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ORIGINAL ARTICLE

Analysis of Asbestos Concentration in 20 Cases of Pseudomesotheliomatous Lung Cancer

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ABSTRACT

Mesothelioma is a rare neoplasm caused by asbestos exposure. The majority of mesotheliomas arise from the pleural lining of the thoracic cavity, but also involve the peritoneal and pericardial cavities. Another type of neoplasm referred to as *pseudomesotheliomatous adenocarcinoma* is rare. Most ''pseudomesotheliomas'' arise in the pleural tissue of the chest cavity and resemble pleural mesotheliomas, macroscopically and histologically. While most arise in the pleura, there are some that metastasize to the pleura from another site. We evaluated asbestos fiber concentrations in 20 cases of pseudomesotheliomatous lung cancer and found a significant number to contain an elevated concentration of asbestos in their lung tissue, which is similar with our study of 55 mesothelioma cases published in 1997. This would provide evidence that some pseudomesotheliomatous lung cancers are caused by asbestos.

Keywords: Asbestos burden, electron microscopy, pseudomesothelioma

Pseudomesotheliomatous lung cancers are macroscopically and histologically similar to epithelioid mesotheliomas. Following a 10-year study which included 53 pseudomesotheliomatous carcinomas consisting of two groups: primary pulmonary carcinomas with florid pleurotropic growth ($n = 47$) of which 34 (70%) were adenocarcinomas; and diffuse carcinomatous involvement of the pleura by metastatic tumor ($n = 6$), Attanoos and Gibbs [1] found that in 76% of their study group, there was a history of asbestos exposure. Harwood and associates [2] defined pseudomesotheliomatous lung neoplasms as ''a variant of peripheral lung cancer'' which closely mimics malignant pleural mesothelioma. Aumann and associates [3] defined pseudomesotheliomatous lung neoplasms as a ''variant of the peripheral adenocarcinoma with involvement of the pleura parietalis, the so-called pseudomesotheliomatous adenocarcinoma of the lung.'' They [3] further emphasized the difficulty of differentiating from epithelioid mesothelioma on ''imaging and cytology, macroscopically as well as histologically.'' The pseudomesothelioma/metastatic tumors considered by some as being from primary lung adenocarcinomas have also been reported with origins including malignant melanoma [4], ''transitional cell carcinoma of the bladder, renal clear cell carcinoma, pancreatic duct adenocarcinoma, prostatic adenocarcinoma and squamous cell carcinoma of the parotid gland'' [1].

Several publications have suggested the importance of differentiating mesothelioma from a pseudomesotheliomatous lung cancer, both for insurance-related claims [3] and as related to medicolegal implications (asbestos related compensation claims) [1]. To our knowledge, the published data regarding concentrations of asbestos fibers and ferruginous bodies within the lung in individuals with pleural tumors other than mesothelioma consists only of an evaluation of lung tissue from three individuals with a diagnosis of ''primary pleural epithelioid haemangio-endothelioma'' [5]. The three cases were referred as part of ''medicolegal compensation claims for asbestos related diseases.'' The tissue burden generated from one of those three cases was interpreted as reflective of the ''occupational history,'' whereas tissue burden from the other two cases was

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determined ''not to show clear-cut increase in asbestos fiber levels.'' While all three cases were ''claimed to have exposure to asbestos,'' the authors found ''typical ferruginous bodies'' in only one case by light microscopy and that case also had asbestos fiber content they considered ''raised in comparison with the range seen in a non-exposed background population.'' The tissue from that case contained 12,300,000 amosite fibers and 29,500,000 crocidolite fibers/g dry weight with no chrysotile fibers being detected. One of the excluded cases contained 400,000 amosite fibers and 200,000 chrysotile fibers/g dry weight of tissue.

Our present study evaluated lung tissue for asbestos from a sub-group of 20 individuals diagnosed as having pseudomesotheliomatous lung carcinoma. The cases had been referred to one of our laboratories (SPH) as part of a medical-legal service. The distinction between mesothelioma and pseudomesothelioma was done via a battery of tests (e.g. histochemistry, immunohistochemistry, and electron microscopy) used in a pathology practice for such distinction and were ultimately evaluated by one of the authors (SPH), who is a member of the U.S.–Canadian Mesothelioma Panel, among other panels. The lung samples were provided as discarded materials to an Analytical Transmission Electron Microscopy (ATEM) laboratory (RFD). The ATEM laboratory data were not used in the medical-legal process and the ATEM laboratory was not compensated for the analysis. Most cases in the study were defined as ''having or suspected of having 'occupational or paraoccupational' histories of exposure'' to asbestos. Previous publications in the literature regarding cases of pseudomesotheliomatous lung cancer raised the issue of asbestos exposure (at least by historical data obtained on some patients), but to our knowledge no correlation with asbestos burden in such cases had been presented in detail until the present study. Findings from this study are compared to findings of tissue burden in previously reported studies by the authors (SPH, RFD), many of which involved cases from the same general region and which were diagnosed as having mesothelioma.

METHODS AND MATERIALS

Tissue samples from a subgroup of 20 individuals diagnosed as having pseudomesotheliomatous lung cancer were randomly selected from a larger group of neoplasms provided by Diagnostic Specialties Laboratory, Inc., P.S., Bremerton, Washington (SPH). The available historical data on individuals in the cohort are shown in Table 1. The initial referrals were neoplasms that had the features of pseudomesotheliomatous lung cancers. Most were subtypes of primary pulmonary adenocarcinomas and other non-mesothelial cancers.

The wet lung tissue submitted to the Tyler laboratory was prepared for evaluation via a digestion procedure using a modified bleach procedure [6]. Dry weights were calculated based on a dry/wet ratio obtained for each digestion pool. All reagents were prefiltered through 0.2 μm pored polycarbonate filter prior to use in the digestion procedure. Control blanks of filters and solution blanks were prepared and evaluated to provide quality assurance within the laboratory. Aliquots of the digestate from each sample were collected on 0.22 μm pored mixed cellulose ester filter for quantitation of ferruginous body content of the tissue samples, while additional aliquots were collected on 0.2 μm pored polycarbonate filters and prepared for evaluation by ATEM for the determination of uncoated asbestos fibers and assessment of core materials of any ferruginous bodies found in the areas evaluated.

The preparation of the mixed cellulose filters used for the determination of ferruginous body content of the tissue consisted of mounting a wedge of each filter on a glass slide, exposing the filter to acetone vapor (making the substrate transparent) and evaluation of the ''cleared'' surface at 100× with magnification increased to 400× via a light microscope if additional morphological clarification was desired. Structures conforming to the definition of a ferruginous body as seen by light microscopy were counted. The definition of such a ferruginous body included elongated/transparent central core and a rust-colored beaded surface.

The polycarbonate filters were prepared for ATEM analysis by a direct method involving collection of the digestate on the filter surface followed by carbon coating of the dried filter. The carbon-coated filter was cut into strips and mounted on 100 mesh copper grids. A modified Jaffe-Wick method [7] was used to remove the filter matrix, leaving the carbon film containing the entrapped fibers, ferruginous bodies, and other particulates. Random areas on the grids were evaluated at 16,000× for uncoated fibers in a JEOL 100CX transmission electron microscope (JEOL USA, Inc., Peabody, MA) which was interfaced with an EDAX DX Prime X-ray analyzer (EDAX, Inc., Mahwah, NJ). An additional scan of other areas on the grids was conducted at 1600× for the presence of additional ferruginous bodies. A fiber as defined in the present study consisted of an elongated entity $\geq$0.5 μm with parallel sides for a majority of its length and an aspect ratio of greater than 5:1. Uncoated fibers as well as the core material of ferruginous bodies were analyzed as to elemental composition by X-ray energy dispersive spectrometry (XEDS) and for crystalline structure by selected area electron diffraction (SAED). The data indicating ferruginous body (FB/g wet or FB/g dry weight of tissue) burden as determined by

TABLE 1. Patient data from 20 pseudomesothelioma cases.

Case #	Age	Sex	Smoking Hx	Occupation	Asbestosis (1982 CAP-NIOSH)	Pleural Plaques	Specimen	Diagnosis	Survival
1	70	M	30-pack years	Insulator helper (5 yrs)	Yes, R & P; Grade 4	Yes, R & P	Autopsy tissue, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural biphasic	8 months
2	74	M	40-pack years	Shipyard worker (29 yrs)	Yes, P; Grade 3/4	Yes, R & P	Autopsy tissue, right and left lungs	Pseudomesotheliomatous adenosquamous carcinoma, right pleural	19 months
3	75	M	45 to 60-pack years	Shipfitter, chipper, caulker, nuclear test director at shipyard	Yes, P; Grade 3/4	Yes, R & P	Autopsy tissue, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural moderately to poorly differentiated; metastases to adrenals	2 months
4	75	M	100-pack years	Shipyard electrician (35 yrs)	Yes, R & P; Grade 3/4	Yes, R & P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural, NOS	7 months
5	76	M	1-pack per day, total pack years unknown	Built kitchen equipment	Yes, P; Grade 3/4	Yes, P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural, acinar	1 month
6	43	M	5+ pack years	Batchmaker, mixer, hod carrier, pipelayer, laborer, maintenance man	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural epithelioid and rhabdoid appearance; metastases to liver and lymph nodes	<1 year
7	69	M	55 to 100-pack years	Steamfitter and plumber (25 yrs)	Yes, P; Grade 3/4	Yes, P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural	20 months
8	61	M	20 to 54-pack years	Electrician (20 yrs)	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural, tubulodesmoplastic type; metastases to liver, adrenal, and lymph nodes	6 months
9	71	M	39-pack years	Wiper & oiler (3 yrs); insulator & pipefitter (35 yrs)	Yes, P; Grade 1	Yes, P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural, papillary (type II pneumocyte by EM); metastases to left lung, omentum, and lymph nodes	14 months
10	62	M	10-pack years	Electrician (45 yrs)	Yes, R	Yes, R & P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural, moderately differentiated; metastases to liver, gallbladder and thyroid	3 months
11	?	M	Unknown	Unknown	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural,	Unknown

(*continued*)

TABLE 1. Continued

Case #	Age	Sex	Smoking Hx	Occupation	Asbestosis (1982 CAP-NIOSH)	Pleural Plaques	Specimen	Diagnosis	Survival
								tubulodesmoplastic; metastases to liver	
12	64	M	Unknown	Shipyard insulator & shipwright (5 yrs); lineman	Yes, P; Grade 2/3	Yes, P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural, NOS; metastases to mesentery, omentum, and other peritoneal surfaces	8 months
13	?	M	Unknown	Unknown	Yes, P; Grade 2	Yes, P	Autopsy, right lung	Pseudomesotheliomatous adenocarcinoma, left pleural, poorly-differentiated; metastases to lymph nodes and thyroid	Unknown
14	59	M	27 to 80-pack years	Shipyard sandblaster & painter (13 yrs); head hooker and furnace manager at a steel plant (18 yrs); laborer	Yes, R	Yes, P	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, right pleural, acinar	6 months
15	67	M	Unknown	Shipyard worker (3 yrs); boatswains mate (9 yrs)	No	Yes, P	Autopsy, right and left lungs	Pseudomesotheliomatous adenosquamous carcinoma, left pleural, poorly differentiated; metastses to liver	2 months
16	?	M	Unknown	Unknown	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural, NOS	Unknown
17	77	M	Unknown	Unknown	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous squamous cell carcinoma, right pleural, moderately differentiated keratinizing; metastases to lymph nodes	Unknown
18	62	M	Unknown	Unknown	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural, NOS; metastases to liver and kidney	6 months
19	64	M	1-pack per day, total pack years unknown	Unknown	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, left pleural, NOS	Unknown
20	53	M	20 to 21-pack years	Laborer, carpenter, service station manager	No	No	Autopsy, right and left lungs	Pseudomesotheliomatous adenocarcinoma, poorly-differentiated pleomorphic	Unknown

P, pathologic
R, radiographic

TABLE 2. Ferruginous body and uncoated fiber data (fiber/g dry).

Case	Total Asbestos	Amosite	Crocidolite	Tremolite	Actinolite	Anthophyllite	Chrysotile	FB/gm dry	FB/gm wet
1L	3,565,000	3,565,000	ND	ND	ND	ND	ND	358,000	60,240
1R	12,685,000	12,220,000	ND	155,000	155,000	ND	155,000	862,000	156,450
2L	7,078,000	1,601,000	253,000	2,696,000	253,000	1,517,000	758,000	23,600	4010
2R	1,190,000	517,000	26,000	388,000	52,000	155,000	52,000	11,600	2340
3L	461,000	346,000	23,000	23,000	ND	69,000	ND	24,000	4640
3R	4,265,000	3,683,000	67,000	268,000	67,000	180,000	ND	16,400	2830
4L	1,136,000	990,000	ND	18,000	ND	73,000	55,000	66,200	15,500
4R	2,862,000	2,531,000	37,000	37,000	ND	110,000	147,000	67,200	15,740
5L	1,588,000	1,037,000	ND	162,000	ND	227,000	162,000	7300	898
5R	726,000	333,000	ND	212,000	30,000	121,000	30,000	1800	239
6L	1,128,000	ND	44,000	44,000	22,000	ND	1,018,000	ND	ND
6R	1,400,000	22,000	67,000	22,000	ND	22,000	1,267,000	ND	ND
7L	1,297,000	1,033,000	198,000	44,000	ND	22,000	ND	328	60
7R	1,160,000	773,000	286,000	50,000	ND	34,000	17,000	2400	578
8L	294,000	261,000	ND	ND	ND	33,000	ND	ND	ND
8R	1,004,000	681,000	ND	97,000	32,000	162,000	32,000	78	10
9L	748,000	332,000	28,000	221,000	28,000	28,000	111,000	20,800	3020
9R	704,000	528,000	ND	59,000	117,000	ND	ND	5700	1160
10L	392,000	178,000	ND	53,000	18,000	143,000	ND	710	160
10R	453,000	349,000	17,000	17,000	ND	70,000	ND	174	40
11L	253,000	113,000	28,000	28,000	ND	ND	84,000	282	40
11R	348,000	174,000	ND	25,000	ND	ND	149,000	2360	380
12L	324,000	116,000	ND	139,000	23,000	23,000	23,000	3080	540
12R	327,000	228,000	ND	33,000	33,000	33,000	ND	3400	620
13R	292,000	ND	ND	80,000	ND	212,000	ND	1460	220
14L	206,000	77,000	26,000	ND	51,000	26,000	26,000	ND	ND
14R	257,000	77,000	ND	26,000	ND	103,000	51,000	769	120
15L	25,000	ND	ND	25,000	ND	ND	ND	380	60
15R	192,000	64,000	ND	ND	ND	128,000	ND	740	99
16L	183,000	ND	ND	46,000	ND	91,000	46,000	ND	ND
16R	25,000	ND	ND	25,000	ND	ND	ND	78	12
17L	58,000	ND	ND	29,000	ND	29,000	ND	433	60
17R	174,000	58,000	ND	58,000	ND	58,000	ND	ND	ND
18L	ND	ND	ND	ND	ND	ND	ND	ND	ND
18R	87,000	29,000	29,000	29,000	ND	ND	ND	ND	ND
19L	69,000	17,000	17,000	ND	ND	35,000	ND	ND	ND
19R	ND	ND	ND	ND	ND	ND	ND	86	20
20L	54,000	ND	ND	ND	54,000	ND	ND	ND	ND
20R	25,000	ND	ND	ND	25,000	ND	ND	ND	ND

L refers to Left Lung and R refers to Right Lung
ND = Nondetectable

light microscopy is reported in Table 2, as are the findings of types and numbers of uncoated asbestos fibers/g of dry tissue. The decision to place both on the same table enables the reader to compare total burden per sample.

RESULTS

Case data

Lung tissue from 20 males diagnosed as having pseudomesotheliomatous lung cancers were used in this study. The age, sex, smoking history, occupational history, diagnosis, and survival of these individuals are listed in Table 1, as well as information concerning whether there was asbestosis and/or pleural plaques. The individuals in this study ranged from 43 to 77 years of age. Thirteen of the twenty individuals had defined smoking histories. The lack of information concerning smoking history and other historical data in Table 1 simply indicates such data were not available. This did not preclude the inclusion of such individuals in this study since the study was not one of the defining asbestos content in occupationally exposed individuals with this type of neoplasm, but rather to show the asbestos content in lung tissue in individuals with pseudomesotheliomatous lung cancer regardless of their occupational exposure history. However, there were fourteen individuals who worked in occupations where asbestos exposures would be expected. Eleven individuals were diagnosed by radiographic and/or pathological findings as having asbestosis based upon 1982 CAP-NIOSH criteria [8] (Table 1). Twelve individuals were identified radiographically and/or pathologically as having

hyaline pleural plaques indicative of prior asbestos exposure. The most common form of this neoplasm defined by the Bremerton laboratory (SPH) was pseudomesotheliomatous adenocarcinoma ($n = 15$). There were two pseudomesotheliomatous adenosquamous cell carcinomas, two tubulodesmoplastic pseudomesotheliomatous lung cancers, and one pseudomesotheliomatous squamous cell carcinoma. The longest defined survival period from diagnosis was 20 months. The average survival rate was six months.

Ferruginous body data

Comparative data as to tissue burden of ferruginous (asbestos) bodies was evaluated from the right and left lung from nineteen individuals and from the right lung in Case 13 (Table 2). Seventeen of the twenty cases (85%) were positive for ferruginous (asbestos) bodies. In 14 cases, at least one or both of the lung samples were found to contain over 20 asbestos bodies per gram wet weight. As a point of reference, Churg and Warnock [9] ''arbitrarily considered 100 bodies to be the division between 'environmental' and 'occupational' exposures.'' However, many individuals considered as being in the ''general population'' in their study contained fewer asbestos bodies per gram of wet tissue, and some appeared to work in blue collar professions where exposure to asbestos could occur. A further comparison of the findings in our study will be made with data from manuscripts in which individuals in the general population with no known history of exposure in occupational or paraoccupational settings were found to have 0–20 ferruginous (asbestos) bodies per gram of wet tissue [10–14].

Cases 6, 18 and 20 were not determined to have ferruginous/asbestos bodies (below detection limit) in either sample, while Sample 16R was determined to have 12 FB/g wet weight of tissue. Case 19 was found to have 20 FB/g wet weight of tissue in one sample (19R) and below the detection limit for ferruginous bodies in the second sample (19L). There were twelve cases with asbestos body concentrations above 20 FB/g wet weight in all samples. Five cases had over 1000 FB/g wet weight of tissue in both samples. The average asbestos body burden for the samples with >20 FB/g wet tissue was 10,800 FB/g wet weight of tissue. The highest number of asbestos bodies was found in Cases 1R (862,000 FB/g dry) and 1A (358,000 FB/g dry). The average asbestos body burden for all samples in the study was 6900 FB/g wet (37,980 FB/g dry) weight of tissue. The average asbestos body concentration for the ''+'' samples was 9650 FB/g wet (52,900 FB/g dry) weight of tissue.

There were 128 ferruginous bodies found in the areas scanned by ATEM. The longest ferruginous body found in the ATEM analysis was formed on an amosite core and was 120 μm. The average length of the ferruginous bodies evaluated by ATEM was 25 μm. The cores of three ferruginous bodies were formed on crocidolite, one on tremolite, and the remaining 124 on amosite cores.

Uncoated fiber burden

Comparative ferruginous body and uncoated fiber burden per each sample is shown in Table 2. Five cases had over one million uncoated asbestos fibers/g of dry tissue in both lung samples. Eighteen cases were positive for asbestos in both samples, while Cases 18 and 19 were positive in one of two samples. The most commonly found asbestos type in the samples was tremolite (77%) followed by amosite (74%), anthophyllite (67%), and chrysotile (46%). Crocidolite and actinolite were found in 38% samples. The highest fiber burden per individual type of asbestos was found for amosite in Case 1 L&R and for tremolite in Case 2L. One way of looking at the fiber burden is on the findings of fiber types and total fiber burden alone. However, another point of interest is to evaluate the fiber lengths that comprise the fiber types in the tissue burden [15,16].

There are two commonly quoted dimensions of fibers to which a fiber burden is compared. These include the ''Stanton fibers'' or ''Index fibers'' [17]. These are dimensions of fibers evaluated in pleural models in animals which were reported to have a higher probability for inducing pleural sarcoma. The specific quote is the ''probability of pleural sarcoma correlated best with the number of fibers measured 0.25 μm or less in diameter and more than 8 μm in length, but relatively high correlations were also noted with fibers in other size categories having diameters up to 1.5 μm and lengths greater than 4 μm.'' The data from our pseudomesothelioma study indicated the following percentage of each fiber type would equate with the most reactive ''Stanton fiber'' (>8 μm length/≤0.25 μm in diameter): amosite – 16.5%, tremolite – 3.6%, actinolite – 4.8%, crocidolite – 4.6%, anthophyllite – 11.2%, and chrysotile – 0% (Table 3).

Another definition of a fiber based on dimensions is the size counted by a phase light microscopy count scheme such as that used in the NIOSH 7400 method in which the fibers counted are >5 μm in length and having a 3:1 aspect ratio [18]. The NIOSH document acknowledges that length is not the only important factor in determining the presence of fibers since, as referenced by the work of Rooker et al. [19] and Upton et al. [20], it is recognized that fibers <0.25 μm in diameter will not be detected by this method. Fibers within lung tissue of these individuals entered the lung via the airways, thus the data were evaluated to determine which population of each type of fiber

TABLE 3. Fibers fitting the Stanton hypothesis (≥8 μm length/<0.25 width).

Fiber type	Percentage
Amosite	16.5
Anthophyllite	11.2
Crocidolite	4.6
Chrysotile	0
Actinolite	4.8
Tremolite	3.6

TABLE 4. Fibers meeting and potentially countable by NIOSH 7400 criteria (>5 μm length/≥0.25 width).

Fiber type	Percentage
Amosite	18.2
Anthophyllite	23.4
Crocidolite	15.9
Chrysotile	0
Actinolite	14.3
Tremolite	9

would have been ''detected'' under the NIOSH 7400 count scheme. The potentially detectable fibers per type were as follows: amosite – 18.2%, tremolite – 9%, actinolite – 14.3%, crocidolite – 15.9%, anthophyllite – 23.4%, and chrysotile – 0% (Table 4). The average fiber length/width from all fibers analyzed was as follows: amosite – 8.6/0.24, tremolite – 3.7/0.34, crocidolite – 9.2/0.24, chrysotile – 6.7/0.05, actinolite – 3.16/0.25, and anthophyllite – 5.2/0.5.

The tissue burden in samples which contained over 60,000 asbestos fibers/g dry weight of tissue contained a mixture of asbestos fiber types. Samples from Case 6 were of interest, in that there were no ferruginous bodies found (within the limits of detection); however, there were over a million uncoated asbestos fibers/g dry weight of tissue in both samples (L and R). The majority type of asbestos was chrysotile and all were fibrils measured in hundredths of a micron in diameter, including 2% that were longer than 10 μm. Thus, even the ''longer'' fibers of chrysotile would not have been ''seen'' by light microscopy or lower magnification by scanning electron microscopy [20].

DISCUSSION

The cohort in our present study of 20 individuals with pseudomesotheliomatous lung cancer has several similarities with that of our earlier study of 55 individuals who died from mesothelioma [21]. The mean age in our pseudomesothelioma study was 65.9 years; whereas in our earlier mesothelioma study group, the mean age was 69.5 years. Most individuals in both groups lived in the Northwestern United States and many in both groups worked at some point in industries where asbestos exposure would have been expected. The majority of individuals in both groups were found to have pleural plaques and while this is a potential indicator of prior asbestos exposure, some individuals in both studies who had plaques did not have pathologically definable asbestosis. While our present group had tumorous involvement of the pleura, selected testing by one of the authors (SPH) indicated the tumors did not meet the criteria of mesothelioma and were classified as ''pseudomesothelioma.'' The ferruginous body cores in the mesothelioma study [21] were predominantly formed on amosite cores (92.7%); similarly, in our pseudomesothelioma study, ninety-seven percent (97%) of the cores were amosite. Six individuals in our pseudomesothelioma study (30%) had asbestos body levels in both samples that were below our expected level found in samples from the general population (0–20 FBs/g wet); whereas in the mesothelioma group, four cases (7.3%) were at this level. In the mesothelioma study, 46 of the 55 cases (83.6%) had >1000 FB/g dry weight of samples evaluated; whereas in our pseudomesothelioma study, there were eight cases where both samples had >1000 FBs/g dry weight of tissue and another two cases where one sample had over 1000 FBs/g dry weight of tissue (46% of samples evaluated).

The combined data from ferruginous body and uncoated asbestos fiber evaluation in the cases of pseudomesotheliomatous lung carcinoma confirmed past exposure to asbestos in all cases. The asbestos body levels were above that expected to be found in the general population (>20 FBs/g wet) [10–14] in both samples in 11 cases, while that level was exceeded in one sample from an additional three cases. As a comparative basis for our findings of uncoated fiber burden in the present study, we offer our publications regarding the findings of uncoated asbestos burden in our studies from the general population [12,13] and from our studies of lung tissue from individuals with mesothelioma [21]. As in the present study, fibers >0.5 μm were evaluated by ATEM in both the general population and mesothelioma study groups. We also provide evaluation of fiber dimensions based on two often quoted count schemes – a ''Stanton fiber'' (>8 in length/ ≤0.25 μm in diameter) and NIOSH 7400 fiber (>0.25 μm in diameter and >5 μm in length). We have not chosen to discuss fiber length as related to pathogenicity since that and other mechanisms associated with reactions with elongated mineral particles has been the subject of a recent EPA/ NIEHS sponsored publication co-authored by one of us (RFD) [16]. We therefore have chosen to include analysis of all fibers and not a selected population of fibers that would not have been acknowledged if applying various selected count schemes to include only a certain population of fibers.

In one study of 33 individuals from the general population [12], the most common form of asbestos found was a non-commercial amphibole. The average number of asbestos fibers per gram of dry tissue was 84,000×. The average length of all types of asbestos was found to be <3 μm. The only fiber type found to qualify as a ''Stanton fiber'' was 5% of the anthophyllite fibers. Ten of the individuals were not found to have asbestos fibers detected within the limit of detection in that given sample. In a second study involving fifteen individual samples from the general population [13], the average fiber burden was determined as 1188 fibers/g dry weight.

The four highest tissue burdens found in our mesothelioma study [21] exceeded the total found in any samples from our pseudomesothelioma study. However, the upper 50% of cases in our pseudomesothelioma study had one or both samples that would fit into the upper 50% of the tissue burdens found in the mesothelioma study [21]. Amosite was the most commonly found amphibole in the mesothelioma cases (96.3%; 53 out of 55 cases) [21]; whereas in our pseudomesothelioma study, 17 of 20 cases (85%) were positive for amosite, 18 (90%) were positive for tremolite, and 15 (75%) were positive for anthophyllite. Twelve cases (60%) in our pseudomesothelioma study were found to have at least one sample positive for chrysotile. Twenty-one of fifty-five mesothelioma cases (38.2%) contained over one million fibers per gram dry weight of tissue [21]; whereas, in the pseudomesothelioma study, there was at least one sample in eight cases (40%) with over one million asbestos fibers/g dry weight of tissue.

Thirty samples of lung tissue in our pseudomesothelioma study were found to contain a combination of asbestos types, including commercial amphiboles (amosite and/or crocidolite). The predominate fiber type in one case (Case 6) was the other and more widely used commercial asbestos – chrysotile. The finding of this fiber in appreciable numbers is of interest in that chrysotile has been reported to clear lung tissue more readily than amphiboles [22] and, in our experience, is often not detected in lung tissue from individuals reported to have had occupational exposures to chrysotile. As seen in Case 6, since no ferruginous bodies were found in either sample, the use of light microscopy for identification of ferruginous bodies as markers for past exposure to a population of longer fibers would have suggested no asbestos marker identified. This is even more of an issue when tissue burden is critical in linking past exposure to causation of an asbestos-related disease since individuals without detectable levels of ferruginous bodies may indeed have an appreciable uncoated fiber burden and simply not coat the fibers, or when the physical composition of the fibers was not suitable for initiating coat formation (too short and/or thin to trigger ferruginous coating). Thus, history of exposure is important in attribution of chrysotile exposure and disease as in a specific example where the lack or limited amounts of chrysotile asbestos in cases with no detectable ferruginous bodies and even low chrysotile burden (following a ''pure chrysotile exposure''). The Helsinki Consensus Report [23] noted that in such cases, other ''compelling clinical or radiological grounds'' may be combined with exposure data in the diagnosis of asbestosis (Case 6 did not have radiologic or pathologic asbestosis). The Helsinki Consensus report also stated ''chrysotile fibers do not accumulate within lung tissue to the same extent as amphiboles because of faster clearance rates; therefore, occupational histories (fiber-years of exposure) are probably a better indicator of lung cancer risk from chrysotile than fiber burden analysis is.'' In our opinion, one must be guarded in placing too much emphasis on the necessity of finding ferruginous (asbestos) bodies in tissue sections or in digested material in linking exposure to causation of disease since there is a wide individual variation in the ratio of uncoated fibers to the number of ferruginous bodies in tissue samples [24]. A comparison of the data regarding ferruginous body counts with that of uncoated asbestos burden in our pseudomesothelioma study reiterates the observation by Pooley and Ranson [25] that when ''using the electron microscope, it is possible to predict the asbestos fiber count that would be obtained by light microscopy, [however,] the reverse prediction cannot be made.''

Another factor emphasized from a publication by Dodson and Atkinson [26] is the importance of recognizing the inherent limitations of instrumentation, count scheme, and tissue preparation technique when evaluating data involving asbestos tissue burden and in order to make appropriate comparisons between publications. As in the findings in our study of tissue burden in 55 mesothelioma cases from the same region [21], the majority of fibers of all types of asbestos in our pseudomesothelioma study were short fibers (<5 μm). The percentage of each type of asbestos fiber meeting the definition of a ''Stanton Index Fiber'' in our pseudomesothelioma study was much smaller than those in the mesothelioma study [21]. These ranged from 31.5% fewer qualifying fibers of amosite in our pseudomesothelioma study and a reduction from 39% of chrysotile fibers in the mesothelioma study [21] versus 0% in the pseudomesothelioma study.

Data from our pseudomesothelioma study reinforce the concern about findings derived in count schemes that inherently exclude a high percentage of the total fiber burden if a count includes only fibers >5 μm and a population of thicker fibers (≥0.25 μ). This is illustrated by the limited number of fibers found in samples in our pseudomesothelioma study that would be counted under a NIOSH 7400 count scheme.

However, it should be recognized that an evaluation of such data must consider the comments of Langer and colleagues [27] that the accepted model was implemented in part for reproducibility and convenience with full knowledge that "short fiber, <5 μm in diameter was the predominate component in the air." Indeed, fibers <5 μm are the predominate fiber type in lung samples and particularly in tissue samples from extrapulmonary sites, including those where asbestos-induced pathological changes occur [16]. In an evaluation of the population of fibers countable under a 7400 Method, there was a drop of 50+% of the fibers per each amphibole type in our pseudomesothelioma study that would be detected in a count of fibers >5 μm by light microscopy when compared to the comparable data per each type of amphibole in the mesothelioma study group [21]. The chrysotile component potentially detectable in the mesothelioma group was reported as 1.4%, whereas none of the chrysotile in our pseudomesothelioma study would have been detected. Of further importance in consideration for detection of inherently thinner fibrils of asbestos structures such as chrysotile and crocidolite in samples from tissue, air, or water is the fact that high magnification ATEM is required for the detection of thin fibers such as illustrated in this study group [20]. The average length of amosite in our pseudomesothelioma study was shorter than those found in tissue from our mesothelioma study group [21] (8.6 versus 12.9), as was the average length per other types of noncommercial amphiboles, e.g.: tremolite, 3.7 versus 8.7; actinolite, 3.2 versus 8.7; and anthophyllite, 5.2 versus 8.5. However, the average length for crocidolite (9.2 versus 8.4) and chrysotile (6.7 versus 6.3) was similar in both studies. The average width of the fibers in our pseudomesothelioma study was also similar to those found on the same type of asbestos in the mesothelioma study [21].

The question as to whether asbestos exposure contributes to the development of pseudomesotheliomatous lung cancers is strongly suggested given some of the occupations within the study cohort since: (1) some feel that these tumors are metastatic from primary adenocarcinomas originating in the lung [1–3] while other scientists believe that many, if not most, arise from epithelial cells (glandular or squamous) in the pleural tissue; and (2) that asbestos is recognized as a lung carcinogen [22]. Interestingly, the occupations provided in many cases would suggest potential asbestos exposure in the workplace with titles such as "Insulators, Pipelayer and Shipyard Workers." Exposure to asbestos was suggested in three individuals listed under the occupation as electricians (Cases 4, 8, and 10), which may or may not be an occupation immediately considered as having asbestos exposure in the workplace dependent on specific work environments. These three individuals were in the top 10 in the pseudomesothelioma study group based on uncoated asbestos burden. The additional information offered in tissue burden data for uncoated fibers was illustrated in Case 8 since ferruginous body burden alone would have suggested ferruginous body levels consistent with those found in samples from the general population (0 FB/g wet weight of tissue in the left lung and 10 FB/g wet weight of tissue in the right lung). Additionally, Case 8 was not found to have either radiologically and/or pathologically defined asbestosis or pleural plaques. A further indication of the importance of tissue burden analysis is shown in Case 5 whose occupation was shown as "Built Kitchen Equipment." The indication in the submission to the pathology laboratory of "asbestos exposure" was validated in that the individual's asbestos and ferruginous body burden was elevated above that expected in individuals from the general population [12]. Included in the asbestos burden were appreciable numbers of the commercial amphibole – amosite. The survival period from diagnosis in the individuals in our pseudomesothelioma study was under two years in most cases. Thus, the prognosis after detection of this neoplasm is poor and similar to that of mesothelioma. Our pseudomesothelioma study indicates that ferruginous body data alone would suggest many of the cases of pseudomesotheliomatous lung neoplasms had past exposures to asbestos that contained a population of longer fibers since such bodies form on fibrous structures that are >10 μm in length. A number of these individuals had levels of ferruginous bodies in lung tissue that exceeded that expected in samples from the general population. However, as shown in our pseudomesothelioma study, even more information regarding past exposure to asbestos can be obtained when tissue samples are evaluated for uncoated fiber burden (including uncoated fiber types, numbers, and dimensions) as well as ferruginous body levels in individuals with pseudomesotheliomatous lung cancer and other diseases potentially induced by asbestos.

With respect to causation, the data we generated from this study would suggest a causal relationship between asbestos exposure and the development of pseudomesotheliomatous lung cancer in some persons with this unique disease and past exposure to asbestos. The question as to the relationship between cigarette smoking and asbestos acting synergistically in the causation of pseudomesotheliomatous lung cancer requires further discussion and was not the subject of this study.

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DECLARATION OF INTEREST

The cases in the present study were submitted to one of the author's laboratory (SPH) for tissue diagnosis as part of a medical and/or legal evaluation and payment was received for said service. Dr. Samuel P. Hammar has served as an expert in litigation cases, predominantly for the plaintiff, where there are claims of injury from asbestos exposure. Dr. Ronald F. Dodson serves as an expert witness at the request of plaintiff and defense firms involved in asbestos litigation. He also occasionally has served as a consultant to the court in such cases. Dodson has and does serve on panels/workshops for federal agencies in programs involving exposure to elongated mineral particles and human health. The authors are solely responsible for the content of this manuscript and the research described in the manuscript.

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