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## SCANNING ELECTRON MICROSCOPIC ANALYSIS OF MINERAL FIBER CONTENT OF LUNG TISSUE IN THE EVALUATION OF DIFFUSE PULMONARY FIBROSIS

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### Abstract

The mineral fiber content of lung parenchyma in 24 cases of diffuse pulmonary fibrosis of unknown cause was determined by scanning electron microscopy and compared with that of 36 autopsy cases of histologically confirmed asbestosis and 20 autopsy cases of patients with normal lungs. Fibers were isolated from the lung using a hypochlorite digestion technique and collected on the surface of a polycarbonate filter. In addition, the types of fibers present (asbestos vs. other mineral fibers) were determined by energy dispersive x-ray analysis (EDXA). When the histologic grade of fibrosis in the cases of asbestosis was compared with the uncoated fiber content by means of linear regression analysis, it was determined that the fiber content of the 24 cases of diffuse pulmonary fibrosis of unknown cause was below the 95% confidence limit for asbestosis in every instance. Furthermore, the majority of fibers analyzed by EDXA were not asbestos in the cases with diffuse pulmonary fibrosis of unknown cause, whereas more than 90% of the fibers from the asbestosis cases were commercial amphiboles (amosite or crocidolite). It was concluded that most patients with advanced pulmonary fibrosis whose tissue samples do not meet histologic criteria for asbestosis do not have asbestos-induced fibrosis, even though there may be some history of exposure to asbestos. In such cases, scanning electron microscopic analysis of mineral fiber content and EDXA of the types of fibers present often provide useful information with regard to the correct classification of these cases.

**Key Words:** Asbestosis, mineral fibers, scanning electron microscopy, idiopathic pulmonary fibrosis, usual interstitial pneumonitis, carcinoma of the lung, parietal pleural plaques.

### Introduction

Asbestosis is defined as pulmonary interstitial fibrosis resulting from inhalation of asbestos fibers, and usually occurs in the setting of prolonged, direct, and often heavy exposure to airborne fibers [24]. Histologic criteria for the diagnosis of asbestosis are well-defined, and include the presence of pulmonary interstitial fibrosis which tends to be distributed in a bronchiolocentric pattern associated with accumulations of asbestos bodies in tissue sections [10]. Various grading schemes have been proposed for the estimation of the severity of fibrosis in patients with asbestosis [10,20,27]. Epidemiologic studies have shown that there is a dose-response relationship between the levels of asbestos exposure and the severity of pulmonary fibrosis (i.e., asbestosis) [34]. Since asbestos fibers tend to accumulate progressively within the lung with continuing exposure, one would expect to find a correlation between the severity of pulmonary fibrosis and the concentration of asbestos fibers which can be recovered from the lung. Indeed, such a correlation has been demonstrated in a number of studies using a variety of techniques to estimate the pulmonary mineral fiber burden [3,8,29,37,42].

However, the correlation between mineral fiber content of the lung and the severity of fibrosis is somewhat imperfect. Ashcroft and Heppleston [3], for example, reported a progressive increase in mineral fiber content from mild to moderate fibrosis but no further increase in patients with severe fibrosis. These authors concluded that factors other than tissue fiber burden must be responsible for the progression to severe fibrosis in patients with asbestosis. Other studies have shown a poor correlation between the severity of asbestosis and the pulmonary mineral fiber content [40]. Since diffuse pulmonary fibrosis of unknown cause (i.e., idiopathic pulmonary fibrosis, also called usual interstitial pneumonitis) is considerably more common than asbestosis [39], and since asbestos workers are not exempt from other fibrotic pulmonary disorders, one possible explanation for these observations is that some of the cases with

severe pulmonary fibrosis and relatively low fiber burdens were not causally related to asbestos exposure.

The problem is further compounded by the reported occurrence of cases of occult asbestosis, that is, diffuse pulmonary fibrosis in which asbestos bodies are not identified in histologic sections but the mineral fiber content of the lung as assessed by electron microscopy is distinctly abnormal [5,16,41]. Such cases would be difficult to distinguish from idiopathic pulmonary fibrosis by histologic evaluation alone. The finding of other markers of asbestos exposure, such as parietal pleural plaques, might be useful in this regard [29]. However, pleural plaques are quite common and may be related to very low level exposure to asbestos [33,38]. Therefore, the mere finding of plaques is no absolute guarantee as to the nature or etiology of diffuse pulmonary fibrosis in a given case.

The author has had the opportunity to examine the mineral fiber content of 36 autopsied cases of asbestosis by means of scanning electron microscopy [29]. Each case satisfied established histologic criteria for the diagnosis of asbestosis, and the severity of fibrosis was assessed using the grading scheme proposed by the College of American Pathologists and the National Institute for Occupational Safety and Health [10]. There was a highly significant correlation between the numbers of fibers 5  $\mu$ m or greater in length per gram of wet lung tissue and the estimated severity of interstitial fibrosis, with  $p < 0.01$ . In addition, there was a suggestion of a threshold burden in order for pulmonary fibrosis to develop [29]. In contrast, a number of cases of diffuse pulmonary fibrosis had been examined which did not satisfy histologic criteria for the diagnosis of asbestosis, even though there had been some history of asbestos exposure. In a few of these cases, there were other clinically relevant explanations for the patient's pulmonary fibrosis. In most of the cases, there was no apparent etiology and mineral fiber analysis was performed to evaluate the possibility of occult asbestosis. Also, in most of these cases, the degree of fibrosis was advanced at the time that the tissue was obtained for histologic evaluation. The purpose of the present study was to determine whether linear regression analysis of the relationship between severity of fibrosis and tissue fiber burden as determined in confirmed cases of asbestosis could provide useful information in the evaluation of cases of diffuse pulmonary fibrosis of unknown cause.

#### Materials and Methods

##### Case Selection.

The study group consisted of 24 cases from the author's consultation files with diffuse pulmonary fibrosis of unknown cause and for which lung tissue was available for analysis of mineral fiber content. In each case, there was some history of exposure to asbestos, although specific details were available for

only 15 patients. In 16 cases, no asbestos bodies were observed in hematoxylin and eosin or iron-stained sections after prolonged searching. In an additional five cases, equivocal asbestos body fragments were noted on an iron-stained section. In two cases, a rare but definite asbestos body was noted on an iron-stained section, and in one case, a rare body was found after prolonged searching of one hematoxylin and eosin and one iron-stained section. These latter three cases (Cases 11, 21, and 22, Table 1) could arguably meet histologic criteria for asbestosis [7]. However, even in these cases, the severity of fibrosis seemed to be far out of proportion to the rare asbestos bodies found, so that further analysis of tissue for mineral fiber content was deemed to be warranted.

##### Grading of Fibrosis.

The grading scheme used in the author's previously reported study of 36 cases of asbestosis [29] was that proposed by the College of American Pathologists and the National Institute for Occupational Safety and Health [10]. Briefly, this scheme involves the assessment of two histologic features: the proportion of bronchioles with fibrosis within their walls (graded on a scale of 0-3), and the most advanced extent of fibrosis on any one slide (graded on a scale of 0-4). To obtain a score for each slide, the scores for each of these features are multiplied together, giving a range of values from 0 to 12. A score for an individual case is then obtained by averaging the scores for all of the slides prepared from that case [10]. In the author's experience using this scale, slides showing grade 3 severity (i.e., fibrosis involving all alveolar septa between two adjacent bronchioles) always show fibrosis involving the walls of a majority of bronchioles (grade 3 profusion). Similarly, slides showing grade 4 severity (i.e., honeycomb changes) always show grade 3 profusion. Thus slides from cases of asbestosis with areas of grade 3 or 4 severity would have scores of 9 or 12, respectively. Therefore, for the purposes of this study, slides from cases of idiopathic pulmonary fibrosis in which all alveolar septa between two adjacent bronchioles were fibrotic were assigned a score of 9, and slides from cases showing honeycomb changes were assigned a score of 12. Grading of the severity of fibrosis using this scheme was available in 20 of the 24 cases included in the present study.

##### Mineral Fiber Analysis.

Tissue mineral fiber content was determined using the sodium hypochlorite digestion procedure, the details of which have been reported previously [31,33]. Briefly, formalin fixed lung parenchyma with a wet weight between 0.25 and 0.35 gm was minced with a clean scalpel blade and digested in 5.25% sodium hypochlorite solution (commercial bleach) with constant gentle agitation. The residue was collected on 0.4  $\mu$ m pore-size Nuclepore® filters, one of which was mounted on a glass slide for asbestos body quantification by light microscopy (LM) at a

magnification of 200x. The other was mounted on a carbon disc with colloidal graphite, sputter-coated with gold, and examined by scanning electron microscopy (SEM). Fibers were counted by SEM using a JEOL JSM 35C scanning electron microscope operated at 20 kV accelerating voltage at a screen magnification of 1000x and a scan rate of 5 sec/frame [30]. Fibers were defined as particles with an aspect ratio (length:diameter) of 3:1 or greater and roughly parallel sides, and particles meeting these criteria and with a length of 5  $\mu\text{m}$  or greater were counted. A total of 100 fields with an approximate cumulative area of 2.53  $\text{mm}^2$  were counted for each sample. From these data, fiber density on the filter surface and numbers of fibers per filter could be determined. Asbestos bodies (asbestos fibers coated with ferroprotein material) were enumerated separately. Results are reported as asbestos bodies or uncoated fibers 5  $\mu\text{m}$  or greater in length per gram of wet lung tissue. Filter blanks were also examined and all reagents prefiltered to avoid contamination [30].

In five cases (Case 3-7, Table 1), only paraffin blocks of lung parenchyma were available for analysis. In these cases, tissue was recovered from the block, deparaffinized in xylene, and rehydrated to 95% ethanol as previously described [32,33]. Digestion was then performed as described above. The Nuclepore® filter was cut in half with a scalpel blade, and one half was mounted on a glass slide for asbestos body quantification by LM whereas the other half was mounted on a carbon disc and examined by SEM. The results were multiplied by a correction factor (0.7) which takes into account the difference in weight between formalin fixed lung and lung which has been processed into paraffin [33]. In addition to the 24 cases of diffuse pulmonary fibrosis described above, asbestos body counts (LM) and uncoated fiber counts (SEM) were determined in 20 adult autopsy specimens with macroscopically normal lungs and no known asbestos exposure.

The chemical composition of mineral fibers was determined by means of energy dispersive spectrometry (EDS) using a KEVEX 7000 detector and an accelerating voltage of 25 kV [30]. In 20 cases with diffuse pulmonary fibrosis, 10 to 25 consecutive fibers were analyzed and classified as asbestos (amosite, crocidolite, tremolite, anthophyllite, actinolite, or chrysotile) or nonasbestos mineral fibers based on their morphology and chemical composition as previously described [30,33]. In two cases, only three (Case 1) and five (Case 19) fibers were analyzed by EDS. In two additional cases (Cases 6 and 7), EDS was not performed.

#### Statistical Analysis.

Linear regression analysis of the relationship between severity of fibrosis and the concentration of uncoated fibers 5  $\mu\text{m}$  or greater in length in 36 autopsy cases of histologically confirmed asbestosis had been reported previously. Ninety-five percent confidence limits were constructed for the regression line using these previously reported

data. The uncoated fiber content of cases of diffuse pulmonary fibrosis of unknown cause as well as autopsied cases with normal lungs were then compared with the linear regression 95% confidence limits constructed from the confirmed asbestosis cases.

#### Results

Demographic information regarding the 24 cases of diffuse pulmonary fibrosis included in this study are summarized in Table 1. The median age for the 20 patients where this information was available was 61 years, with a range of 42 to 85 years. All were men with the exception of Case 8. Specific information regarding the patients' occupations were available in 15 cases (Table 1). Smoking history was available in only 10 cases: eight patients were smokers (Cases 4-7, 11, 17, 21 and 23) with a range of 20 to 70 pack years, and two were nonsmokers (Cases 14 and 20).

The pathologic findings in the 24 cases are summarized in Table 1. One patient had interstitial lung disease the onset of which coincided with clinically diagnosed polymyositis (Case 17) [30]. Another patient had familial pulmonary fibrosis and cirrhosis of the liver (Case 19), and his brother was diagnosed with an identical condition. Case 23 developed diffuse pulmonary fibrosis following x-ray therapy for carcinoma of the lung [18]. Case 6 had an open lung biopsy which showed changes indicative of desquamative interstitial pneumonitis [19]. The remaining 20 cases were classified histologically as idiopathic pulmonary fibrosis or usual interstitial pneumonitis (U.I.P.) [19]. Grading of the severity of fibrosis was performed in 20 cases, and was assessed as grade 9 in four cases and grade 12 in 16 (Table 1). Seven patients also had carcinoma of the lung (Cases 4, 8, 13, 16, 20, 23 and 24), and three had parietal pleural plaques (Cases 17, 21 and 23). Tissue was obtained for histology and mineral fiber analysis from surgical specimens in 11 cases and from autopsies in 13 cases.

The mineral fiber content of the lung in all 24 cases is provided in Table 1, and is compared with that of 76 cases of asbestosis [29] and 20 normal lungs in Table 2. The median asbestos body count in the 24 cases as determined by light microscopy was 24 AB/gm. In 15 cases, asbestos body content was below the limit of detection by SEM (i.e., 440 AB/gm for a 0.3 gm sample). In only one case (Case 15) was there a greater than expected discrepancy between the asbestos body content as determined by LM vs. SEM. The median uncoated fiber content for the 24 cases was 13,300 fibers per gram of wet lung tissue. The uncoated fiber content overlapped with that of 20 patients with normal lungs in 15 of 24 cases. In 9 of the 24 cases, the uncoated fiber content overlapped with that of the 76 patients with asbestosis. However, in every autopsied case of asbestosis with 46,000 or less uncoated fibers per gram, fibrosis was confined to the walls of respiratory bronchioles

Table 1. Demographic, Histopathologic, and Mineral Fiber Content Data on 24 Cases of Diffuse Pulmonary Fibrosis of Unknown Cause

Case No.	Age/ Sex	Diagnosis	Fibrosis grade	PPP	Occupation	AB/gm (LM)	AB/gm (SEM)	UF/gm (SEM)
1	70/M	U.I.P.	12	--	NA	< 3	< 440	< 440
2	62/M	U.I.P.	12	--	Farmer	< 3	< 440	29,000
3	51/M	U.I.P.	12	--	NA	< 5	< 440	6,900
4	55/M	U.I.P., large cell, ca., lung	9	--	Welder, sheet metal worker, shipyard	< 6.8	< 440	3,260
5	60/M	U.I.P.	NA	--	Brake mechanic (34y)	< 22	< 440	42,800
6	42/M	D.I.P.	NA	--	Brake repair (5y)	< 30	< 440	14,600
7	58/M	U.I.P.	12	--	Brake mechanic	< 95	< 440	24,000
8	74/F	U.I.P., adenoca., lung	12	--	NA	3.1	< 440	6,340
9	70/M	U.I.P.	NA	--	NA	6.7	< 440	4,200
10	?/M	U.I.P.	12	--	NA	11	< 440	9,370
11	71/M	U.I.P.	12	--	Construction worker (20y)	14	< 440	13,300
12	?/M	U.I.P.	12	--	NA	23	< 440	9,500
13	62/M	U.I.P., bronchogenic ca. with osteoclast like giant cells	12	--	Military laundry 20+ y ago	25	440	8,790
14	63/M	U.I.P.	9	--	Engr. maintenance chem. co. (35y)	36	< 440	6,800
15	43/M	U.I.P.	12	--	Welder (remote past)	55	2,700	18,900
16	65/M	U.I.P., squamous cell ca., lung	12	--	NA	118	< 440	24,000
17	58/M	Polymyositis I.L.D.	12	+	Draftsman shipyd, (28y)	140	960	800
18	57/M	U.I.P.	12	--	NA	148	600	18,000
19	47/M	Familial U.I.P., cirrhosis	12	--	Insulation work one summer (20+ yr ago)	180	< 440	7,940
20	59/M	U.I.P., BACA, Renal cell ca.	12	--	Merchant marine, deck maint., and boilermaker (32y)	360	1,300	20,400
21	?/M	U.I.P.	12	+	Shipyd. worker	450	320	16,100
22	?/M	U.I.P.	9	--	NA	470	2,850	26,500
23	75/M	Diffuse pulm. fibrosis, s/p XRT; Ca. of lung	NA	+	Sheet metal worker, shipyd. (24yr)	730	495	45,700
24	85/M	U.I.P., Adenoca., RUL	9	--	Brakeline grinder, many yrs.	7,740	7,800	13,300

Ab/gm=asbestos bodies per gram of wet lung tissue; adenoca.=adenocarcinoma; BACA=bronchioloalveolar cell carcinoma; ca.=carcinoma; chem. co.=chemical company; D.I.P.=desquamative interstitial pneumonitis; Engr.=engineer; ILD=interstitial lung disease; LM=light microscopy; maint.=maintenance; NA=not available; PPP=parietal pleural plaques; SEM=scanning electron microscopy; s/p XRT=status post x-ray therapy; UF/gm=uncoated fibers 5  $\mu$ m or greater in length per gram of wet lung tissue; U.I.P.=usual interstitial pneumonitis.

(Grade 3 or less). Thus even in the overlap areas, the degree of fibrosis in the diffuse pulmonary fibrosis cases (grade 9 or 12) was far out of proportion to the mineral fiber content. Furthermore, the asbestos body counts in the diffuse pulmonary fibrosis cases overlapped with those in the asbestosis series in only two cases (Cases 23 and 24) (Table 2).

The relationship between severity of fibrosis and uncoated fiber content of the lung for 36 cases of asbestosis [29], 20 cases of diffuse pulmonary fibrosis, and 20 cases with normal lungs is illustrated in Figure 1. The calculated regression line and the 95% confidence limits

for the asbestosis cases show that the fiber content for the diffuse pulmonary fibrosis cases of unknown cause is below the lower confidence limits for asbestosis in every case. Also of interest is the observation that the uncoated fiber content for the control cases with normal lungs is below the lower confidence limit for zero (i.e., absence of) fibrosis in 17 out of 20 cases.

The results of energy dispersive x-ray analysis of more than 1500 fibers from patients with asbestosis [29] and more than 250 fibers from patients with diffuse pulmonary fibrosis of unknown cause are summarized in Table 3.



# Mineral Fibers and Pulmonary Fibrosis

Table 2. Mineral Fiber Content of Lungs in Patients with Asbestosis, Idiopathic Pulmonary Fibrosis (U.I.P.), and Normal Lungs<sup>+</sup>

	N	Age	AB/gm (LM)	UF/gm (SEM)
Asbestosis (all cases)*	76	63 (37-87)	37,800 (600-1,600,000)	330,000 (18,000-12,500,000)
Grades 1-3	14	57 (46-70)	27,000 (3,670-1,600,000)	306,000 (22,100-2,890,000)
Grades 3-6	21	64 (48-75)	33,600 (700-1,400,000)	441,000 (78,000-7,810,000)
Grades 6-9	13	62 (51-79)	101,000 (1,190-481,000)	526,000 (18,500-12,500,000)
Grades 9-12	4	64 (48-65)	13,240 (840-234,000)	201,000 (116,000-3,600,000)
U.I.P. <sup>++</sup>	24	61 (42-85)	24 (<3-7,740)	13,300 (<440-45,700)
Normal Lungs	20	64 (28-85)	2.9 (0-21.9)	3,130 (<420-16,900)

+ Values presented are medians with ranges indicated in parentheses underneath.

\* Histologic grading of asbestosis was available in 52 of 76 cases.

++ This category includes a few cases of other diffuse interstitial lung disease (See Table 1).

These show that more than 90% of the fibers analyzed in patients with asbestosis are commercial amphiboles (amosite or crocidolite), whereas more than half of the fibers analyzed in patients with diffuse pulmonary fibrosis of unknown cause are not asbestos. The types of non-asbestos mineral fibers identified include talc, silica, rutile, various aluminum silicates and other silicates, iron rich (presumably iron oxide), fiberglass, and organic (probably amorphous carbon) [6,28]. In only six of the 22 cases of diffuse pulmonary fibrosis were the majority of fibers analyzed a commercial form of asbestos (i.e., amosite, crocidolite, or chrysotile). Thus if the uncoated fiber count is corrected so that only asbestos fibers are included, the discrepancy between the asbestosis cases and the other diffuse pulmonary fibrosis cases shown in Figure 1 would be even greater.

## Discussion

The relationship between the concentration of uncoated fibers 5  $\mu$ m or greater in length as assessed by SEM and the severity of fibrosis in asbestosis as assessed histologically shows considerable scatter of the data, with the 95% confidence limits extending over two orders of magnitude (Figure 1). Nonetheless, the present study shows that SEM analysis of mineral fiber content of lung tissue can provide useful information in the evaluation of diffuse pulmonary fibrosis of unknown cause. As a group, the 24 cases of diffuse pulmonary fibrosis with some history of asbestos exposure but failing to meet histologic criteria for the diagnosis of asbestosis (i.e., fibrosis and asbestos bodies) are distinctly different from the cases of histologically confirmed asbestosis (Figure 1 and Table 2). There are several possible explanations for these observed differences.

The first possibility is that most or all of the 24 cases studied are not asbestosis;

i.e. asbestos is an unlikely cause for the fibrosis observed. This seems to be the most reasonable interpretation in at least 18 of the 24 cases presented. In 13 cases, the uncoated fiber count was within the range of values found in patients with normal lungs, and in 11 of 12 cases where EDS was performed, the majority of the fibers analyzed were not a commercial form of asbestos (Cases 1, 3, 4, 6, 8-14, 17 and 19). In an additional five cases, the uncoated fiber count was moderately elevated, but the majority of fibers analyzed were not a commercial form of asbestos (Cases 2, 15, 16, 18 and 20). In two of these 18 cases, a diagnosis of asbestosis was felt to be unlikely on clinical grounds (Cases 17 and 19). However, in the remaining 16 cases, there was no apparent clinical explanation for the patient's interstitial lung disease other than a possible exposure to asbestos.

Another possibility is that some or all of the 24 cases are examples of asbestosis due to exposure to a predominate fiber type differing from that of the cases with histologically confirmed asbestosis. Since chrysotile is more readily degraded and cleared from the lungs than amphibole fibers and is therefore less likely to form asbestos bodies, this possibility must be seriously considered [4]. However, in studies of chrysotile miners and millers with asbestosis, it has been shown that asbestos bodies are readily detected histologically and that these bodies for the most part have chrysotile asbestos cores [21]. In addition, studies of asbestos bodies recovered by bronchoalveolar lavage from chrysotile friction product and cement workers show similar numbers of bodies as recovered from individuals exposed to amphiboles [17]. Furthermore, the bodies recovered from the chrysotile workers have chrysotile cores. The author's own unpublished observations of chrysotile textile workers with asbestosis indicate that asbestos bodies are readily detected histologically in these cases.

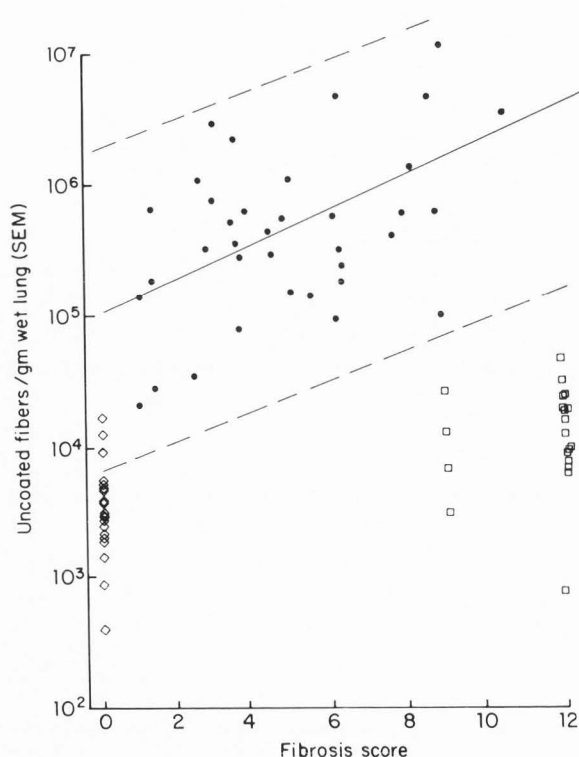


Figure 1. Linear regression analysis of the relationship between the severity of fibrosis and the pulmonary concentration of uncoated fibers 5  $\mu$ m or greater in length as determined in 36 autopsied cases of asbestosis by SEM. The regression equation is given by  $\log y = 0.117x + 5.05$  and is indicated by the solid black line. Dashed lines mark the 95% confidence limits. Asbestosis (●), diffuse pulmonary fibrosis of unknown cause (□), and normal autopsied lungs (◇).

Thus differences in predominate fiber type exposure between patients with confirmed asbestosis and the 24 cases of diffuse pulmonary fibrosis of unknown cause are an unlikely explanation for the observed differences between these two groups.

A third possibility is that some of the cases are examples of asbestosis due to exposure primarily to short (< 5  $\mu$ m) chrysotile fibers. Such fibers would not form asbestos bodies and would not be counted using the methodology in the present study. This possibility would be particularly applicable to Cases 5-7, whose occupations involved exposure to brake linings and brake shoe dust. However, brake dust has a very low fibrogenic potential since asbestos comprises less than 1% of the dust by weight, the fibers present are ultrashort chrysotile (<1  $\mu$ m in length), and most of the fiber has been structurally altered by the heat generated during the braking process [43]. Furthermore, there is a large body of experimental data which indicates that short-fibered asbestos (<5  $\mu$ m

in length) is nonfibrogenic [1,2,11-14,22,36,44]. EDS of ten fibers from Case 5 showed that six were chrysotile and two were non-commercial amphiboles (tremolite and anthophyllite). Unfortunately, EDS was not performed in Cases 6 and 7. The author considers that a link between brake dust exposure and diffuse pulmonary fibrosis remains unproven in these cases.

In some of the cases in the present study, the correct classification as idiopathic pulmonary fibrosis or asbestosis remains in doubt even after careful histologic assessment and SEM analysis of mineral fiber content. Case 23 was a patient who had diffuse pulmonary fibrosis first manifest clinically following therapeutic radiation for carcinoma of the lung [18]. Although the mineral fiber content in this case is below the 95% confidence limits for diffuse (Grade 9) asbestosis, it is well within the range for milder asbestosis which may have been obscured by superimposed radiation-associated fibrosis. Case 24 was an 85 year old man who developed fatal pulmonary fibrosis some 20 years after retirement from his occupation as a brakeline grinder in a manufacturing plant for many years. Although the uncoated fiber content was disappointing, the high asbestos body count by LM and SEM makes it difficult to exclude asbestosis in this case. The majority of the fibers analyzed by EDS were amosite. A useful piece of information would have been the appearance of a chest x-ray at the time of his retirement at age 65, but this information was unavailable. Cases 21 and 22 also had distinctly elevated asbestos body counts, rare asbestos bodies in histologic sections, and a majority of the fibers analyzed by EDS were commercial amphiboles. In addition, Case 21 was a shipyard worker and also had parietal pleural plaques. Although these cases could conceivably be examples of asbestosis, the discrepancy between the uncoated fiber content and severity of fibrosis remains unexplained.

An interesting finding in this study is the observation that only three of 20 patients with normal lungs at autopsy had uncoated fiber counts within the 95% confidence limits for grade 0 fibrosis. This observation is interpreted as further evidence for a threshold level for asbestos exposure which is necessary before interstitial fibrosis can be identified histologically. This interpretation is supported by the identification of substantial numbers of patients with parietal pleural plaques, a history of asbestos exposure, and an uncoated fiber content intermediate between that of normal controls and the lower limit for asbestosis, but who lack any appreciable interstitial fibrosis [33,38]. These data are not consistent with a linear dose-response, no threshold model for asbestosis [9,15].

There are several possible explanations for the amount of scatter in the data regarding fiber content and degree of fibrosis in asbestosis as illustrated in Figure 1. One possibility is variation in host response, with some individuals generating a greater fibrogenic reaction to a given fiber burden than others.

Also, some variation in severity of fibrosis may have been due to histologic sampling. Some investigators have reported that the severity of fibrosis correlates better with total surface area of retained asbestos fibers than with fiber concentration [23,35], although this observation has not been confirmed by others [8]. Finally, the dilutional effect of fibrosis upon fiber concentration must be considered, since the reporting of data in terms of fibers per gram results in a decrease in fiber concentration as the weight of the lungs increases consequent to the deposition of collagen and the accumulation of inflammatory cells and proteins [25,26]. This dilutional effect of fibrosis can be accounted for by multiplying the fiber concentration per unit lung weight by the total weight of both lungs and reporting the data as total fibers within the lungs. The effect this dilutional factor has on the regression line is to flatten the slope and thus minimize the incremental increase in fibrosis which occurs with increasing total lung fiber burden. This correction was not used in the present study since eleven of the 24 cases were based on the analysis of surgical specimens and total lung weight was therefore not known. This dilutional effect does not account for the differences in fiber burden between the asbestosis and diffuse pulmonary fibrosis cases since comparisons between these two groups were stratified by severity of fibrosis. In this manner, the dilutional effect of fibrosis should be the same for cases with, for example, grade 9 severity regardless of whether the patient has asbestosis or idiopathic pulmonary fibrosis.

A novel conclusion of this study is that most patients with advanced pulmonary fibrosis whose tissue samples do not meet histologic criteria for asbestosis do not have asbestos-induced fibrosis, even though there may be some history of exposure to asbestos. Although cases of occult asbestosis (e.g., Cases 21 and 22) probably do occur, they are distinctly uncommon. These observations reinforce the importance of adhering to strict criteria for the histologic diagnosis of asbestosis, specifically, the requirement for finding asbestos bodies as well as fibrosis in histologic sections. In cases of diffuse pulmonary fibrosis where asbestos bodies are not observed in spite of some history of asbestos exposure, scanning electron microscopic analysis of mineral fiber content and energy dispersive x-ray analysis of the types of fibers present often provide useful information with regard to the correct classification of these cases.

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Table 3. Energy Dispersive Spectrometry of 1530 Fibers from 76 Patients with Asbestosis and 269 Fibers from 22 Patients with Idiopathic Pulmonary Fibrosis

Case No.	No. Fibers Analyzed	Commercial Amphiboles	Non-Commercial Amphiboles	Chrysotile	NAMF
U.I.P.					
1	3	--	--	--	< 440
2	10	--	8,700	--	20,300
3	10	--	1,380	--	5,520
4	10	330	--	--	2,930
5	10	--	12,200	36,700	12,200
8	10	630	2,540	--	3,170
9	10	--	--	--	4,200
10	10	940	940	--	7,500
11	10	1,330	6,650	--	5,320
12	10	--	--	--	9,500
13	10	--	--	--	8,790
14	10	--	--	--	6,800
15	16	4,720	5,910	--	8,270
16	10	9,600	9,600	--	4,800
17	20	520	40	--	240
18	10	5,400	--	1,800	10,800
19	5	3,180	1,590	--	3,180
20	25	4,080	--	--	16,600
21	10	9,660	1,610	--	4,830
22	20	17,200	3,980	1,320	3,980
23	20	38,800	4,570	--	2,280
24	20	9,310	--	--	3,990
TOTAL	269	85 (32%)	34 (12.6%)	8 (3.0%)	142 (53%)
Asbestosis					
1	20	12,500,000	--	--	--
2	50	9,310,000	--	--	--
3	20	7,800,000	--	--	--
4	20	4,840,000	--	--	--
5	20	4,790,000	--	--	--
6	20	3,600,000	--	--	--
7	10	3,400,000	--	--	--
8	20	2,920,000	--	--	--
9	20	2,890,000	--	--	--
10	20	2,300,000	--	--	--
11	20	1,890,000	--	--	--
12	20	1,400,000	--	--	--
13	20	1,260,000	--	--	--
14	20	1,220,000	--	--	--
15	50	1,220,000	--	--	--
16	20	1,100,000	--	--	--
17	20	1,060,000	--	--	56,000
18	10	1,020,000	--	--	--
19	20	882,000	49,000	--	49,000
20	20	826,000	--	--	--
21	20	823,000	43,300	--	--
22	20	752,000	--	--	--
23	20	716,000	--	--	--
24	20	687,000	--	--	33,200
25	20	621,000	--	--	--
26	20	603,000	--	--	31,800
27	20	596,000	--	--	31,400
28	20	593,000	--	--	--
29	20	547,000	--	--	--
30	20	523,000	--	--	27,600
31	20	516,000	--	--	--
32	10	432,000	108,000	--	--
33	20	431,000	--	--	--
34	20	416,000	--	--	--
35	20	407,000	--	--	--
36	10	373,000	--	--	--
37	20	371,000	--	--	--

# Mineral Fibers and Pulmonary Fibrosis

38	10	360,000	--	--	540,000
39	20	317,000	--	--	--
40	20	314,000	16,500	--	--
41	20	314,000	--	--	--
42	20	291,000	32,300	--	--
43	20	284,000	--	--	--
44	10	282,000	--	--	--
45	20	281,000	14,800	--	--
46	20	272,000	16,000	32,000	--
47	20	272,000	--	--	--
48	20	246,000	--	--	--
49	20	226,000	--	--	--
50	20	193,000	--	--	34,000
51	25	191,000	--	--	16,600
52	50	189,000	--	--	--
53	10	169,000	--	--	--
54	20	160,000	--	--	8,440
55	20	152,000	--	8,000	--
56	20	149,000	--	--	16,600
57	10	141,000	--	--	--
58	20	139,000	7,300	--	--
59	20	134,000	8,900	--	35,600
60	20	116,000	15,400	7,700	15,400
61	20	113,000	--	--	37,800
62	20	110,000	--	5,800	--
63	20	91,800	5,400	--	10,800
64	20	79,200	9,900	4,950	4,950
65	10	70,200	7,800	--	--
66	20	60,800	--	--	--
67	20	60,000	--	--	15,000
68	20	48,000	6,000	--	66,000
69	50	46,400	17,400	--	81,200
70	20	35,400	4,170	--	2,080
71	20	21,400	--	--	14,300
72	25	20,500	3,150	--	15,800
73	20	15,700	1,850	--	920
74	10	14,900	--	--	9,920
75	10	10,200	7,650	--	7,650
76	10	4,170	--	--	1,790
TOTAL	1,530	1,389 (91%)	33 (2.2%)	6 (0.4%)	102 (6.7%)

Commercial Amphiboles: Amosite and Crocidolite

Non-Commercial Amphiboles: Tremolite, Anthophyllite, and Actinolite

NAMF = Non-asbestos mineral fibers: Talc, silica, rutile, aluminum silicates, potassium aluminum silicates, sodium aluminum silicate, sodium calcium silicate, iron, aluminum-iron, fiberglass, and organic (Z<11).

\*U.I.P. (usual interstitial pneumonitis) category includes a few cases of other diffuse, interstitial lung diseases (See Table 1).

= Not detected.

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#### Discussion with Reviewers

L.E. Stettler: Many long (greater than 5  $\mu\text{m}$ ) chrysotile fibers found in lung tissues have diameters less than 0.1  $\mu\text{m}$ . Given the relatively low magnification used (1000x), how efficient is your method for counting these very small diameter fibers?

R.F. Dodson: Would the author indicate approximately what percentage of the total chrysotile, amphibole and other fibers were excluded by using 0.4  $\mu\text{m}$  filters (by passing through the pores), an SEM magnification of 1000x, and a counting regimen including only fibers 5 micrometers or longer?

P. Dumortier: What is the minimum diameter of the fibers detected with counting methodology (SEM 1000x)? Could you visualize long fibers with very small diameters (e.g. 0.05  $\mu\text{m}$ )? Please comment on the influence of the results on fiber counts especially for chrysotile.

J.L. Abraham: If the counting is done at a magnification of 1000 and only fibers greater than 5 micrometers in length are counted, some comment on the undercounting of chrysotile or very thin amphiboles is necessary.

Author: At the relatively low magnification of 1000x, detection of the thinnest fibers ( $\leq 0.25 \mu\text{m}$ ) is partly dependent upon fiber length. The thinnest fibers that are detectable at this magnification are approximately 0.1  $\mu\text{m}$  in diameter. This method is therefore very inefficient for detecting chrysotile or amphibole fibers with diameters less than 0.1  $\mu\text{m}$ . Structures suspected to be fibers at 1000x are confirmed by centering in the field of view and examining at 10,000x. Although the total fiber count will be underestimated by omitting fibers greater than 5  $\mu\text{m}$  long but less than 0.1  $\mu\text{m}$  in diameter, this is unlikely to affect the conclusion on an individual case. A case of asbestosis would be missed by this method only if the vast majority of fibers 5  $\mu\text{m}$  or greater in length had diameters less than 0.1  $\mu\text{m}$ , and the numbers of fibers 5  $\mu\text{m}$  or greater with diameters greater than 0.1  $\mu\text{m}$  were near or slightly greater than our normal range. TEM could detect such cases, but the author is aware of no such report in the medical literature.

L.E. Stettler: Why do you use the 0.4  $\mu\text{m}$  pore size filter instead of a smaller pore size? Is fiber loss through these larger pores a problem?

## Mineral Fibers and Pulmonary Fibrosis

Author: A study published by O'Sullivan et al. (Environ Res 43: 97, 1987, coauthored by R.F. Dodson) has shown that a significant percentage of a stock solution of amosite fibers passes through a 0.4  $\mu$ m pore size Nuclepore filter. However, as noted by these authors the great majority of the fibers in the stock preparation were less than 5  $\mu$ m in length. Furthermore, there was an inverse correlation between fiber length and potential for being drawn through the pore. Thus whether there is a significant loss of fibers of the size counted by SEM at 1000x magnification due to use of 0.4  $\mu$ m pore-size filters is unknown. It is unlikely that any loss would affect the comparisons made in this study between asbestosis and idiopathic pulmonary fibrosis cases. Because of the organic residues often present in severely fibrotic lungs, the 0.4  $\mu$ m pore-size is used to facilitate filtration of the sample.

R.F. Dodson: Would the authors state how many square centimeters of tissue sections should be examined from patients with confirmed interstitial fibrosis before the presence or absence of bodies can be used for clarifying the diagnosis (i.e., asbestosis?).

Author: A previous study from the author's laboratory (Ref. 29) indicated that there were 5 or more asbestos bodies per  $\text{cm}^2$  of iron-stained histologic section in 95% of our cases of asbestosis, and 2 or more bodies per  $\text{cm}^2$  in all 76 cases (100%).

J.E. Craighead: The author has previously published quantitative correlative studies comparing the asbestos body counts of a tissue section with the asbestos body content of the corresponding tissue. These data are frequently quoted. Realizing that the experimental approaches in this study differed, has he confirmed the results of these earlier studies? It is not possible for me to answer this question from the data provided.

Author: Rare asbestos body fragments were observed in histologic sections of three cases with 470, 450, and 14 asbestos bodies per gram of wet lung tissue. No definite bodies were identified in sections from 20 other cases with counts ranging from 0 (none detected) to 730 asbestos bodies per gram. These observations are consistent with our previously reported sensitivity of histologic sections for the detection of asbestos bodies (i.e., they are generally not detected when the concentration is less than 200 bodies per gram). The one surprise was my inability to find bodies in histologic sections from case 24 with a digestion determined concentration of more than 7000 bodies per gram of wet lung.

P. Dumortier: Asbestos bodies were not detected in the sections of case 24 (7800 AB/gm wet lung tissue), excluding the histological diagnosis of asbestosis. Unpublished results from our laboratory obtained on 17 cases show that at least one section among six contain two or more

asbestos bodies for all the cases having more than 1000 AB/gm wet lung tissue. Could you please comment?

Author: See response to previous question.

R.F. Dodson: I would suggest that asbestos bodies should be reported as ferruginous bodies until a percentage from each patient has been analyzed and the cores proven to be asbestos.

Author: Studies done by Churg and Warnock have shown that ferruginous bodies isolated from human lungs that have a thin, translucent core virtually always have an asbestos core. Nonasbestos ferruginous bodies generally have black cores or yellow "sheet-silicate" type cores, and these are enumerated separately by the author as pseudoasbestos bodies (data not shown). The only exception is the zeolite (erionite) body which also has a thin translucent core, but these have not yet been reported from North America. In all of the cases in this series with detectable bodies by SEM (i.e., > 440 per gram), asbestos cores were identified by EDXA in each instance.

R.F. Dodson: Would the author discuss in more detail the comments made by Drs. Warnock and Churg in references #41 and #7, respectively, concerning the likelihood or reduced likelihood of finding asbestos bodies in tissue sections from individuals with asbestosis?

Author: Dr. Churg in Ref. #7 suggests that in the setting of diffuse pulmonary fibrosis, the finding of a single asbestos body in a histologic section is sufficient to make a diagnosis of asbestosis. The analysis of tissue mineral fiber content in cases 11, 21, and 22 at least raises the question as to whether that approach is in fact correct. Warnock and Wolery in Ref. 41 reported that in some cases where asbestos bodies were scarce in histologic sections, extracts of asbestos fibers yielded more than 500,000 total amphibole fibers per gram of dry lung and suggested that this figure should be the indicator to signify that interstitial fibrosis in an individual case is due to asbestos. This value is roughly equivalent to 50,000 fibers per gram of wet lung and includes all fibers 0.25  $\mu$ m or greater in length (with an unknown but probably small percentage of fibers greater than 5  $\mu$ m in length). These authors did not perform the types of linear regression and statistical analyses which are reported in the present study.

R.F. Dodson: Summary length and width histograms for the various fiber types would be useful.

J.L. Abraham: What are the dimensions of fibers? A distribution of length-diameter and aspect ratio would help put the data in perspective with other reported data.

Author: Unfortunately, these data are not available on a fiber by fiber basis. As noted above, all fibers counted were five microns or greater in length. Most of the commercial amphibole and chrysotile fibers were greater than 10  $\mu$ m long and less than 0.5  $\mu$ m in diameter.

Most of the nonasbestos mineral fibers were less than 10  $\mu$ m long and greater than 0.5  $\mu$ m in diameter.

J.L. Abraham: What are the detection limits for the analysis? What does one fiber counted represent in numbers of fibers per gram of tissue for each case? How many fields or fibers are counted and what size filter is used?

Author: The detection limit is approximately 140 fibers per filter. The number of fibers per gram that each fiber represents will depend on the amount of tissue digested per filter, but for a typical case (0.30 gm sample) would be 440 fibers (or bodies) per gram wet weight. The protocol I use involves counting 200 fibers or 100 fields, whichever comes first. The filter is 25 mm in diameter.

J.L. Abraham: A detection limit of 440 fibers per gram for a sample weighing 0.3 gram means the fraction of the filter scanned is approximately 0.0075. Please confirm these calculations.

Author: Correct.

J.L. Abraham: In case 17 how were the numbers determined? The table reports that the number of asbestos bodies per gram was 960 and the number of fibers per gram was 800. Were these the same or different analyses? If one fiber equals 440 fibers per gram how does the number of 960 result?

Author: The relationship of one fiber counted equals 440 fibers per gram applies only to 0.30 gram sample size. In this one case, the sample size was substantially larger than 0.30 gram.

J.L. Abraham: In table 1 cases 16-24 had asbestos body content above the background limits of 100 per gram wet lung. Please comment on this in relationship to the conclusion that the fibrosis was determined to be unrelated to asbestos in most of those cases.

Author: All of the 24 cases had uncoated fiber counts which fell below the 95% confidence limits for bona fide asbestosis cases for the severity of fibrosis present. Cases 17 and 19 had uncoated fiber counts within the range of values observed in our reference population with normal lungs. Cases 16, 18 and 20 had modestly elevated uncoated fiber counts, but the majority of fibers analyzed were not a commercial form of asbestos. The correct classification of cases 21-24 is uncertain (See the 5th paragraph under Discussion), but if these cases are examples of asbestosis, the discrepancy between the fiber content and severity of fibrosis remains unexplained.

P. Dumortier: Do you consider that it is possible to formally exclude asbestos induced fibrosis in a case of chrysotile exposed worker whose lungs are examined long after exposure and do not show high concentrations of fibers?

What would be your medicolegal position in such a case?

Author: The author has examined lung tissue from a patient who made asbestos blankets, gaskets and brake-linings exclusively from chrysotile for 7 years, with no additional exposure for 43 years prior to admission. Open lung biopsy showed mild asbestosis (Grade 3) with asbestos bodies present in histologic sections. Analysis of lung tissue showed large numbers of high aspect ratio tremolite fibers and some long chrysotile fibers as well. In the hypothetical case you described, if histologic criteria for asbestosis are not satisfied and analysis does not show high concentrations of fibers, I would say that from a pathologic perspective, a diagnosis of asbestosis can not be established to a reasonable degree of medical certainty.

R.F. Dodson: The author should clearly state his conclusions pertain only to those fibers  $\geq 5 \mu$ m in length and  $\geq 0.1 \mu$ m in width (based on his limit of detection at 1000x).

Author: The rationale for the author's belief that fibers less than 5  $\mu$ m in length are unlikely to contribute importantly to the pathogenesis of asbestosis are outlined in the 4th paragraph of the Discussion section. Fibers less than 0.1  $\mu$ m in diameter account for only a small proportion of fibers, even in a stock solution of amosite consisting mostly of short fibers (O'Sullivan et al., Environ. Res. 43: 97, 1987).

P. Dumortier: As case 24 was also exposed in brake lining operations, it must be cited along with cases 5-7.

Author: Case 24 was discussed separately (5th paragraph under Discussion) because the patient worked as a brakeline manufacturer rather than as a brake repairman, large numbers of asbestos bodies were found by digestion, and amosite was the predominant fiber detected by EDS. Asbestosis cannot be excluded with certainty in this case.

J.E. Craighead: I am confused regarding the author's interpretation of Results regarding tremolite fibers. Are they a "marker" of chrysotile asbestos exposure? Can the counts of tremolite be used to assess past chrysotile exposure? Are tremolite fiber size considerations important? Does the work reported here provide information relevant to this important issue?

Author: There is considerable data in the literature to indicate that tremolite fibers are a marker of chrysotile asbestos exposure and that the counts of tremolite can be used to assess past chrysotile exposure. The author believes that as is the case for any other mineral fiber, fiber dimensions of tremolite are important determinants of pathogenicity. I do not believe that the work reported here provides information relevant to this issue.



## Mineral Fibers and Pulmonary Fibrosis

P. Dumortier: What are the types of fibers encountered in the control subjects? Is there any similarity with those reported for the subjects with UIP?

Author: Nearly 80% of the fibers 5µm or greater in length isolated from the control subjects' lungs were nonasbestos mineral fibers of similar types to those found in the UIP cases. Only 5% were commercial amphiboles. The remaining 15% were non-commercial amphiboles (mostly tremolite) and chrysotile.

J.L. Abraham: Is there any further information on the fraction of patients with parietal pleural plaques who have had lung parenchyma analyzed and who have grade 0 fibrosis or asbestosis? How many of these types of cases have actually been studied?

Author: The author has analyzed lung parenchyma asbestos content in 40 cases with parietal pleural plaques but without evidence of parenchymal asbestosis. These cases will be reported separately. The median asbestos body count by light microscopy was 1450 bodies/gm wet lung, and the median fiber count by SEM was 26,000 fibers/gm. More than half of the fibers analyzed in these cases by EDXA were commercial amphiboles, amosite or crocidolite.

J.E. Craighead: Does the author believe selected area electron diffraction has any role in the crystallographic evaluation of suspect asbestos fibers?

Author: In the author's opinion, asbestos fibers can be distinguished from nonasbestos mineral fibers from human lung samples in most instances on the basis of morphologic features and EDXA results. Electron diffraction is useful in some instances, particularly in distinguishing between anthophyllite asbestos and fibrous talc or between anthophyllite asbestos and chrysotile.

L.E. Stettler: Have you analyzed any of the nonfibrous particles in your specimens? Considering the lack of correlation between fibrosis and asbestos fiber content in the 24 lungs, could this fibrosis be related somehow to the lungs' nonfibrous mineral content?

Author: I have not investigated the relationship between fibrosis and the nonfibrous mineral content of the lung in these 24 cases.

P. Dumortier: Figure 1 includes data from 36 patients with asbestosis, whereas, Table 2 summarizes information regarding tissue asbestos content in 76 cases of asbestosis. Please comment.

Author: The author has examined the tissue asbestos content in 76 patients with asbestosis, including surgical and autopsy samples. Only those cases with sufficient histologic sampling (i.e., autopsies) were included in the construction of Figure 1.

R.F. Dodson: It would be helpful to add one column to Table 1 for smoking data in pack years.

Author: Smoking history was available in only 10 of the 24 cases. Therefore, it seemed more reasonable to merely summarize these data in the first paragraph of the Results section rather than to display this limited information in tabular form.

J.E. Craighead: Did any of the cases of alleged UIP and asbestosis have the bronchiolitis obliterans form of pulmonary fibrosis? If so, what did the quantitative data tell us about these cases?

Author: None of these cases had the bronchiolitis obliterans form of pulmonary fibrosis.