yr	6060	6060	8080	<1010
12	44 F	EPl	Dry cleaning plant assembler	ND	5440	5440	680	<680
13	64 M	BPl	School administrator	ND	3280	4580	3930	<660
14	58 F	SPl	Teacher's aide	18 yr	4330	4330	6930	<870

B, biphasic; E, epithelial; ND, no data; Pl, pleural mesothelioma; S, sarcomatoid; TAA, tremolite + actinolite + anthophyllite.
[a]Father, toolgrinder; mother, glass plant worker.
[b]Husband, shipyard worker.
[c]Father and mother, shipyard workers.

Table 4. Multivariate analysis of tremolite, non-commercial amphibole, talc and chrysotile concentrations[a]

Dependent variable	Independent variable	Coefficient	F statistic	P value	Deviance (%)[b]
log(tremolite)	log(talc)	0.355	240	<0.0001	42
	log(chrysotile)	0.502	89	<0.0001	16
log(TAA)	log(talc)	0.347	170	<0.0001	36
	log(chrysotile)	0.457	58	<0.0001	12

TAA, tremolite + actinolite + anthophyllite.
[a]Fiber levels determined by scanning electron microscopy; log transformation of values (see text for details).
[b]Percent of deviance of dependent variable that is explained by the independent variable.

that were ≥5 μm in length. Furthermore, chrysotile fibers split longitudinally *in vivo* to produce long fibers that are <0.1 μm in diameter (Roggli and Brody, 1984; Coin *et al.*, 1994). Such long, thin fibers would likewise be missed at the screening magnification that we use. Since chrysotile content is poorly detected by SEM and fiber burden is a poor indicator of total chrysotile exposure, other information must be sought to address this question.

The best indicator of chrysotile exposure is an occupational history (Henderson *et al.*, 1997). In this regard patients in our series with elevated tremolite fiber burdens typically had exposures to both commercial amphibole fibers (especially amosite) and chrysotile. A substantial proportion of the cases occurred among shipyard workers, pipefitters/welders, electricians, plumbers/construction workers, steamfitters/boiler workers and US Navy/merchant marine seamen. Household contacts of asbestos workers and building occupants accounted for six and three cases, respectively. The latter individuals in particular are more likely to be exposed to chrysotile (Gaensler, 1992).

Another source of information that sheds some light on this question is published data on tremolite and chrysotile lung fiber burdens as determined by transmission electron microscopy. This method is more sensitive in detecting very fine chrysotile fibers and chrysotile fibrils. In a study of five chrysotile miners and millers with malignant pleural mesothelioma, Churg *et al.* (1984) noted a ratio of tremolite to chrysotile ranging from 3.9 to 12 (median 5.5). In a study of 83 shipyard workers and insulators with mesothelioma, Churg and Vedal (1994) found a geometric mean tremolite to chrysotile ratio of 12.8. There was a strong correlation between tremolite and chrysotile fiber concentrations ($r = 0.37$, $P < 0.001$ for the entire series of 144 patients). These findings suggest little, if any, removal of tremolite from end-products containing chrysotile. Furthermore, a recent study by Tossavainen *et al.* (2001) using SEM detected tremolite contamination of all six samples of processed Chinese chrysotile that were analyzed (range 0.002–0.310% tremolite by weight).

Of particular interest are cases of mesothelioma in which non-commercial amphiboles are the only fiber

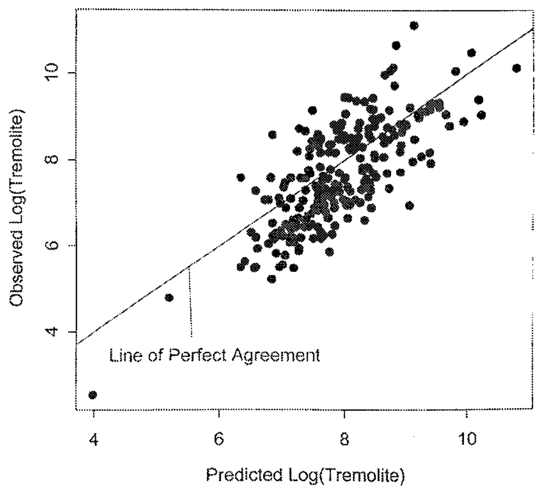

Fig. 3. Regression analysis for the relationship between the observed concentration of tremolite (*y*-axis, log transformation) and the predicted concentration of tremolite based on talc and chrysotile concentrations (*x*-axis). The straight line indicates the line of perfect agreement.

type found in excess in lung tissue samples. Three such cases in our series occurred among individuals with environmental exposure to tremolite/actinolite in Turkey. When these cases are excluded, mesotheliomas associated with non-commercial amphibole fibers alone accounted for ~3.5% of our cases (11 of 312). These included six men and five women ranging in age from 44 to 73 yr (median age 58 yr). The exposure duration ranged from 8 days to 34 yr. Five had occupational exposures to asbestos, three were household contacts of asbestos workers and three were occupants of buildings with asbestos-containing materials.

We made no attempt in the present study to discriminate between true fibers and cleavage fragments. All fibers counted were at least 5 μm in length and almost all had aspect ratios of 5 or greater. These are the fibers believed to be most active in the induction of mesothelioma. Similarly, we did not identify non-fibrous particles in this study, so we cannot comment upon the lung content of vermiculite. However, it seems unlikely that exposure to vermiculite would explain a substantial portion of the tremolite burden in our patient population, which had such widespread exposure to chrysotile asbestos.

Chrysotile was not detectable in nearly 90% of our cases and yet there was a highly significant correlation between chrysotile and tremolite content by multivariate analysis. This is partly due to an artifact of the analysis, since undetected chrysotile was recorded as half the detection limit for the purposes of the analysis and tremolite when present was often at the detection limit of our methodology. Also, the association between TAA and talc or chrysotile may be trivial, since most of the TAA fibers detected in our study were tremolite. In many of our cases the lungs had a high burden of commercial amphibole fibers so that the detection limits were unusually high. When one excludes cases in which the detection limit for tremolite exceeded our upper limit of background range for tremolite, then tremolite is present in 74% of cases (166 of 225) and exceeds our background in 36% (81 of 225).

In conclusion, evidence from our series indicates that tremolite and other non-commercial amphibole fibers are present in the lungs of a substantial proportion of patients with mesothelioma. In some patients these fibers appear to be the likely cause of the disease. The data are consistent with a derivation of the pulmonary tremolite burden from both talc and chrysotile asbestos. The findings do not support the concept that most or all of the tremolite is removed from chrysotile during the milling process.

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