Analysis of Asbestos Fiber Burden in Lung Tissue from Mesothelioma Patients

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Mesothelioma is a rare neoplasm that occurs most frequently in individuals with previous asbestos exposure. Differences for risk of development of asbestos-related mesothelioma and lung cancer have been attributed to the various types of asbestos, as well as to the dimension of the inhaled fibers. In the present study, 55 individuals with the pathological diagnosis of mesothelioma were evaluated as to ferruginous body and fiber content in lung tissue. The procedures used in the analysis included tissue digestion and analysis of the collected material for ferruginous bodies by light microscopy and for uncoated fibers by analytical transmission electron microscopy. Forty-six of the samples had ferruginous body concentrations of over 1000/per gram dry weight of lung tissue. The majority of the cores of these ferruginous bodies were amosite. Likewise, the most common uncoated asbestos fiber in the tissue was amosite. Only a small percentage of each type of asbestos would have been visible by light microscopy or even potentially by electron microscopy if the magnification was not sufficient to detect those with thin (<0.2 μm) diameters. The consistent finding in most of the cases was a considerable presence of asbestos, often of mixed types.

Keywords asbestos, electron microscopy, ferruginous bodies, mesothelioma

The relationship between exposure to asbestos and the risk of developing mesothelioma was established through the observations of Wagner in South Africa [1]. Additional reports involving other asbestos-exposed cohorts have further supported this observation. These diverse exposures occur during mining operations [2–4], to individuals working with asbestos-containing products, or with environmental exposures to asbestos-containing dust [2, 5–8]. The most common site for development of this rare tumor is in the pleural cavity, although approximately 10% arise in the peritoneal cavity [9]. In spite of greater worldwide awareness of risks from asbestos exposure, recent reviews pointed out that asbestos-associated cancers continue to increase in incidence [10, 11].

Chrysotile is the most widely used asbestos, accounting for 97% of the world's production in 1976 [12]. It is reasonable, therefore, that various exposure levels from commercial applications should most often be to this type of asbestos. Even so, it has been suggested that in the risk for development of mesothelioma, differences exist between exposures to various amphiboles and to chrysotile [2]. Crocidolite has been reported as having the most potential for causing tumors, followed by amosite, with chrysotile being the least tumorigenic [1, 2, 4]. An increased awareness of the presence of the amphibole tremolite as a component of some chrysotile veins, as well as other mineral deposits, has raised the issue that it may have a major role in the development of mesothelioma following exposure to products made from these minerals [13, 14]. These concepts have given rise to an "amphibole hypothesis" as a basis for explaining the risk of developing an asbestos-related malignancy [15]. In reality, many workplace exposures have been to a mixture of asbestiform minerals, as well as to other fibrous and nonfibrous dusts [16].

The potential for developing lung cancer from asbestos exposure is often confounded with environmental exposures such as cigarette smoking. The risk of mesothelioma induction in humans and animals is more exclusively linked to the inhalation of asbestos fibers. Considerable data on the risks of developing mesothelioma from occupational settings have come from historical reviews of various exposed cohorts. Likewise, tissue burden analysis

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and a correlation of these findings have occurred with groups in which asbestos exposure was part of their daily work activities, such as miners, millers, or workers involved with the manufacture of asbestos-containing products. Much less is known about the quantification of ferruginous bodies and uncoated asbestos fibers in individuals with mesothelioma where exposures were to asbestos-containing end products in the workplace.

The data from tissue analysis are further limited because few studies have been carried out by transmission electron microscopy (TEM) at a sufficiently high magnification to permit a counting scheme that includes short (≥0.5 μm) as well as long, thin fibers. The inclusion of such fibers is necessary for an accurate assessment of uncoated fiber burden. Numbers of chrysotile fibrils, as well as thinner amphibole fibers, could also be ignored by TEM if the counting protocol includes only longer fibers (≥5 μm) and does not use a sufficiently high magnification to compensate for these thin "fibrils." This may be particularly important in mesothelioma, since these are the geometrically defined fibers shown to reach extrapulmonary sites, including the lymph nodes and pleural plaques [17].

The primary purpose of the present study is to characterize the fiber burden in tissue in a series of mesothelioma cases. Unlike most previous studies, this group had diverse exposures ranging from shipyards, where some amphibole exposures would be expected, to vocations where the more likely exposure would be to chrysotile such as cement production and brake shoe factories [12]. With this mixed cohort, the questions to be answered are (1) What relationship exists between the chrysotile and amphibole burden in individuals with mesothelioma? (2) Which amphibole was most commonly encountered? and (3) What are the length and width characteristics of the asbestos fibers in these cases of mesothelioma? This final question is of particular interest, since a recent report suggests there may be a relationship between low-aspect-ratio amosite fibers and mesothelioma [18]. This is in contrast with the theory that long, thin fibers [12, 19–23] are reported to be the most tumorigenically active of the inhaled fibers, and raises further issues as to the importance of the shorter fibers as contributors to such disease.

The data collected in this study also provided an opportunity to evaluate other aspects of mesothelioma, including latency period, length of survival, and influence of histologic type on survival time. In addition, each case was evaluated for whether asbestosis was present, and this was correlated with asbestos body and asbestos fiber concentrations in lung tissue.

MATERIALS AND METHODS

A cohort of 55 individuals with a pathological diagnosis of mesothelioma (made by one of the co-authors, SH) was selected for analysis and characterization as to the types of asbestos fibers within their lung tissue. All of the cases included in this study either had complete or partial autopsies (47 cases) or had lungs and pleural tissue harvested (8 cases).

The diagnosis of mesothelioma in each case was made by established criteria, including immunohistochemistry, and electron microscopy [24, 25]. Histologically, the mesotheliomas were grouped into four major categories: (1) epithelial, (2) biphasic, (3) sarcomatoid, and (4) desmoplastic, though other histologic subtypes of mesothelioma have been recognized [24, 25].

Lung tissue from patients was evaluated by one of the authors (SH) for pathologic asbestosis and graded according to the College of American Pathologists and the National Institute for Occupational Safety and Health criteria [26]. An approximate 5-g sample from a site in each lobe was also collected for a digestion procedure in the Bremerton Laboratory [27] and determination of ferruginous bodies per gram wet lung.

All cases in the cohort were from the Pacific Northwest and most had exposures associated with shipyard-related activities. The historical data listed in Table 1A include sex, age, smoking history, and occupation; Table 1B has other medical history; and Table 1C lists dates of first and last exposure and dates of diagnosis and death.

The lung tissue preparation for light and electron microscopy as carried out in the Tyler Laboratory consisted of the Williams' modified bleach procedure [28]. Multiple sites from tumor-free, formalin-fixed lung parenchyma were sectioned from each lung for quantitative analysis. Two separate digest pools were done on each case, one from the right lung and one from the left. The right digest pool contained an average of 0.3478 g dry weight of lung tissue (range 0.8403 to 0.0970) and the left digest pool contained an average of 0.3276 g dry weight of lung tissue (range 0.6798 to 0.0801). These pools were processed through the modified bleach (9% sodium hypochlorite) digestion procedure of Williams et al. [28]. Dry weights were calculated based on a wet/dry ratio obtained for each digest pool. All reagents were prefiltered through 0.2-μm pored polycarbonate filters. Aliquots of the pools were collected on 0.2-μm pored polycarbonate filters for analytical transmission electron microscopy (ATEM) and on 0.22-μm pored mixed cellulose ester filters for quantitation of ferruginous bodies by light microscopy.

Mixed cellulose ester filters were used to evaluate the ferruginous body content. These filters contained an average of 0.0329 g dry weight of lung tissue (range 0.0660 to 0.0068). One-fourth of the filter was collapsed in acetone vapor (making it transparent) and evaluated by light microscopy at ×100, ×200, and ×400. The ferruginous bodies that were noted as being typical in morphology (beaded coat, reddish-brown color, and clear core) were

TABLE 1A Historical data for mesothelioma cases

Assay number	Sex	Age[a]	Packs/year[b]	Asbestosis/grade	Work history[c]
L-1	M	64	NS	Y/1	Shipfitter, insulator asbestos exposed
L-2	M	69	U	N	Unknown, asbestos exposed
L-3	M	58	32	Y/1	Shipyard insulator
L-4	M	82	50	Y/1	Electrical engineer
L-5	M	73	NS	Y/1	Contractor (23), sheet metal (7), shipyard (1)
L-6	M	62	NS	N	Machinist (32), clerk (3), Navy (2)
L-7	M	85	13	N	Shipyard painter (25), railroad brakeman (25)
L-8	M	63	29	Y/1	Carpenter (28), Navy (1), machinist (1)
L-9	M	57	NS	Y/1	Electrical contractor (24), boilermaker (6), Army other (4)
L-10	M	60	2	N	Drywall, janitor
L-11	M	U	37	Y/1	Asbestos pipe/brick (20), boilermaker (3)
L-12	M	75	38	Y/1	Painter
L-13	M	71	50	N	Tool engineer/machinist/plant manager/mechanic (50), other (4)
L-14	M	67	43	Y/1	Shipyard (2)
L-15	M	73	4	Y/1	Electrician, laborer
L-16	M	U	NS	N	Civil and structural engineer
L-17	M	76	7	Y/1–3	Shipyard WWII, pipe fitter
L-18	M	60	S(pipe)	N	Contractor (28), seaman/master (9)
L-19	M	72	20	Y/1	Boilermaker
L-20	M	U	10	N	Sheet metal/shipyard (5), sales
L-21	M	71	30	Y/1	Unknown
L-22	M	76	S(pipe)	N	Shipyard inspector, construction
L-23	M	70	28	Y/1	Railroad boilermaker
L-24	M	51	U	Y/1	Plumber/pipe fitter (13), construction (4)
L-25	M	72	NS	N	Ship refrigeration/Asbestos removal (28)
L-26	M	62	50	N	Shipyard worker (2)
L-27	M	74	30–50	Y/1–3	Steel mill sales (15), boilermaker/railroad (6)
L-28	M	74	NS	Y/1	Merchant Marine
L-29	M	83	16	Y/1	Shipfitter
L-30	M	65	16	N	Orchard work, boiler room welder (1.5)
L-31	M	71	100	N	Cement mason (25)
L-32	F	67	23	N	Electronic components assembler (11)
L-33	M	70	35	U	Insulator (36)
L-34	M	69	35	N	Shipyard, ship building and repair
L-35	M	65	U	N	Unknown
L-36	M	67	NS	N	Shipyard pipe fitter
L-37	M	73	74	N	Packing plant (used asbestos wool)
L-38	M	55	U	N	Spraying asbestos
L-39	M	75	U	Y/1–2	Unknown
L-40	M	72	15	Y/1	Pipe fitter (1), machinist/shipyard (26)
L-41	M	43	13	N	Laborer/construction
L-42	M	62	NS	Y/1	Construction (14), concrete (6), asbestos tile (3)
L-43	M	82	NS	Y/1	Sheet metal worker, furnace installer
L-44	M	74	4	Y/1–2	Shipyard insulator and pipe coverer
L-45	M	79	52	Y/1	Electrician (4)
L-46	M	65	60	N	Shipyard machinist
L-47	M	87	NS	N	Shipyard rigger, crane operator
L-48	M	82	7–10	Y/1–2	Shipyard electrician (23)
L-49	M	66	22	Y/1	Pipefitter/shipfitter
L-50	M	77	2	Y/1	Shipyard painter
L-51	M	60	S	Y/3	Unknown
L-52	M	75	45	N	Navy/shipyard (20)
L-53	M	66	11	Y/1	Electrician
L-54	M	74	53	N	Unknown
L-55	F	74	15	N	Brake shoe factory (2)

Note. U and None, unknown or unavailable; Y, yes; N, no.

[a]Age at time of death.

[b]NS for nonsmoker, U for unknown, S for smoker listed as number of packs/year.

[c]Job and (number of years worked).

TABLE 1B Historical data for mesothelioma cases

Assay number	Location of mesothelioma	Histological type[a]	Parietal plaque	Location of metastases	Medical history, other
L-1	Left pleura	Epith.	Y	Peritoneum, peripancreatic, lymph node	Cardiovascular, pneumonia
L-2	Right pleura	Mixed biph./sarco.	Y	Mesentery	Thymoma, prostate adenocarcinoma
L-3	Peritoneum	Epith.	Y	Umbilicus	
L-4	Left pleura	Mixed biph./sarco.	Y	Peritoneal	Cardiovascular
L-5	Peritoneum	Epith.	Y	Abdominal wall	None
L-6	Right pleura	Biph.	N	Liver, pericardium, Right lung	Hypertension, pericarditis
L-7	Right pleura	Epith.	Y	Left visceral pleura, Left lung, lymph nodes	Cardiovascular, diverticulosis
L-8	Left pleura	Sarco.	Y	None	Bronchitis, pneumonia
L-9	Right pleura	Epith.	Y	Peritoneal, periaortic	Bronchitis, pneumonia
L-10	Right pleura	Epith.	N	Left lung, lymph nodes	
L-11	Left pleura	Sarco.	U	U	Bronchitis, pneumonia
L-12	Left pleura	Mixed desmo./sarco.	Y	Abdominal serosa	Cardiovascular
L-13	Left pleura	Epith.	Y	U	Chronic obstructive pulmonary disease
L-14	Peritoneum	Epith.	Y	None	Pulmonary thromboembolic
L-15	Right pleura	Sarco./desmo.	Y	U	Bronchitis, prostate adenocarcinoma
L-16	Right pleura	Poorly differentiated epith.	U	U	
L-17	Right pleura	Mixed desmo./sarco.	Y	None	Chronic obstructive pulmonary disease, diabetes
L-18	Right pleura	Sarco.	Y	None	Fibrosis, cardiovascular
L-19	Right pleura	Epith.	Y	Lymph nodes, serosa of stomach	
L-20	Right pleura	Sarco.	Y	U	Bronchitis, pneumonia
L-21	Left pleura	Biph.	Y	None	Bronchitis, pneumonia, alveolar damage
L-22	Right pleura	Mixed sarco./desmo	U	None	Bronchitis, pneumonia, alveolar damage
L-23	Left pleura	Variable differentiation	Y	Nodes, liver, spleen, Right Lung	Emphysema, atherosclerosis
L-24	Right pleura	Variable differentiation	Y	Right lung	None
L-25	Peritoneum	Mixed epith./sarco.	N	None	None
L-26	Right pleura	Sarco.	Y	Mesentery, adrenals, Right pleura	Bronchitis, pneumonia, arteriosclerotic/ cardiovascular
L-27	Right pleura	Sarco.	Y	Liver, peritoneum, spleen, node	Bronchitis, pneumonia, emphysema
L-28	Left pleura	Epith.	U	Right lung, lymph nodes	Arteriosclerotic/ cardiovascular
L-29	Left pleura	Mixed pleomorphic	Y	Left lung, pericardium, liver, spleen	Arteriosclerotic/ cardiovascular
L-30	Right pleura	Epith.	Y	Lymph nodes, Left chest wall	Arteriosclerotic/ cardiovascular
L-31	Right pleura	Epith.	Y	Lymph nodes, Left pleura, Left Lung	Arteriosclerotic/ cardiovascular
L-32	Right pleura	Pleomorphic	U	U	None
L-33	Right pleura	Epith.	Y	Left visceral and parietal pleura	Arteriosclerotic/ cardiovascular
L-34	Left pleura	Epith./ tubulopapillary	Y	Lymph nodes	Arteriosclerotic/ cardiovascular
L-35	Left pleura	Poorly differentiated	U	Right lung, epicardium	None
L-36	Right pleura	Biph.	Y	Left lung, liver, adrenal, kidneys	None
L-37	Left pleura	Epith.	Y	Hilar/bronchopulmonary	None
L-38	Peritoneum	Epith.	Y	None	None
L-39	Right pleura	Epith.	Y	Left lung, pleura, liver, lymph nodes	None

TABLE 1B *(Continued)*

Assay number	Location of mesothelioma	Histological type	Parietal plaque	Location of metastases	Medical history, other
L-40	Right pleura	Mixed sarco./biphasic	U	None	None
L-41	Right pleura	Sarco.	N	U	None
L-42	Right pleura	Mixed sarco./desmo.	U	Epicardium	None
L-43	Left pleura	Epith.	Y	Left and Right lung, abdominal cavity	None
L-44	U	Epith.	U	U	None
L-45	Right pleura	Sarco.	Y	Pancreas, liver, subcut. tissue	None
L-46	Left pleura	Biph.	Y	Lymph node, pericardium, myocardium	None
L-47	Right pleura	Biph.	Y	Hilar, lymph nodes, Right lung	None
L-48	Right pleura	U	Y	U	None
L-49	Left pleura	Epith.	Y	Left Lung	None
L-50	Right pleura	Sarco.	Y	Adrenal gland, heart, mesentery	None
L-51	Right pleura	Epith.	Y	U	None
L-52	Right pleura	Epith.	Probable	U	None
L-53	Left pleura	Epith.	Y	Hilar, lymph nodes, mesentery	None
L-54	Left pleura	Mixed desmo./sarco.	Y	Omentum, myocardium, lymph nodes	Emphysema
L-55	Left pleura	Epith./tubulopapillary	N	Left lung, vertebra, psoas muscle	None

Note. Y, yes; N, no; U, unknown or unavailable.
Biph., biphasic; Sarco., sarcomatoid; Desmo., desmoplastic; Epith., Epithelial.

considered asbestos bodies and thus a component of the asbestos burden. For each case, results from right and left sides were averaged and reported as ferruginous bodies per gram dry weight of lung tissue.

The polycarbonate filters contained an average of 0.00338 gram dry weight of lung tissue (range 0.0132 to 0.0019). They were examined by light microscopy at ×100, ×200, and ×400, and ferruginous bodies were evaluated. Filters were prepared via direct method for ATEM. By this method, the filters were carbon coated, cut into 3-mm^2 pieces, and mounted on 100-mesh copper grids. A modified Jaffe–Wick method was used to remove the filter matrix, leaving a carbon film containing the entrapped fibers, ferruginous bodies, and other particulates [29]. Fiber quantitation and ferruginous body core analysis were done by ATEM. Quality-control procedures, in addition to the prefiltering of reagents, included analysis of selected filters from the lot used in the procedures, as well as reagent/filter blanks. The data from these analyses were used to establish background for comparative purposes.

The grids were evaluated for continuity and overall quality of the films at ×20,000 or at a lower magnification of ×2,500 in a JEOL 1200EX, which was interfaced with either a TN-5500 X-ray analyzer or an EDAX NX-2 X-ray analyzer. Uncoated fibers ≥0.5 μm long having parallel sides for a majority of their length and with a length/width ratio greater than 3:1 were counted. Either the first 100 fibers in a completed opening or all the fibers in 10 openings were counted from each of 3 grids, which is equivalent to 1.32 mm^2. In addition, the remainder of the grids were scanned at the lower magnification for ferruginous bodies. When ferruginous bodies were found, the cores were analyzed by ATEM. These were characterized by X-ray energy dispersive spectrometry (XEDS) and selected area electron diffraction (SAED). Length/width, composition, and various ratios were noted for the asbestos fibers. Results were determined as fibers per gram dry weight of lung tissue for total asbestos, as well as for individual fiber types. To provide further compensation for random sampling, the data are reported as a weighted average obtained from the two sides (Table 2).

Fiber concentrations were found to have highly skewed distributions, as illustrated in Figure 1, for all fiber types; therefore, medians were used as the usual summary measures of central tendency. Fiber lengths, widths, and aspect ratios were all summarized in terms of geometric means. The nonnormal distributions of the data dictated that nonparametric statistical methods, such as Fisher's exact for 2 × 2 tables, and the Wilcoxon and Mann–Whitney tests for comparisons of fiber concentrations, lengths, widths, and aspect ratios, be used.

TABLE 1C Historical data for mesothelioma cases

Assay number	Year of first exposure	Year of last exposure	Date of diagnosis[a]	Latency period (years)[b]	Date of death[a]	Survival from date of diagnosis (months)
L-1	1956	1975	07-91	35	11-92	16
L-2	U	U	11-91	U	12-92	13
L-3	1954	1962	07-92	38	11-92	4
L-4	1942	1973	01-92	50	05-92	4
L-5	1937	1946	07-91	54	10-91	3
L-6	1945	1984	10-90	45	04-91	7
L-7	1921	1971	07-91	70	11-91	4
L-8	1945	1976	10-91	46	10-91	4
L-9	1960	1990	12-90	30	10-91	10
L-10	1959	1960	01-90	31	04-91	14
L-11	1942	1958	08-92	50	01-93	5
L-12	1941	1976	04-91	50	04-91	1 wk
L-13	1939	1978	01-92	53	05-93	16
L-14	1951	1953	01-93	42	03-93	2
L-15	1940	1970	09-92	30	01-93	4
L-16	1955	1992	03-92	37	12-92	9
L-17	1941	1945	01-93	52	04-93	3
L-18	1938	1988	07-92	54	04-93	9
L-19	1939	1972	01-93	54	07-93	6
L-20	1942	1947	01-93	51	05-93	4
L-21	1941	1946	10-86	45	10-87	12
L-22	1941	1945	08-87	46	10-87	2
L-23	1940	1954	04-90	50	11-90	7
L-24	1961	1974	11-90	29	08-91	8
L-25	1946	1982	11-90	44	02-91	2½
L-26	1946	1965	03-91	45	05-91	2
L-27	1939	1980	04-90	51	09-90	5½
L-28	1943	1951	10-90	47	10-91	12
L-29	1941	1949	08-90	49	08-91	12
L-30	1942	1975	05-88	46	01-90	19
L-31	1945	1981	09-85	40	11-87	26
L-32	1951–1959	1970	12-87	36	02-89	12
L-33	1947	1983	05-87	40	12-87	7
L-34	1942	1974	12-85	43	02-88	25
L-35	U	U	U	U	07-84	U
L-36	1945	1980	02-87	42	11-87	9
L-37	1937	1938	09-87	50	01-88	5
L-38	1948	1948(3 Mon)	07-87	41	01-88	6
L-39	U	U	U	U	05-88	U
L-40	1941	1974	03-93	52	09-93	5
L-41	1966	1980	09-91	25	10-92	12
L-42	1961	1987	12-87	46	04-88	4
L-43	1916	1971	05-87	71	07-88	14
L-44	1942	1949	10-87	45	03-88	5
L-45	1941	1945	08-86	45	07-87	12
L-46	1954	1980	03-89	35	06-89	3
L-47	1940	1947	05-89	49	09-89	4
L-48	1942	1955	03-90	48	02-91	10
L-49	1943	1971	10-88	45	06-90	20
L-50	1941	1970	03-90	39	04-90	1
L-51	U	U	02-87	U	10-87	8
L-52	1945	1965	07-87	42	08-87	2
L-53	1942	1944	02-87	U	10-87	8
L-54	1935	1946	01-89	54	06-89	5
L-55	1929	1931	03-86	57	06-87	15

Note. U, unavailable.
[a]Month-year.
[b]Latency period = date of diagnosis minus date of first exposure.

TABLE 2 Total uncoated asbestos fibers and ferruginous bodies per case

Total fibers/ g dry	FB/g dry[a]	FB/g wet[a]	Fiber/ FB's ratio[b]	Assay number
68,950,000	1,122,333	201,416	61	L-33
63,417,431	1,248,686	170,985	51	L-38
32,812,500	683,269	114,788	48	L-44
13,625,806	1,008,031	195,419	14	L-3
6,875,000	227,787	31,739	30	L-48
5,701,613	150,670	29,077	38	L-9
4,136,364	170,082	27,780	24	L-11
3,999,548	85,783	13,114	47	L-19
3,989,464	34,456	4,903	116	L-14
2,925,000	38,930	6,832	75	L-52
2,722,222	45,114	7,219	60	L-17
2,581,967	33,704	5,234	77	L-22
2,230,392	83,524	14,116	27	L-53
2,119,141	97,618	15,523	22	L-36
1,894,587	181,712	26,464	10	L-15
1,682,692	21,077	4,100	80	L-43
1,563,333	21,278	3,325	73	L-23
1,439,394	43,627	8,391	33	L-7
1,422,764	38,447	3,952	37	L-34
1,045,300	2,686	458	389	L-40
1,014,493	4,635	528	219	L-42
928,030	17,984	2,968	52	L-51
923,261	8,851	1,536	104	L-37
899,306	3,620	546	248	L-20
855,263	81	13	10,559	L-35
758,808	3,571	547	212	L-28
740,385	4,304	699	172	L-41
698,357	107,211	18,968	7	L-1
694,444	3,089	488	225	L-55
665,205	9,564	2,114	70	L-21
606,436	134,634	17,677	5	L-50
588,235	268	40	2,195	L-26
559,764	7,849	971	71	L-24
505,556	17,891	3,338	28	L-5
490,924	13,425	1,631	37	L-39
446,697	2,867	400	156	L-16
433,559	4,115	759	105	L-47
391,791	36,266	6,074	11	L-49
352,011	1,938	279	182	L-30
331,439	8,591	1,409	39	L-46
318,627	7,718	1,148	41	L-29
291,667	16,609	2,542	18	L-12
239,726	3,993	737	60	L-4
222,458	0	0	0	L-18
217,662	1,764	299	123	L-45
159,091	2,082	325	76	L-32
150,538	1,240	240	121	L-31
131,579	965	160	136	L-54
112,179	347	60	323	L-27
72,016	3,762	756	19	L-8
62,500	9,699	1,737	6	L-2
43,532	281	46	155	L-6
43,532	60	10	726	L-10
22,096	365	60	61	L-25
0	0	0	0	L-13

[a]FB/g determined on digestion pool from multiple sites of right and left lung by Tyler laboratory.
[b]Based on dry weight per gram.

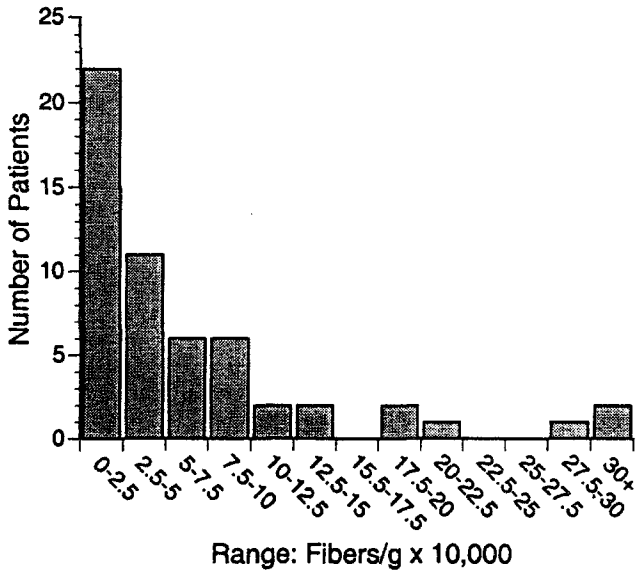

FIG. 1 *Tremolite fiber loads: the number of patients having fiber loads* (fibers per g) *of tremolite for a range of fiber loads.*

RESULTS

Because of the extensiveness and complexity of the data, the sections are discussed as Medical Evaluation (Tables 1A–C), Ferruginous Body (FB) Data (Table 2), and Uncoated Fiber Data (Table 3). When the term "ferruginous body" is used in the text to describe structures as seen by light microscopy, it implies a morphology consistent with an asbestos body.

Medical Evaluation

Table 1A

There were 53 males and 2 females in the study and the mean age at time of death was 69.5 years (range 43 to 87). Thirty-eight were smokers, 11 were nonsmokers, and smoking history was unavailable for 6. The majority of the cases had occupational exposures related to shipyard activities. The specific type of job and number of years at that job are listed on Table 1A.

Table 1B

The majority of the cases (49) had pleural mesothelioma; (30 were right pleura and 19 were left pleura), and there were 5 cases of peritoneal mesothelioma. Location of tumor was unavailable on 1 of the cases. Pleural plaques were found in 39 of 44 cases. This information was unavailable in 11 cases. The mesothelioma had metastasized in 35 cases. No metastases were found in 9 cases and data were unavailable in 11 cases. The histological type of the tumor is listed in Table 1B.

Table 1C
Latency period for the purposes of this study was defined as the difference between date of diagnosis and date of first exposure. While the latency period ranged from 25 to 71 years, the mean was 45 years, with 45 and 50 years being most frequent. Latency information was not available in 6 cases. Survival from date of diagnosis ranged from 1 week to 26 months and had a mean of 8.3 months. Survival information was unavailable in 2 cases.

Survival was also determined according to histologic type of mesothelioma. Twenty-five epithelial mesotheliomas, which included four peritoneal mesotheliomas, had a mean survival of 10.64 months, with a range of 2–26 months. When excluding the four peritoneal mesotheliomas, the mean survival was 11.95 months. Eleven sarcomatoid mesotheliomas had a mean survival of 6.14 months, with a range of 1–12 months. Eight biphasic mesotheliomas had a mean survival of 6.81 months, with a range of 2.5–12 months. Four mesotheliomas exhibited variable differentiation, showing more than four histologic patterns. These four mesotheliomas had a mean survival of 7.38 months, with a range of 2.5–12 months. One of the mesotheliomas showing variable differentiation was a peritoneal mesothelioma. When that case is excluded, the mean survival was 9 months, with a range of 7–12 months. Six desmoplastic mesotheliomas had a mean survival of 3.04 months, with a range of 1 week to 5 months. Five peritoneal mesotheliomas (four epithelial, one showing variable differentiation) had a mean survival of 3.5 months, with a range of 2–6 months.

Ferruginous Body Data From Tyler Laboratory

As described in methodology, aliquots from the right and left lung were evaluated for ferruginous bodies. To compensate for random sampling, the data represent an average between the 2 aliquots from each patient (Table 2). Forty-six of the 55 patients (83.6%) were found to have an average ferruginous body burden of greater than 1,000 ferruginous bodies per gram of dry weight of lung tissue, a concentration often quoted as representing occupational levels of tissue burden [30]. This is clearly above the <20 ferruginous bodies per gram of wet lung tissue reported for the general population in studies by Roggli et al. [31, 32], Breedin and Buos [33], and Dodson et al. [34]. The highest concentration was 1,248,686 ferruginous bodies per gram dry of lung tissue (case L-38), while the mean in this group of 46 cases was 125,567 ferruginous bodies per gram dry of lung tissue. A subpopulation of the group (4 out of 55, 7.3%) was found to have ferruginous body levels within the range reported in general populations (<20 ferruginous bodies per gram wet weight of lung tissue) [33, 34]. The tissue from 6 patients contained a range of from 60 to 965 ferruginous bodies per gram dry weight of lung tissue. Tissue from 2 individuals (L-13, L-18) did not have ferruginous bodies as defined within the limit of detection inherent in the methods used in the light microscopy analysis (10–35 ferruginous bodies per gram of wet lung tissue and 85 to 240 ferruginous bodies per gram of dry lung tissue).

Core analysis of the 841 ferruginous bodies found by ATEM indicated that 781 (92.9%) were formed on amosite, 24 (2.9%) on crocidolite, 8 (1.0%) on tremolite, 3 (0.4%) on anthophyllite, 3 (0.4%) on actinolite, and 1 (0.1%) formed on chrysotile. In addition, 11 (1.3%) were formed on nonasbestos fibers, and 10 (1.2%) were either totally coated or successful analysis of the core material could not be achieved. There was considerable variation in the ratio of uncoated fiber to ferruginous body burden. These ranged from 5:1 to as high as 10,559:1 (Table 2).

Uncoated Fiber Data

The uncoated fiber data were based on analysis by XEDS, SAED, as well as measurement of length and width of 3,244 asbestos fibers. The total uncoated asbestos burden in the patients ranged from a high of 68,950,000 fibers per gram dry weight of lung tissue to a low of 22,096 fibers per gram dry weight of lung tissue. The latter was represented by an amosite burden. While the numbers of uncoated fibers can vary with the techniques used in the counting scheme [35], a synopsis of the data on the subject is included in a recent review [36]. Within the limits of detection used in the study (17,100 to 350,000 fibers per gram dry weight of lung tissue), no asbestos fibers were found in samples from one patient, case L-13 (Table 3). There were no significant right/left side differences as to concentration or prevalence of fiber types. The same analysis indicated no statistical differences for fiber types per left versus right side for lengths, widths, and aspect ratios. Peritoneal tumors had significantly thinner amosite fibers than did pleural tumors ($p < .04$).

As shown in Figure 2, the most common commercial amphibole was amosite in that 53 of the cases (96.4%) were found to have detectable levels. The highest concentration of amosite was 67,550,000 fibers per gram dry weight of lung tissue with 18 of the patients (32.7%) having over one million amosite fibers per gram dry weight of lung tissue. A total of 39 patients (70.9%) had amosite levels greater than 200,000 fibers per gram dry weight of lung tissue. Uncoated amosite was found in lengths up to 137 μm; however, the majority was less than 13 μm in length. The geometric mean of the length of all amosite fibers measured was 12.9 μm and the geometric mean of the width of the measured amosite fibers was 0.244 μm (Table 4). The geometric mean of the aspect ratios for the

TABLE 3 Uncoated fiber data per case (fibers/g dry)

Total asbestos	Amosite	Crocidolite	Tremolite	Actinolite	Anthophyllite	Chrysotile	Nonasbestos	Assay number
68,950,000	67,550,000	1,400,000	0	0	0	0	2,100,000	L-33
63,417,431	29,434,251	28,899,083	0	0	0	0	0	L-38
32,812,500	31,506,530	326,493	163,246	0	326,493	489,739	2,122,201	L-44
13,625,806	13,412,903	0	0	0	0	212,903	1,064,516	L-3
6,875,000	6,593,750	156,250	31,250	0	0	93,750	500,000	L-48
5,701,613	4,770,161	28,226	169,355	56,452	56,452	620,968	1,552,419	L-9
4,136,364	4,003,788	0	0	0	0	132,576	2,280,303	L-11
3,999,548	3,651,762	223,577	0	0	0	124,210	596,206	L-19
3,989,464	3,453,065	134,100	134,100	234,674	0	33,525	804,598	L-14
2,925,000	2,800,000	50,000	75,000	0	0	0	475,000	L-52
2,722,222	2,527,778	0	21,605	21,605	21,605	129,630	1,123,457	L-17
2,581,967	2,175,546	23,907	47,814	47,814	23,907	262,978	2,486,339	L-22
2,230,392	2,144,608	0	64,338	0	0	21,446	150,123	L-53
2,119,141	1,936,849	22,786	45,573	45,573	0	68,359	250,651	L-36
1,894,587	1,346,154	0	448,718	0	49,858	49,858	698,006	L-15
1,682,692	1,383,547	56,090	37,393	130,876	0	74,786	616,987	L-43
1,563,333	1,143,333	0	46,667	0	46,667	326,667	233,333	L-23
1,439,394	1,060,606	0	303,030	18,939	37,879	18,939	1,193,182	L-7
1,422,764	853,659	0	106,707	71,138	177,846	213,415	960,366	L-34
1,045,300	613,546	45,448	204,515	68,172	22,724	90,896	2,863,214	L-40
1,014,493	855,978	0	63,406	31,703	0	63,406	475,543	L-42
928,030	839,646	0	22,096	66,288	0	0	66,288	L-51
923,261	776,379	62,950	0	41,966	0	41,966	440,647	L-37
899,306	753,472	97,222	0	24,306	0	0	388,889	L-20
855,263	614,035	0	65,789	87,719	87,719	0	1,162,281	L-35
758,808	23,713	23,713	284,553	165,989	260,840	0	1,067,073	L-28
740,385	673,077	0	0	22,436	0	44,872	291,667	L-41
698,357	492,958	41,080	0	61,260	20,540	82,160	246,479	L-1
694,444	300,926	0	46,296	23,148	0	324,074	509,259	L-55
665,205	614,035	34,113	0	0	17,057	0	447,583	L-21
606,436	462,046	0	28,878	0	0	115,512	0	L-50
588,235	392,157	0	73,529	0	0	122,549	588,235	L-26
559,764	382,997	29,461	29,461	0	117,845	0	353,535	L-24
505,556	427,778	0	0	0	0	77,778	194,444	L-5
490,924	404,290	0	57,756	0	28,878	0	231,023	L-39
466,697	262,763	0	52,553	0	52,553	78,829	367,868	L-16
433,559	374,437	39,414	0	0	19,707	0	256,194	L-47
391,791	348,259	43,532	0	0	0	0	195,896	L-49
352,011	176,006	0	125,718	0	0	50,287	678,879	L-30
331,439	287,247	0	0	0	44,192	0	44,192	L-46
318,627	220,588	0	73,529	0	24,510	0	171,569	L-29
291,667	72,917	0	97,222	0	121,528	0	145,833	L-12
239,726	199,772	0	19,977	19,977	0	0	739,155	L-4
222,458	49,435	0	0	0	74,153	98,870	568,503	L-18
217,662	195,896	0	21,766	0	0	0	544,154	L-45
159,091	159,091	0	0	0	0	0	70,707	L-32
150,538	37,634	37,634	18,817	56,452	0	0	188,172	L-31
131,579	109,649	0	21,930	0	0	0	87,719	L-54
112,179	56,090	28,045	28,045	0	0	0	84,135	L-27
72,016	36,008	0	0	0	0	36,008	252,058	L-8
62,500	62,500	0	0	0	0	0	395,833	L-2
43,532	21,766	0	0	0	0	21,766	108,831	L-6
43,532	0	0	0	21,766	0	21,766	261,194	L-10
22,069	22,069	0	0	0	0	0	22,096	L-25
0	0	0	0	0	0	0	66,794	L-13

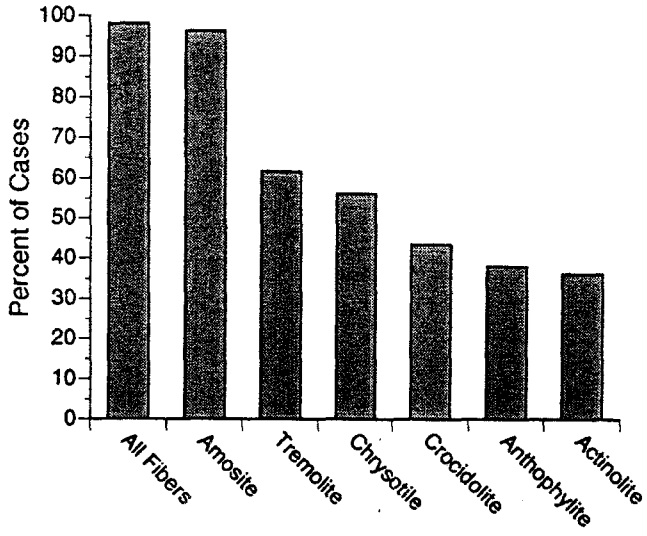

FIG. 2 *Percentage of cases with fibers present, by fiber type.*

amosite was 43.6. A comparison of geometric means is shown by the boxplots in Figure 3.

Crocidolite fibers were found in 22 (40.0%) of the cases, with none detected in the remaining 33 (60.0%) cases. The concentration of crocidolite was similar to that of amosite in 3 of the cases (5.5%) (Table 3). In all 22 cases, where both fiber types of commercial amphiboles were found, amosite was generally the predominant type by ratios varying from 1:1 to as high as 169:1. There were 31 additional cases (56.4%) where amosite was present with no crocidolite detected. In those cases, the amosite ranged from 21,770 fibers to over 13,413,000 fibers per gram dry weight of lung tissue. The length as determined by geometric mean of the crocidolite fibers was 8.46 μm, while the width was 0.194 μm. The crocidolite fibers were, therefore, shorter and thinner when compared to the same parameters for amosite. A further comparison as shown in Figure 3 shows that crocidolite had a slightly higher aspect ratio. Case L-38, which had the highest concentration of uncoated crocidolite, and an approximately equal amount of amosite, was found to have ferruginous bodies formed on both amphiboles. The average length of ferruginous bodies formed on crocidolite was 28.6 μm, while that for amosite was 37.4 μm.

TABLE 4 Fiber characteristics: geometric means

Fiber type	Length (μm)	Width (μm)	Aspect ratio
Amosite	12.90	0.244	43.6
Crocidolite	8.46	0.194	51.6
Chrysotile	6.36	0.076	103.6
Tremolite	6.25	0.285	27.6
Actinolite	8.71	0.328	31.4
Anthophyllite	8.51	0.564	23.1

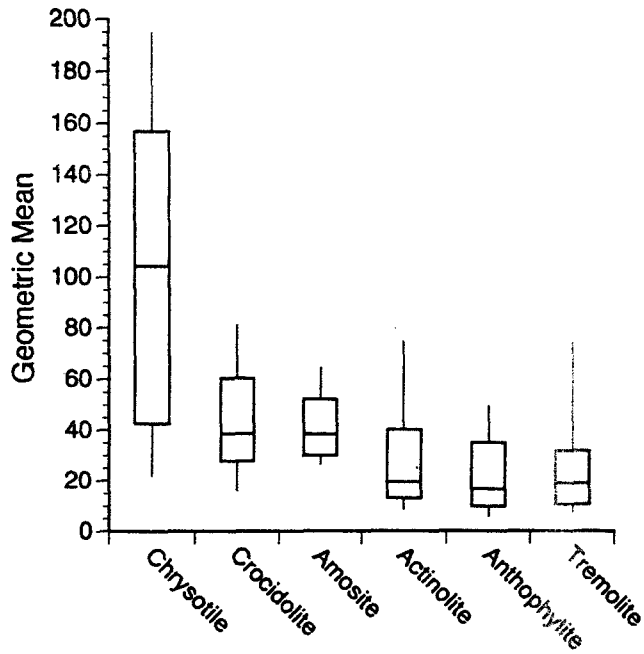

FIG. 3 *Aspect ratio by fiber type. The top, bottom, and interior lines of the boxes correspond to the 75th percentile* (top quartile), *25th percentile* (bottom quartile), *and 50th percentile* (median) *respectively. The whiskers extend from the 10th percentile* (bottom) *and the 90th percentile* (top).

Chrysotile was either found in equal concentration with amphiboles or as the majority asbestiform mineral in only three of the patients (cases L-6, L-8, and L-10). These individuals had an overall low asbestos burden. No chrysotile was detected in 24 (43.6%) cases. The chrysotile concentration ranged from 18,900 to 621,000 fibers per gram dry weight of lung tissue. As has been reported in previous studies, chrysotile fibers were generally short, with the length (based on geometric means) being 6.36 μm and a very thin width of 0.076 μm.

The most common noncommercial amphibole was tremolite, which was found in 33 cases (60.0%). Tremolite fibers were similar in length (geometric mean of 6.25 μm) to chrysotile, but in diameter (geometric mean of 0.285 μm) were more similar to amosite. The average aspect ratio for tremolite based on geometric mean was 27.6. The longest tremolite fiber observed was 26 μm. Several studies have suggested that tremolite, as a contaminant of chrysotile, is important in the risk of developing mesothelioma among chrysotile workers [37], as well as in miners and millers [38, 39]. In the present study, 11 of the patients (20.0%) with chrysotile did not have detectable tremolite. Conversely, 13 patients (23.6%) with detectable levels of tremolite had no chrysotile detected. Thus, the authors do not believe the present data could support an argument that the tremolite exposure occurred from tremolite contamination in chrysotile.

Even though there is a mild correlation between chrysotile fibers per gram versus tremolite fibers per gram (0.23 = p value of <.05), there is an even stronger correlation between amosite and chrysotile ($p = .45$, $p < .0001$). If one chooses to make the argument that tremolite presence was attributable to chrysotile, then a stronger argument could be made that chrysotile accounts for the presence of amosite. The latter point has not been suggested in the literature as a naturally occurring link.

Lung tissue from 21 (38.2%) cases had detectable limits of actinolite. The length (geometric mean) of actinolite fibers was 8.71 μm, with the aspect ratio (geometric mean) being 31.4. The other noncommercial amphibole, anthophyllite, was found in 21 (38.2%) cases. Anthophyllite fibers, as with the actinolite findings, tend to occur more frequently in the individuals with the overall higher fiber burdens. The anthophyllite fibers were similar in length (geometric mean, 8.51 μm) to the actinolite fibers. The aspect ratio of the anthophyllite fibers was 23.1.

While no ferruginous bodies were found in patient L-18, the individual did have uncoated asbestos fibers (222,500 fibers per gram dry weight of lung tissue). The number of uncoated chrysotile fibers in this patient, when compared with those of the other individuals, indicated there were only 12 patients (21.8%) with higher chrysotile burden. Patient L-18 had the sixth lowest amosite burden and the seventh highest concentration of anthophyllite. Patient L-13, reported as having no ferruginous bodies based on detectable limits used in the study, had no uncoated asbestos fibers, but did have nonasbestos fibers in the tissue.

There have been several reports in the literature that longer fibers are more tumorigenic than shorter fibers. While it should be appreciated that considerable variation occurred in total numbers of the types of uncoated fibers, nevertheless, the question was asked as to what percentage of the fiber population from each type could be considered "long" as per Stanton dimensions. Thus, a comparison was made of the fibers in each type that conform to the Stanton hypothesis [19, 20, 40], that is, those fibers being ≥8 μm long and <0.25 in diameter (Table 5A). Using this Stanton definition, 53 of the 55 (96.4%) patients had long, uncoated asbestos fibers. Fifty-two of 55 patients (94.5%) had long amosite fibers, while 21 of 55 patients (38.2%) had long chrysotile fibers. Long fibers of crocidolite were present in 16 of 55 (29.1%) patients. The noncommercial amphiboles (tremolite, actinolite, and anthophyllite) were found as long fibers in 17, 13, and 18 patients, respectively.

Since environmental air monitoring under the NIOSH-582 (National Institute for Occupational Safety and Health) counting scheme is based on fibers longer than 5.0 μm, a determination of this "definition" of short and long (or regulated) fibers was made using this standard. The percentage of the fibers in each type of asbestos that could have been potentially counted as "long" fibers (≥5.0 μm, per NIOSH counting criteria) are shown in Table 5B. The NIOSH counting standard is based on the use of phase-contrast light microscopy; thus, the fibers shorter than 5μm and those longer than 5μm, but thinner than the functional detectable limit of the light microscope (<0.25 μm) would have appreciably changed the fibers per category. Under these conditions, far fewer fibers would be counted (see Table 5B).

The geometric mean concentration of fibers ≥5.0 μm is, in decreasing order; amosite > crocidolite > chrysotile > anthophyllite > actinolite > tremolite. For fibers ≥8.0 μm (per Stanton hypothesis), the order is amosite > crocidolite > chrysotile > tremolite > actinolite > anthophyllite. The total number of "short" fibers per asbestiform was also compared. The decreasing concentrations for those <5.0 μm or <8.0 μm were amosite > crocidolite > chrysotile > tremolite > actinolite > anthophyllite (Table 6).

Comparisons: Pathologic Asbestosis With FB/Asbestos Fiber Data

Cases with pathologic asbestosis and those without pathologic asbestosis were compared with respect to asbestos bodies per gram of wet lung tissue (Bremerton Laboratory [BL]), as well as a multisite quantitative analysis of ferruginous bodies per gram of wet lung tissue (Tyler Laboratory

TABLE 5A Fibers fitting the Stanton hypothesis

Fiber type	Percentage
Amosite	48
Anthophyllite	46
Crocidolite	41
Chrysotile	39
Actinolite	34
Tremolite	16

Note. Length ≥8.0 μm and diameter <0.25 μm.

TABLE 5B Fibers meeting and countable by NIOSH criteria

Fiber type	Percentage >5 μm	Percentage detectable by light microscopy[a]
Amosite	70	33.0
Crocidolite	67	16.0
Anthophyllite	67	53.7
Actinolite	60	37.9
Chrysotile	55	1.4
Tremolite	36	27.6

[a]>5 μm length and ≥0.25 μm diameter.

TABLE 6 Geometric mean of the concentrations of asbestos

≥5 μm	Amosite >	Crocidolite >	Chrysotile >	Anthophyllite >	Actinolite >	Tremolite >
≥8 μm	Amosite >	Crocidolite >	Chrysotile >	Tremolite >	Actinolite >	Anthophyllite >
<5 μm	Amosite >	Crocidolite >	Chrysotile >	Tremolite >	Actinolite >	Anthophyllite >
<8 μm	Amosite >	Crocidolite >	Chrysotile >	Tremolite >	Actinolite >	Anthophyllite >

[TL]), used for comparison with total asbestos fibers per gram of dry lung tissue (Tables 7A and 7B). As stated under the Materials and Methods, the numbers listed as "ferruginous bodies per gram wet lung—BL" were determined by routine light microscopic examination at the Bremerton Laboratory of 5 g of digested peripheral lung tissue. The numbers listed under the designation "ferruginous bodies per gram wet lung—TL" represented a combined digest pool from multiple sites of the right and left lungs that were obtained as part of the quantitative analysis of bodies and fibers at the Tyler facility. Over 97% of the ferruginous bodies analyzed by ATEM were asbestos bodies.

Twenty-nine cases showed pathologic asbestosis, and were graded according to the criteria of the College of American Pathologists and the National Institute for Occupational Safety and Health [26]. As shown in Table 7A, 22 of 29 patients showed pathologic grade 1 asbestosis which was usually focal (extent A). The mean asbestos body concentration per gram of wet lung tissue in patients with pathologic asbestosis as determined by routine digestion and light microscopic evaluation in the Bremerton analysis was 6,122, with a range between 422 and 25,000 and a median of 3,440. In contrast, mean ferruginous body concentration per gram of wet lung tissue in the 26 cases that did not show pathologic asbestosis was 1,569, with a

TABLE 7A Cases with pathologic asbestosis

Assay number	Pathologic grade of asbestosis	Extent of asbestosis	FBs/g wet[a]	FBs/g wet[b]	Total asbestos fibers/g dry
L-1	1	A	12,140	18,968	107,211
L-3	1	A	25,000	195,419	13,625,806
L-4	1	A	712	737	239,726
L-8	1	A	422	756	72,016
L-9	1	B	1,852	29,077	5,701,613
L-11	1	B	12,771	27,780	4,136,364
L-12	1	A	1,320	2,542	291,667
L-14	1	A	20,100	4,903	3,989,464
L-15	1	A	11,200	26,464	1,894,587
L-17	1–3	C	5,620	7,219	2,722,222
L-19	1	B	25,000	13,114	3,999,548
L-21	1	A	1,980	2,114	665,205
L-23	1	A	3,440	3,325	1,563,333
L-24	1	A	1,260	971	559,764
L-27	1–3	B	1,560	60	112,179
L-28	1	A	850	547	758,808
L-29	1	A	4,220	1,148	331,439
L-33	1–2	B	5,700	201,416	68,950,000
L-39	1–2	B	813	1,631	490,924
L-40	1	A	1,088	458	1,045,300
L-42	1	A	694	528	1,014,493
L-43	1	A	643	4,100	1,682,692
L-44	1–2	B	ND	114,788	32,625,692
L-45	1	A	2,975	299	217,662
L-48	1–2	B	13,000	31,739	6,875,000
L-49	1	A	11,600	6,074	391,791
L-50	1	A	2,450	17,677	134,634
L-51	3	C	496	2,968	928,030
L-53	1	A	2,521	14,116	2,230,392

[a]Ferruginous bodies/g wet lung as determined by light microscopy of 5 g of digested lung from site where blocks taken for grading by light microscopy (Bremerton Laboratory).

[b]Ferruginous bodies/g wet lung determined on digestion pool from multiple sites of right and left lung as used in quantitative analysis by light and electron microscopy (Tyler Laboratory).

TABLE 7B Cases without pathologic asbestosis

Assay number	Ferruginous bodies/g wet[a]	Ferruginous bodies/g wet[b]	Total asbestos fibers/g dry
L-2	1,572	1,737	62,500
L-5	5,190	3,338	505,556
L-6	220	46	43,532
L-7	2,390	8,391	1,439,394
L-10	117	10	43,532
L-13	117	0	0
L-16	382	400	446,697
L-18	144	0	222,458
L-20	108	546	899,306
L-22	1,541	5,234	2,581,967
L-25	840	60	22,096
L-26	540	40	588,235
L-30	1,580	279	352,011
L-31	57	240	150,538
L-32	327	325	159,091
L-34	1,221	3,952	1,422,764
L-35	0	13	855,263
L-36	2,382	15,523	2,119,141
L-37	678	1,536	923,261
L-38	13,109	170,985	63,417,431
L-41	360	699	740,385
L-46	968	1,409	331,439
L-47	4,420	759	433,559
L-52	929	6,832	2,925,000
L-54	59	160	131,579
L-55	183	488	694,444

[a]Ferruginous bodies/g wet lung as determined by light microscopy of 5 g of digested lung from site where blocks taken for grading by light microscopy (Bremerton Laboratory).

[b]Ferruginous bodies/g wet lung determined on digestion pool from multiple sites of right and left lung as used in quantitative analysis by light and electron micrsocopy (Tyler Laboratory).

range between 0 and 13,109 and a median of 540 (Table 7B).

Ferruginous bodies per gram of wet lung tissue in the 29 cases with pathologic asbestosis as determined from the Tyler analysis ranged from 60 to 201,416, with a mean of 25,505 and a median of 4,100. In contrast, the 26 cases without pathologic asbestosis had a mean ferruginous body count per gram of wet lung tissue of 8,918, with a range of 0 to 170,985, and a median of 546 (Table 7B).

Of the 29 cases with pathologic asbestosis, mean total asbestos fibers per gram of dry lung tissue was 5,426,123, with a range of 72,016 to 68,950,000, and a median of 1,045,300 (Table 7A). In contrast, the 26 cases without pathologic asbestosis had a mean asbestos fiber concentration per gram of dry lung tissue of 3,258,705 with a range of 0 to 63,417,431 and a median of 505,556 (Table 7B).

DISCUSSION

Medical

Mesothelioma, a rare neoplasm, occurs most frequently in individuals with previous asbestos exposure. The life expectancy of patients with different histologic types of mesothelioma reported herein is similar to what has been reported in the literature [24]. Patients with epithelial mesothelioma have a longer mean survival than those with sarcomatoid, desmoplastic, and biphasic mesotheliomas and mesotheliomas showing variable differentiation. The reason for this difference in survival is not clear. The five peritoneal mesotheliomas reported in this study had a mean survival of 3.5 months, which is consistent with what we have found in a much larger group ($n = 70$) of patients with peritoneal mesothelioma (average survival = 4.2 months; range <1 month–18 months; unreported data collected by SH).

In this study, we found that those patients whose lung tissue showed pathologic asbestosis had higher mean asbestos body and asbestos fiber concentrations than those whose lung tissue showed no asbestosis. However, our data have shown there was an extensive range in asbestos body and fiber concentration in patients with and without pathologic asbestosis, making it impossible to predict the presence of pathologic asbestosis based on lung asbestos body/fiber concentration. Our observations are similar to those of Warnock and Isenberg [41], who demonstrated that the lungs from some patients with lung cancer had a heavy burden of asbestos without showing fibrosis, whereas some with the same concentration of asbestos did have asbestosis. Warnock and Isenberg suggested that fibrosis may depend on factors besides total fiber burden.

Leigh et al. [42] found a statistically significant trend between lung fiber content and mesothelioma cell type from epithelial (low fiber content) through mixed to sarcomatous (high fiber content). This trend was stated to be most apparent for total uncoated fibers and crocidolite. A higher lung fiber content was found in peritoneal mesotheliomas versus pleural mesotheliomas. In our study, pleural epithelial mesotheliomas had a mean total asbestos fiber concentration per gram of dry lung tissue of 6,275,651 (range 0–68,950,000). The biphasic mesotheliomas ($n = 7$) had a mean total asbestos fiber concentration per gram of dry lung tissue of 532,485 (range 43,532–2,119,141). The sarcomatoid ($n = 10$) had mean total asbestos fiber concentration per gram of dry lung tissue of 133,625 (range 134,634–4,136,364). The mesotheliomas that had a predominantly desmoplastic histologic appearance ($n = 5$) had a mean asbestos fiber concentration per gram of dry lung tissue of 1,524,404 (range 131,579–2,722,222). The mesotheliomas showing variable differentiation ($n = 5$) had a mean asbestos fiber count per gram of dry lung tissue of 693,778 (range 159,091–1,563,333). The peritoneal mesotheliomas ($n = 5$) had a mean asbestos fiber concentration per gram of dry lung tissue of 16,312,071 (range 22,096–63,417,431).

Our findings are similar to those reported by

Leigh et al. [42], with respect to peritoneal mesotheliomas having a higher lung fiber burden than pleural mesotheliomas. Our findings were different than those reported by Leigh et al. [42], with respect to mean fiber concentration and histologic type of pleural mesothelioma. In decreasing order of total mean asbestos fiber burden, the histologic types of pleural mesothelioma were ranked as follows: (1) epithelial, (2) desmoplastic, (3) variable differentiation, (4) biphasic, and (5) sarcomatoid. The total number of cases is small and with a larger sample, the results could obviously be different.

Analytical

While the amphibole crocidolite has been linked with the development of mesothelioma [12, 43] in the studies of Sluis–Cremer et al. [4], amosite has also been suggested to be important in the production of this tumor [18, 44, 45]. In the present study, amosite is the most commonly found amphibole, while only 40.0% of the patients had crocidolite. This prevalence of amosite in the present study (96.4%) compares favorably with the observation by Churg and Vedal [18], where amosite was found in all lungs of shipyard workers and insulators from the Pacific Northwest [18]. The occurrence of amosite in the present study of mesothelioma is higher than reported by Roggli et al. [45] where 81% of the cases contained amosite. Roggli et al. reported 58% of all fibers ≥5 μm were amosite, while amosite accounted for 64.3% of such fibers in the present study.

The length/width ratios of amosite burden in work of Churg and Vedal [18] led to the suggestion that an association existed between low-aspect-ratio amosite fibers and mesothelioma. This contrasts with the present data in which the amosite population is represented by fibers with a higher aspect ratio (as based on uncoated fibers data). This shift would be further toward higher aspect ratios if the lengths/widths of amosite fibers that were cores of the ferruginous bodies were included in the overall amosite data. In our study, the only asbestiform mineral found (based on geometric mean of the length) to have the majority of fibers less than 5 μm was tremolite (64% < 5.0 μm).

Since the work histories suggest chrysotile exposure, its absence in tissue digests requires an explanation. For example, chrysotile is often inhaled as a shorter fiber than amphiboles. In 16 patients, there were longer chrysotile fibers (≥5.0 μm) in the autopsy tissue, while all patients had populations of short amphiboles. The absence of chrysotile in some samples thus could have resulted from (1) a greater preferential clearance of short chrysotile while sparing the equivalent length amphiboles, or (2) an initial disproportionate inhalation of amphiboles had occurred.

Tremolite has been suggested as an important component of chrysotile dust and a contributor to the development of mesothelioma among miners [13, 14]. In the present study, if one chooses to emphasize the slight correlation between the levels of chrysotile and tremolite and conclude that an exposure to chrysotile involves a resultant exposure to tremolite, the stronger correlation between chrysotile and amosite must also be appreciated. We suggest the exposure was to mixed asbestiform minerals and the combination in the tissue most likely reflects such exposures to the various asbestiform minerals rather than a contaminant of one asbestos containing product with another type of asbestos—i.e., tremolite in chrysotile.

The absence of tremolite fibers in 22 of the 55 patients (40.0%) is in contrast with the suggestion of Srebro and Roggli [13] that this asbestiform is "nearly ubiquitous and represents the most common amphibole fiber in the lungs of urbanites." While the data in the present study cannot be used to speculate where tremolite exposures may have occurred, there is no disagreement with Srebro and Roggli's conclusion [13] that "moderate exposures may produce malignant pleural mesothelioma in some patients." The same argument, however, can be made from the present study for each asbestiform at lower exposures as it may impact on individual susceptibility for developing mesothelioma.

Ferruginous bodies are a marker of past asbestos exposure. Among this group of individuals with mesothelioma, 83.6% of the patients would have been classified as having had occupational exposures based on the concentrations of ferruginous bodies (≥1000 ferruginous bodies per gram of dry weight of lung tissue). Two of the patients (3.6%) were found to have no ferruginous bodies (L-13, L-18). These individuals could, therefore, be considered to have "control" levels by this standard. However, by electron microscopy, the tissue from patient L-18 was found to have uncoated amosite, anthophyllite, and chrysotile fibers. The critical question in such cases is that if their work history clearly indicated repetitive exposures to asbestos, why were their tissue burdens within control levels for FBs?

Rates of clearance and toxicity of different dusts vary as per individual susceptibility. Likewise, the development of ferruginous bodies in individuals vary and some are poor "coaters" even if appropriate asbestos fibers are present [34, 46]. Conversely, some individuals, such as L-2, appear to be very efficient "coaters" since there are occupational levels of ferruginous bodies (>1000 FBs/g dry) and reasonably low numbers of uncoated fibers.

The impact of clearance could be offered to explain the lack of chrysotile burden in numbers of the patients, even though logic would dictate their workplace exposures (defined by the levels of amphiboles) surely should have included appreciable chrysotile. It should be noted that 31 of the patients (56.4%) did have chrysotile burdens, with portions

of that burden (55%) being made up of fibers longer than 5 μm.

An explanation for the development of mesothelioma in individuals with low fiber burden could always be offered that disease had spontaneously developed, even though there was a history of a known past exposure, suggesting asbestos as a contributor. Another explanation is based on Churg's suggestion that mesothelioma appeared at a much lower amosite burden than that required for asbestosis, which could apply to all types of asbestos [18]. Thus, combining this concept with the impact of clearance efficiency in some individuals, a result could be the reduced or very low tissue burden at the time of death.

Exposure to the noncommercial asbestiform anthophyllite has been reported to have a high potency for causing lung cancer, but suggested as a low potency in the tumorigenicity of mesothelioma when compared with other types of asbestos [47]. In part, these differences may have some basis in that a much greater emphasis has been placed on evaluating human exposures to commercial types of asbestos. However, one of the few studies of cohorts with appreciable exposure to the noncommercial asbestos anthophyllite reported the occurrence of mesotheliomas [48]. Concern for exposure and resultant risks from noncommercial amphiboles is not a trivial issue since, in the present study, the noncommercial types of asbestos are found in some patients at higher levels than the combination of the commercial types. For example, in cases such as L-12 and L-18, anthophyllite is the predominant amphibole. When all noncommercial forms (tremolite, actinolite, and anthophyllite) are combined, the majority of all asbestos fibers in L-12 and L-28 were noncommercial types.

The concentration of fibers, per asbestos type, is dependent on the quality of exposure for each cohort. Thus, while tremolite was in greater concentration than other asbestiforms (≥5.0 μm) in asbestos miners [39], our observations indicate amosite as being the prevalent fiber in these cases. However, the underlying conclusion in the present study, as well as that of Dufresne et al. [39], is that individuals with mesothelioma have in most cases a confirmed asbestos burden and that most of these burdens are above general population levels for either ferruginous body and/or uncoated fiber burden.

The risk for mesothelioma appears to be clearly tied in most cases to the exposure to asbestos. The relationship of longer amphibole fibers to the risk of developing mesothelioma may, as suggested by Churg and Vedal [18], simply constitute the major component at time of autopsy due in part to its reduced rate of clearance. While chrysotile burden among the patients in the present study was overshadowed by the amphibole burdens, 55% of the chrysotile burden is made up of fibers longer than 5 μm. Once again, either indicating a selective inhalation of longer chrysotile fibers to the exclusion of shorter fibers or more likely the resultant impact of clearance on the short fiber burden. This observation should not be used to exclude the composite importance of shorter fibers as contributors to the disease, nor, from the present data, can arguments be offered in support of varying levels of risk for mesothelioma from exposure to the various types of asbestos. In fact, the present data offer the opposite argument. The common link in these mesothelioma patients appears to be the presence of asbestos of various types and variable lengths.

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