Malignant Mesothelioma Associated with Low Pulmonary Tissue Asbestos Burdens: A Light and Scanning Electron Microscopic Analysis of 18 Cases

Sharon H. Srebro, Victor L. Roggli, and Gregory P. Samsa

Department of Pathology and Center for Health Services Research in Primary Care, Durham Veterans' Administration and Duke University Medical Centers, Durham, North Carolina

Most malignant mesothelioma cases are associated with pulmonary asbestos body (AB) counts significantly greater than those of the general population. However, the question remains whether malignant mesothelioma cases associated with "normal" AB counts (*i.e.*, indistinguishable from the general population) represent background incidence levels or are, actually, asbestos related. We performed AB counts (by light microscopy) and mineral fiber analysis (by scanning electron microscopy) in 18 mesothelioma cases with AB counts within our normal range (0 to 20 AB/g wet lung) and in 19 "control" cases. Our study demonstrated that approximately one-third (6 of 18) of the mesothelioma cases have asbestos fiber burdens greater than 95% of the control levels. These results suggest that these six mesothelioma cases may be asbestos related despite AB counts similar to those of the general population. An asbestos etiology was suggested in three additional cases, but too few amphibole fibers were identified in these cases to be certain of a value above background. The remaining nine cases showed no evidence of an asbestos etiology. Electron microscopic analysis of pulmonary mineral fibers may be required to differentiate asbestos-related mesotheliomas from nonasbestos-related cases when AB counts are within the range of background values.

Key words: Asbestos, Mesothelioma, Fiber analysis, Asbestos bodies, Amosite, Tremolite, Scanning electron microscopy.

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There is indisputable evidence regarding mesothelioma development and occupational exposure to asbestos, and most mesothelioma cases are associated with pulmonary asbestos body (AB) counts significantly greater than those of the general population. However, a substantial proportion (20 to 50%, depending on the study) of mesothelioma cases do not have a history of asbestos exposure (1–4). There is considerable controversy over whether these cases represent asbestos-related mesotheliomas. Some authors report that asbestos-induced mesotheliomas require a threshold asbestos dose and that nonoccupational-associated mesotheliomas represent a background level as a result either of "spontaneous" occurrence or of nonasbestos causes (5, 6). Other authors report a linear dose-response relationship with no identifiable threshold level for asbestos (7–9), and some contend that all mesotheliomas are asbestos related (10).

This report presents a comparison of data for 18 mesothelioma cases with AB counts (by light microscopy [LM]) within our "normal" range *versus* data for 19 "control" cases with normal lungs at autopsy. Our normal range is 0 to 20 AB/g wet lung, based on 84 cases with no history of asbestos exposure or evidence of asbestos-related disease (4, 11, 12). The purpose of this study is to compare the results of scanning electron microscopy (SEM) analyses to determine whether some mesothelioma cases with AB counts within this normal range are possibly asbestos related.

MATERIALS AND METHODS

Case Selection

The 18 cases selected for the current study were obtained from one of the authors' (V. L. R.) consultation files, which contain data on more than 500 mesothelioma cases. The diagnosis of malignant mesothelioma was confirmed by one of the authors (V. L. R.) using our previously published criteria (13), which include gross distribution of the tumor, histologic appearance, and histochemical and immunohistochemical findings. Histochemical studies using periodic acid-Schiff, with and without diastase predigestion, and Alcian blue, with and without hyaluronidase predigestion, and/or immunohistochemical studies using antibodies directed against cytokeratins, carcinoembryonic antigen, or Leu M1 were performed in most cases, and ultrastructural studies were performed in a few cases (14). In all cases, the diagnosis of mesothelioma was made independently of asbestos exposure history or tissue mineral fiber content.

We had the opportunity to do AB counts (by LM) for 153 mesotheli-

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oma cases and mineral fiber analyses (by SEM) for 120 cases. The LM analysis indicated that 22 of these 153 cases (14%) had AB counts within our normal range of 0 to 20 AB/g wet lung. Four of these cases were excluded from the current study (three because no additional lung tissue was available for SEM analysis and one because the filter was not available for fiber-type analysis). The remaining 18 cases included 16 autopsy specimens and 2 surgical specimens of lung parenchyma. For comparison purposes, SEM analysis was performed on 19 control cases with no documented history of asbestos exposure and no evidence of asbestos-related disease. These cases were a subset of the 84 cases representing the general population for which AB counts had been previously determined by LM (4, 11, 12). Furthermore, these 19 cases fit the criterion of macroscopically normal lungs at autopsy, as described in earlier reports (11, 15). Originally, there were 20 control cases that met the criteria defined above. However, one of these cases had amosite counts an order of magnitude higher than all the other control cases in this study. An extensive search through this patient's medical records and two phone calls to surviving relatives revealed that his employment history included installing furnaces, an occupation associated with asbestos exposure (16). Therefore, this case was excluded from the control group for this analysis. Demographic information was also collected, when available, for all 37 cases and included age, sex, diagnosis at autopsy, occupation, and smoking history.

Tissue Digestion Technique and AB (by LM) Analysis

Formalin-fixed lung parenchyma was prepared for analysis by digestion in a 5.25% sodium hypochlorite solution (commercial bleach), as detailed in previous reports (11, 17, 18). AB counts for all 19 control cases and for 6 mesothelioma cases (Cases 2 and 13 to 17; Table 1) were quantified using the technique of Smith and Naylor (18, 19) for approximately 5-g samples of lung tissue. In six mesothelioma cases (Cases 3, 5, 6, 7, 10, and 18), limited lung tissue (<1.0 g) was available. For these cases, our laboratory developed a hypochlorite digestion procedure [modified from Williams *et al.* (20)], which is suitable for smaller sample sizes (0.1 to 0.4 g wet weight) (11, 17, 18). Our laboratory determined that, for small sample sizes, AB counts obtained by these technique are, on average, within 10% of values determined by the Smith and Naylor procedure (11).

Digested lung tissue was collected on 0.4-μm pore size polycarbonate filters. One filter was mounted on a glass side, and the entire filter was examined by LM (at a magnification of 200×) for AB. Ferroprotein-coated fibers with broad, yellow cores or black cores were considered to be nonasbestos ferruginous bodies (pseudo-AB) and were not counted in these analyses (21). The detection limit of this analysis was 1 AB/filter. The equivalent detection limit in asbestos bodies per gram of wet lung depends on the weight of the sample but, in most cases, corresponds to approximately 3.0 AB/g (*i.e.*, for an average 0.3-g sample of lung tissue).

In six cases (Cases 1, 4, 8, 9, 11, and 12; Table 1), Formalin-fixed lung tissue was not available. For these cases, the tissue was obtained from paraffin blocks by dissolving the paraffin in xylene and rehydrating to 95% ethanol, as described in earlier reports (4, 11). Tissue digestion and microscopic analyses were performed as described above. The results were multiplied by 0.7 to correct for the wet weight difference between Formalin-fixed lung and paraffin-embedded lung (11, 18).

Mineral Fiber Analysis (by SEM)

All samples except one were prepared for SEM analysis by digesting lung tissue (0.25 to 0.35 g wet weight) in hypochlorite using the method developed by our laboratory for small samples, as discussed above (11, 17, 18). The remaining case (Case 2) was prepared using the Smith and Naylor technique (18, 19). The residue was then collected on 0.4-μm pore size polycarbonate filters. The filter was mounted on a carbon disc with colloidal graphite and then sputter-coated with gold. A JEOL JSM 35C SEM (JEOL, USA, Peabody, MA) operated at 20 kV accelerating voltage, a screen magnification of 1000×, and a scan rate of 5 sec/frame was used to count total uncoated fibers (UF) and AB (ferroprotein-coated asbestos fibers) (22). Fibers were defined as particles with an aspect ratio (length/diameter) of at least 3:1 and approximately parallel sides. Only particles that met both of these criteria and had a length of 5 μm or greater were counted. At a screen magnification of 1000×, most of the fibers counted were 0.2 μm in diameter or greater. A total of 100 fields, approximately 2.53 mm^2 in area, was counted for each sample. The detection limit for this SEM analysis was 140 AB or UF per filter, which corresponds to approximately 440 AB or UF per gram of wet lung for an average 0.3-g sample. Blank filters were also examined, and all reagents were prefiltered to avoid contamination with fibers (12, 22).

The chemical composition of mineral fibers (asbestos and nonasbestos) was determined by energy-dispersive x-ray analysis (EDXA). The asbestos fibers were classified as amosite, crocidolite, tremolite, anthophyllite, actinolite, or chrysotile based on the x-ray spectra (11, 12, 15). Nonasbestos mineral fibers were classified based on their elemental content by x-ray spectra and included, among others, talc, rutile, silica, miscellaneous silicates, and fibrous glass (15, 23). For the mesothelioma cases, 5 to 25 fibers (average of 11 fibers) were classified by EDXA. Five fibers were classified for each of the control cases.

Statistical Methods

Single-variable linear-regression analysis (24) was used to examine the relationship between the AB counts (by LM) and five sets of SEM fiber analysis results (*i.e.*, total UF, amosite fibers, tremolite/actinolite/anthophyllite fibers, chrysotile fibers, and nonasbestos mineral fibers). This method was also used to determine

TABLE 1. DEMOGRAPHIC, PATHOLOGIC, AND OCCUPATIONAL INFORMATION AND RESULTS OF LUNG TISSUE ANALYSES

Case	Age/ sex	Diagnosis[a]	Occupation[b]	Smoke[c]	AB/g[d]	Total	Uncoated fibers (UF/g)[e]			
							AMOS[f]	TAA[g]	CHRYS[h]	NAMF[i]
Mesothelioma cases										
1	56/M	BPL-R	Painter/spackler	70 PY	<15.0	101,000	<4060	16,160	<4060	84,800
2	60/M	EPE	Shipyard worker (WWII)	5	14.4	600	60	60	<60	400
3	/M	EPL-R	Brake repair		14.0	7220	1440	2170	720	2890
4	60/M	BPE	Truck driver (vermiculite)	132 PY	<12.4	3400	1368	<680	<680	2040
5	68/F	DPL-L	No history of exposure		9.8	3300	<660	1980	<660	1320
6	52/M	SPL-R	Ship engine room/brake repair	40 PY	7.1	2760	280	550	<280	1930
7	65/M	EPL-R	Navy	52 PY	6.4	21,300	1070	7490	4280	9630
8	77/M	SPL-R	Sales		<5.2	3930	<390	1570	<390	2360
9	68/M	EPL-L	Railroad machinist	35 PY	<5.0	5130	<510	<510	<510	5130
10	67/M	BPL-R	Merchant Marine	NS	<3.0	5400	<1080	1080	<1080	4320
11	61/M	EPL-R	Carpenter	20 PY	<3.0	1560	<310	<310	<310	1560
12	65/M	BPC	Weigh station employee		2.8	6920	<690	690	<690	6230
13	58/F	DPL-L	Teacher's aide (building exposure)		2.8	13,000	<870	4330	<870	8660
14	66/M	BPL-L	Brake repair	XS (16 yr)	2.6	1220	120	240	<120	850
15	57/F	PL	Wife of shipyard worker	40 PY	2.0	24,300	<4860	9720	<4860	14,600
16	45/M	PE	Attorney (building exposure as student)	24 PY	1.0	6120	<1220	1220	<1220	4900
17	53/M	EPL-L	Accountant (building exposure)		<0.2	6370	<640	1270	640	4460
18	45/M	EPL-R	No history of exposure		<3.0	<440	<440	<440	<440	<440
Control cases										
19	64/M	MI	Hospital, farmer	NS	22.0	4970	<990	<990	<990	4960
20	76/M	ALL	Manual labor		19.6	8830	<1770	1770	<1770	7060
21	40/M	GBM	Manual labor		9.7	420	<100	210	<100	210
22	61/M	Esophageal cancer	Truck driver		8.9	2020	<400	400	<400	1620
23	64/M	Melanoma	Air Force	NS	7.4	2830	<570	<570	<570	2830
24	64/M	Alzheimer's	NA		5.4	12,700	<2540	2540	<2540	10,160
25	59/M	Gastric cancer	Guard	NS	3.5	2350	<470	470	<470	1880
26	53/M	ABE	Air Force	NS	3.0	3800	<760	<760	<760	3800
27	71/M	CLL	Music	50 PY	3.0	1480	<300	890	<300	590
28	61/M	CAD	Garage owner		2.8	870	<170	<170	<170	870
29	51/M	Cirrhosis	Manual labor		2.2	4980	<1000	<1000	1000	3980
30	53/M	Hepatoma	Spinning mill		2.2	3270	<650	1310	<650	1960
31	28/M	ALL	Air Force	NS	1.0	4800	<960	960	<960	3840
32	36/M	Pancreatitis	NA		1.0	3930	<790	<790	<790	3930
33	67/M	GBM	NA		0.8	2150	<430	<430	<430	2150
34	71/M	ESRD	Business supply store	XS (pipe)	0.4	2550	<510	<510	510	2040
35	64/M	MI	Electrical engineer	NS	0.4	1860	<370	370	<370	1490
36	85/M	CVD	Manual labor	XS	0.2	3000	<600	<600	<600	3000
37	63/M	AAA	Hospital aide		<0.2	2990	<600	<600	<600	2990

[a] AAA, abdominal aortic aneurysm; ABE, acute bacterial endocarditis; ALL, acute lymphoblastic leukemia; B, biphasic; CAD, coronary artery disease; CLL, chronic lymphocytic leukemia; CVD, cardiovascular disease; D, desmoplastic; E, epithelial; ESRD, end-stage renal disease; GBM, glioblastoma multiforme; L, left; MI, myocardial infarct; PC, pericardial; PL, pleural; PE, peritoneal; R, right; S, sarcomatous.

[b] NA, not available.

[c] NS, nonsmoker; PY, pack-years; S, smoker (unknown duration/quantity); XS, ex-smoker.

[d] AB/g, asbestos bodies/gram of wet lung by light microscopy (LM).

[e] UF/g, total uncoated fibers ≥ 5 μm (in length) per gram of wet lung by scanning electron microscopy (SEM).

[f] AMOS, amosite.

[g] TAA, tremolite, anthophyllite, actinolite.

[h] CHRYS, chrysotile.

[i] NAMF, nonasbestos mineral fiber.

whether smoking history (in pack-years) was linearly related to total UF.

A statistical package (DISFIT, developed at Duke University, Durham, NC) (25) was used to determine whether the AB (by LM) and UF (by SEM) results were consistent with log normal unimodal or bimodal distribution. This software is based on a maximum likelihood algorithm, which generates the parameters of the log normal distribution and then uses a chi-square test to compare the observed values with expected values.

The Wilcoxon test, a nonparametric assigned-rank method (24), was used to compare AB (by LM) and fiber counts (by SEM) between the 18 mesothelioma cases and the 19 control cases. For all statistical analyses, a P value of 0.05 or less was considered statistically significant.

RESULTS

AB counts (by LM) were conducted for 153 mesothelioma cases. The distribution of AB

counts is presented in Figure 1. These data are consistent with a log normal bimodal distribution (P = 0.14, *i.e.*, the observed distribution does not differ significantly from a log normal bimodal distribution) (25). The hypothesis of a unimodal distribution was rejected with a probability of $P < 0.001$. This observation suggests the presence of two distinct populations (*i.e.*, probable nonasbestos cases and probable asbestos-related cases for the first and second distributions, respectively). Sixteen percent of the cases are represented in the first distribution, which has a geometric mean of 1.4 AB/g and a geometric SD of 1.6 AB/g (or 0.14 with an SD of 0.21 on the $\log_{10}$ scale). The second distribution represents approximately 84% of the cases, with a geometric mean of 1020 AB/g and a geometric SD of 15.5 AB/g (or 3.01 with a SD of 1.19 on the $\log_{10}$ scale). The "50% point" (*i.e.*, the value that is an equal number of SDs from the mean of either distribution) is 3.7 AB/g wet lung, which corresponds to 0.57 on the $\log_{10}$ scale. The hatched area in Figure 1 represents the cases with normal-range AB counts (*i.e.*, less than 20 AB/g wet lung). This area included data that overlapped with the lower end of the second distribution, suggesting that some mesothelioma cases with normal AB counts may be asbestos related. This observation is the basis for the study presented here. To determine whether any of the mesothelioma cases with normal AB counts (*i.e.*, the hatched area in Fig. 1) were possibly asbestos related, mineral fiber types were characterized (by EDXA) for each case (except four cases, as discussed above) and compared with fiber types from the control cases.

The age range of the mesothelioma cases (Table 1, Cases 1 to 18) was 45 to 77 yr with a median of 60 yr. The age range of the control cases (Table 1, Cases 19 to 37) was 28 to 85 yr with a median of 62 yr. Women represented 17% (3 of 18) of the mesothelioma cases in this study. None of the control cases in this study were women.

The diagnoses at autopsy also are shown in Table 1. The control cases died from a variety of nonpulmonary causes. The mesothelioma cases included 14 pleural, 3 peritoneal, and 1 pericardial mesothelioma.

Smoking histories were available for 11 of 18 (61%) of the mesothelioma cases and for 9 of 19 (47%) of the control cases. The mesothelioma cases included eight smokers (with 20 to 132 pack-year histories), one smoker (unknown duration/quantity), one ex-smoker (quit 16 yr), and one nonsmoker. The nine control cases for whom smoking histories were available included six nonsmokers, one smoker (50 pack-years), one ex-smoker, and one pipe ex-smoker (quit 16 yr).

The arithmetic means and the median values for the AB counts, total UF counts, and various fiber types for cases and controls are presented in Table 2. A one-tailed Wilcoxon test (24) revealed that the amosite and tremolite fiber counts were statistically different between the control and the mesothelioma groups (P = 0.006 and 0.004, respectively). A one-tailed Wilcoxon test (24) suggested that the total UF distributions also were different, although this finding did not reach statistical significance (P = 0.059). The AB, chrysotile, and nonasbestos mineral fiber counts revealed no statistical differences between the control group and the mesothelioma group (P = 0.74, 0.49, and 0.20, respectively). The percentages of fiber types detected for the mesothelioma cases and the controls are reported in Table 3. Previously reported values for 94 mesothelioma cases representing AB counts from 0 to 1,600,000 AB/g wet lung are also included for comparison (26).

A single-variable linear-regression analysis (24) of AB (by LM) *versus* SEM data (*i.e.*, total UF, amosite, tremolite/actinolite/anthophyllite, chrysotile, and nonasbestos mineral fibers) was performed for the 18 mesothelioma cases in this study. This analysis indicated that the AB counts correlated best with amosite counts (R = 0.46), although this correlation did not reach statistical significance ($P > 0.05$). The other correlations yielded r values less than 0.10 ($P > 0.05$).

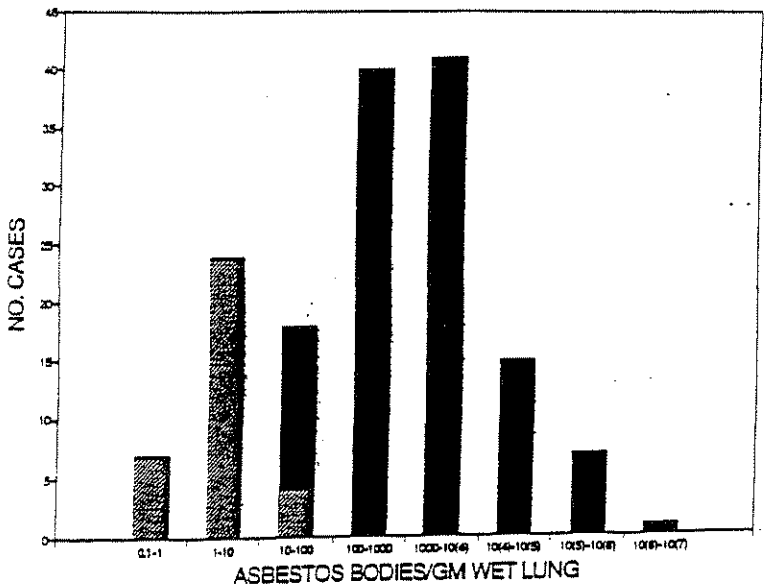

Figure 1. Distribution of pulmonary AB counts (by LM) for 153 malignant mesothelioma cases. Our normal range is 0 to 20 AB/g wet lung. Note the logarithmic scale, with value ranging from 0.1 (10^{-1}) to 10 million (10^{7}) AB/g. The data are consistent with a bimodal log normal distribution (P = 0.14), with 16% of the cases having a geometric mean of 0.14 and a geometric SD of 0.21; 84% of the cases had a geometric mean of 3.01 and a geometric SD of 1.19. The first distribution (*hatched area*) has values overlapping with those of the general population. The 50% point (*i.e.*, the value that is an equal number of SDs from the mean of either distribution) is 3.7 AB/g wet lung (0.57 on the log scale), which lies within the range of our laboratory's normal values.

TABLE 2. MEDIAN AND MEAN VALUES LM AND SEM RESULTS[a]

	Median	Mean
Mesothelioma cases (n = 18)		
Asbestos bodies (LM)	2.8	5.0
Total uncoated fibers (SEM)	5400	12,600
Amosite	ND[c]	270[b]
Tremolite/actinolite/anthophyllite	1180	2810[b]
Chrysotile	ND	310
Nonasbestos mineral fibers	605	9170
Control cases (n = 19)		
Asbestos bodies (LM)	2.9	3.5
Total uncoated fibers (SEM)	2990	4330
Amosite	ND	ND
Tremolite/actinolite/anthophyllite	ND	470[b]
Chrysotile	ND	75
Nonasbestos mineral fibers	2500	2970

[a] All results are reported per gram of wet lung tissue. Mean values are arithmetic means.
[b] The mean values for amosite fibers and tremolite/actinolite/anthophyllite fibers were significantly different from corresponding values for the controls (P = 0.006 and 0.004, respectively).
[c] ND, below limits of detection.

TABLE 3. PERCENTAGE OF MINERAL FIBER TYPES IDENTIFIED BY EDXA[a]

	Control cases (%)[b]	Mesotheliomas current study[c] (%)	Mesotheliomas previous study (26)[c] (%)
Amosite	12.1[d]	10.3	58.0
Crocidolite	0.0	0.0	3.0
Tremolite	11.6	20.0	10.0[e]
Actinolite	2.1	1.1	—[e]
Anthophyllite	0.0	0.0	—[e]
Chrysotile	2.1	3.2	3.0
Nonasbestos[f]	83.2	65.4	25.0

[a] Percentage of fibers greater than or equal to 5 μm in length (coated and uncoated).
[b] 18 cases with AB counts (by LM) from 0 to 20 AB/g wet lung.
[c] 94 cases with AB counts (by LM) from 0 to 1,600,000 AB/g wet lung.
[d] Coated fibers only; no uncoated amosite fibers were detected.
[e] Tremolite/actinolite/anthophyllite fibers were not reported separately for the earlier study (26) but instead were reported as a combined total percentage.
[f] Nonasbestos mineral fibers, including talc, rutile, silica, aluminum silicates, other silicates, iron oxides, aluminum oxides, iron-chromium, and fibrous glass.

Uncoated amosite fibers (>5 μm) were not detected in any of the 19 control cases but were detected in 6 of the mesothelioma cases (Cases 2, 3, 4, 6, 7, and 14; Table 1). Occupational histories for these six mesothelioma cases indicated that two were employed as brake repairmen, one was a vermiculite truck driver, one was employed in a ship engine room (and later as a brake repairman), one worked on ships in WW II (duties unspecified), and one was in the Navy (duties unspecified). One of these six cases (Case 7) had tremolite and chrysotile levels greater than those of all the control cases. One other case (Case 3) had chrysotile levels greater than those in 95% of the control cases (but not greater than those in all the controls). Chrysotile fibers (>5 μm) were not detected in the other four cases.

Pulmonary tremolite levels for three mesothelioma cases (Cases 1, 13, and 15; Table 1) exceeded values for all the control cases. Tremolite fibers were the only asbestos fibers (>5 μm) detected in these three cases; there were no actinolite or anthophyllite nor any amosite, crocidolite, or chrysotile fibers detected. The highest tremolite count was observed in a man previously employed as a painter/spackler. The other two cases were apparently nonoccupational exposures. One was a housewife of a Navy ship worker, and the other was employed as a teacher's aide in an asbestos-containing building for 18 yr. This latter case is discussed in an earlier report (27). Fiber analysis of acoustical plaster from the building where she was employed revealed tremolite fibers. Pulmonary noncommercial amphibole counts for one other mesothelioma case (Case 7, Table 1) also were greater than all the control values. However, this case was discussed above because uncoated amosite fibers were also detected.

Only one mesothelioma case (Case 17, Table 1) demonstrated a chrysotile level greater than 95% of the control values, but the value was not greater than all of the control levels. Two other mesothelioma cases (Cases 3 and 7) had chrysotile levels greater than 95% of the control levels, but these cases also revealed uncoated amosite fibers and are discussed above.

DISCUSSION

The present study demonstrates that, based on tissue mineral fiber analyses, approximately one-third

(6 of 18) of the mesothelioma cases with normal AB counts have asbestos fiber counts greater than 95% of control levels. These results suggest that these six cases may be asbestos related despite AB counts (by LM) within our normal range. In particular, the higher percentage of noncommercial amphibole fibers detected in the cases in this study (as compared with all mesotheliomas in our previous studies) indicates that fiber-type analysis may be required to differentiate asbestos-related mesotheliomas from nonasbestos cases when AB counts (by LM) are less than 20 AB/g wet lung. It is of interest to compare our results with those of Gibbs *et al.* (28), who studied the mineral fiber content of 84 patients with mesotheliomas. Their selection criteria differed from ours in that their study population consisted of patients for whom the asbestos exposure history was either absent, vague, or ill defined, whereas our cases were selected on the basis of an AB count within our normal range. In spite of the vague or absent exposure history, a substantial number of the cases reported by Gibbs *et al.* had a tissue burden of commercial amphibole fibers (all sizes, as detected by transmission electron microscopy) greater than 1 million fibers/g dry lung, including 22 of 40 patients with household, neighborhood, or no known exposure. Such cases are presumably asbestos related.

The commercial amphibole fiber amosite is a potent inducer of mesothelioma (26), and, in six of our cases, uncoated amosite fibers were detected *versus* none detected for any of the control cases. In two of these cases (Cases 3 and 4), amosite levels clearly exceeded background levels and were the probable cause of the mesothelioma. In three additional cases (Cases 6, 7, and 14), amosite fibers were detected but were not clearly above background level because the calculated values were based on a single fiber detected (*versus* none detected in controls). In one additional case (Case 2), amosite was also detected but at levels below the detection limit for controls because a larger sample size (5 g) was used for this case only. The presence of amosite fibers in three of these cases (Cases 3, 4, and 14) was unexpected because the occupations (two brake repair employees and one vermiculite truck driver) are not normally associated with amosite exposure. One possible explanation for these exceptional cases is incomplete exposure histories, with undocumented low-level amosite exposure. Tremolite is a common contaminant of vermiculite (29, 30) but was not detected in the vermiculite truck driver case presented here.

In four cases (Cases 1, 7, 13, and 15), pulmonary tremolite levels were greater than those of all of the control cases, and, in three of these, no other asbestos fiber types were detected. These four cases had exposure histories to materials that are associated with potential tremolite contamination. Tremolite is a common contaminant of chrysotile (31), the most common commercial asbestos in the United States. The highest tremolite count was detected in a man previously employed as a painter/spackler (Case 1), who may have been exposed to tremolite contamination from the chrysotile released from spackling during the sanding process. The second highest tremolite count was found in a man who was employed in the Navy, and a single amosite fiber was also detected in this case (Case 7). A third case (Case 15) was the wife of a shipyard worker. Although chrysotile asbestos was frequently used at shipyards, amosite (a major fiber type found in shipyards) was not detected in this case. This could mean that amosite was present but below detecting limits in this one case or that her tremolite exposure derived from some other source. The fourth patient was employed in a building that contained tremolite in the acoustical tile, as discussed in a previous report (27). Another potential source of exposure for these latter two cases was cosmetic talc, which also in the past was frequently contaminated with tremolite asbestos (32).

One additional case (Case 17) demonstrated a chrysotile fiber count greater than 95% of the levels for the controls but not greater than all of the control values. However, SEM is not as sensitive at detecting chrysotile fibers, which are frequently less than 5 μm in length and thinner than 0.1 μm (33). We are uncertain regarding the role of asbestos in this particular case.

Nine of the 18 mesothelioma cases (Cases 5, 8 to 12, and 16 to 18) had normal AB counts (by LM) and tissue asbestos contents (by SEM) that were less than or equal to control levels. In addition, AB counts for these nine cases are in the first group of the aforementioned bimodal distribution (Fig. 1). Together, these results strongly support the hypothesis that these nine cases are spontaneous mesotheliomas or are nonasbestos related, despite the fact that three of these cases (Cases 9 to 11) had occupations associated with potential asbestos exposure. Three additional cases (Cases 2, 6, and 14) had detectable amosite but at levels not clearly different from those in controls. These cases are considered to be of uncertain etiology.

Fiber-type distributions (fibers >5 μm in length) for the current study and controls are presented in Table 3. The distribution was significantly different for the mesothelioma cases with normal AB counts (*i.e.*, <20 AB/g wet lung) compared with the distributions for the 94 previously reported cases (26). For example, amosite represented 16% of the fibers detected in the normal-range mesothelioma cases in the current study *versus* 60% of fibers detected in the 94 mesothelioma cases reported earlier (26). Conversely, the noncommercial amphibole percentage was higher for the current study (21% *versus* 10%). Crocidolite fibers greater than 5 μm in length were not detected in either the controls or the normal-range mesothelioma cases in the current study but represented 3% of the fibers in the overall series of mesothelioma cases. The chrysotile percentages were approximately the same for all three populations. The nonasbestos fiber percentages for the normal-range mesothelioma cases in this study and the controls (65% and 79%, respec-

tively) were considerably higher than the 25% nonasbestos fibers reported for the 94 earlier mesothelioma cases. There is no evidence for an increased mesothelioma risk associated with these nonasbestos mineral fibers (32).

Peritoneal mesotheliomas constitute 9% (45 of 458) of the mesotheliomas represented in the author's (V. L. R.) consultation files. This figure is in close agreement with the 10% prevalence noted in a large study of 4710 mesothelioma cases by Hillerdal (34). However, the current study includes a disproportionate number of peritoneal mesotheliomas (3 of 17, 18%). Prior studies have suggested that peritoneal mesotheliomas require a higher asbestos burden for initiation than do pleural mesotheliomas (1, 35, 36). Nevertheless, peritoneal mesotheliomas have previously been reported in patients with brief exposures to asbestos (35). We have also observed that the duration of asbestos exposure was similar and, in fact, statistically indistinguishable for peritoneal and pleural mesothelioma cases (unpublished observations). One of the three peritoneal mesotheliomas in this study (Case 4) was considered to be asbestos related because the amosite levels exceeded control levels. Although it is possible that this is a case of nonasbestos-related peritoneal mesothelioma that just happens to have an elevated amosite content, this explanation seems less likely to us because of the strong association between amosite asbestos exposure and peritoneal mesothelioma and the recognized occurrence of these tumors after relatively brief or low-level exposures. In one other case (Case 2), amosite was detected but at levels below the detection limit for the controls.

Women represent 17% (3 of 18) of the mesothelioma cases in this study compared with 6% (9 of 136) of the mesothelioma cases with AB counts greater than 20 AB/g. This larger share is expected because most mesotheliomas in women are associated with nonoccupational exposure and, thus, with lower AB burdens [McDonald and McDonald (3) estimated that less than 5% of mesotheliomas in North American women are occupationally related]. Two of these three cases are considered to be probable asbestos-related mesotheliomas because the noncommercial amphibole counts exceeded control levels. None of the control cases in this study were women. Therefore, we could not determine whether women in the general population have noncommercial amphibole levels greater than men.

Smoking histories were available for 11 of 18 of the mesothelioma cases and for 9 of 19 of the control cases (Table 1). As mentioned earlier, 9 of these 11 patients with mesotheliomas were smokers (plus one ex-smoker), although only 3 of these 9 control cases had positive smoking histories (one 50 pack-year smoker, one ex-smoker, and one pipe ex-smoker). The total UF, amosite, and noncommercial amphibole counts were different between the mesothelioma and the control groups (P = 0.059, 0.006, and 0.004, respectively). The development of mesothelioma is not associated with cigarette smoking (1, 37). However, cigarette smoking can diminish the lung's ability to clear foreign particles (38–40) and is a possible contributing factor to the higher fiber counts noted for the mesothelioma cases. Furthermore, the smoking histories revealed that four of the six mesothelioma cases that are probably asbestos related had smoking histories greater than 40 yr; whereas only one of the remaining 12 cases did. For the 20 cases with available smoking histories, a linear-regression analysis (24) of pack-years *versus* total UF indicated no evidence of a linear relationship (R = 0.33, $P > 0.05$). (Ex-smokers were excluded from the analysis because pack years were not available, and a value of zero was used for nonsmokers.) Thus, we could not confirm a statistically significant effect of smoking on pulmonary fiber burdens in this small series.

It should be noted that the elevation in fiber content relative to background in six of our mesothelioma cases is small. In addition to differences in smoking history and sex, there is also a potential difference in ambient asbestos levels in the localities from which these patients with mesotheliomas came compared with those in the reference population. Although the patients with mesotheliomas as a group had a statistically significant elevation in amosite and tremolite content, it is conceivable that this difference may merely reflect differences in ambient asbestos levels.

As mentioned earlier, the cases represented in this study were selected from one of the authors' (V. L. R.) consultation files. Therefore, these cases represent a subset of United States medicolegal mesothelioma cases and are not necessarily representative of all mesotheliomas in the United States.

In summary, some malignant mesothelioma cases associated with AB counts that are indistinguishable from the general population may nonetheless be asbestos related. These cases may be differentiated from nonasbestos-related cases by electron microscopic analysis of uncoated pulmonary mineral fibers. Our findings further suggest that, with respect to mesothelioma, some individuals seem to be susceptible to rather low levels of amphibole asbestos exposure.

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Address reprint requests to: Victor L. Roggli, M.D., Department of Pathology, Box 3712, Duke University Medical Center, Durham, NC 27710.

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