

THE ROLE OF ANALYTICAL SEM IN THE DETERMINATION OF CAUSATION IN MALIGNANT MESOTHELIOMA

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Asbestos is a group of mineral fibers that share certain characteristics making them useful in the manufacture of a variety of products. There are two large groups of asbestos fibers: serpentine, of which chrysotile is the only asbestiform member, and amphiboles, which include five distinct mineral species. Amosite and crocidolite are amphibole fibers that were used commercially, whereas tremolite, actinolite, and anthophyllite have had limited commercial usage. These noncommercial amphiboles, however, are important as contaminants of other mineral species. A variety of non-asbestos mineral fibers may also be identified within human lung samples. The analysis of the mineral fiber content of lung tissue by means of scanning electron microscopy (SEM) has provided useful information regarding the causation and pathogenesis of malignant mesothelioma. The following is an abbreviated summary of the author's experience in this regard during the past 24 years.

Tissue Selection. In most circumstances, formalin-fixed lung tissue is utilized, although fresh specimens work just as well. In some instances, paraffin-embedded tissue is all that is available. Such samples can be deparaffinized and rehydrated. A correction factor must be applied to equate the values obtained from paraffin blocks to those obtained from formalin-fixed tissue. In the author's laboratory, the correction factor has been determined to be approximately 0.7. Areas of consolidation, congestion, or tumor should be avoided as much as possible. Since there is some site to site variation of mineral fiber content within the lung, the more tissue that is available for analysis the better. Ideal specimens include autopsy or pneumonectomy specimens, with analysis of multiple sites. In the authors' laboratory, two or three samples are typically analyzed from a pneumonectomy specimen, whereas four sites (upper and lower lobes of each lung) are sampled when both lungs are available at autopsy. Samples usually include lung parenchyma abutting against the visceral pleura, with each sample typically weighing 0.25-0.35 gm (wet weight). However, analyses may be performed on as little as 0.1 gm of wet tissue. Transbronchial biopsy specimens are unlikely to be representative.

Digestion Technique. Techniques for mineral fiber analysis generally involve three basic steps: dissolution and removal of the organic matrix material in which the fibers are embedded, recovery and concentration of the mineral fibers, and analysis of fiber content by some form of microscopy.¹ Digestion is accomplished with sodium hypochlorite solution (commercial bleach). Once digestion is complete, the inorganic residue may then be collected on a polycarbonate filter, with a pore size of 0.2 to 0.45 μ m. Use of a pore size which is too large in relation to the size of the fibers to be analyzed can result in significant loss of fibers and underestimation of the mineral fiber content of the sample.

Fiber Identification and Quantification. Conventional light microscopy is ideal for the quantification of asbestos bodies, which are counted at a magnification of 200-400x. The results are reported as numbers per gram of wet lung tissue. Alternatively, a piece of lung tissue adjacent to the one actually analyzed can be dried to constant weight to obtain a wet-to-dry weight ratio, and the results reported as asbestos bodies per gram of dry weight. As a rule of thumb, one fiber/gm wet lung = one fiber/cm³ = ten fibers/gm dry lung. SEM has utility for the

quantification of fibers within the lung and identification of fiber types. The latter can be accomplished by coupling the SEM with energy dispersive x-ray analysis (EDXA) to determine the elemental composition of individual fibers. This information can be used to classify a fiber as asbestos or non-asbestos and to determine the specific asbestos fiber type. Sample preparation for SEM is relatively simple, requiring only that the filter be mounted on a suitable substrate and then coated with an appropriate conducting material.

Variability of Results. Interlaboratory comparison trials demonstrate that striking differences can occur among laboratories even when the same sample is analyzed. Some asbestos bodies and fibers may be lost during the preparation process, and some of the smallest fibers are difficult to recognize and count in a reproducible fashion. Nonetheless, there is evidence for internal consistency within individual laboratories, with similar ranking of samples among different laboratories from the lowest to the highest tissue fiber concentration. Still, one must use caution in comparing results between laboratories, bearing in mind any differences in the analytical procedures employed.¹

Intralaboratory variation can occur due to variation in fiber content from one site to another within the lung. In the author's experience, paired samples have asbestos body and fiber concentration values ranging from identical to within a factor of two or three. Rarely, two samples from the same patient may differ by as much as a factor of 10 or more. There is a growing consensus that the fiber burden that persists in the lung is the primary determinant of subsequent disease.¹

Malignant Pleural Mesothelioma. The author has analyzed the asbestos content of the lung in 396 patients with malignant mesothelioma. The median asbestos body count for pleural mesothelioma cases that also had asbestosis is much higher than cases that had parietal pleural plaques (PPP) without asbestosis (12,400 AB/gm vs. 845 AB/gm), which in turn is much higher than cases that had neither plaques nor asbestosis (105 AB/gm). A similar trend is observed for uncoated fibers as measured by SEM. The asbestos body content was within our normal range of 0-20 AB/gm in 74 cases, or 20% of the total. In 27 of these 74 cases, the fiber content was found to be elevated by SEM. Hence, the asbestos content was indistinguishable from that of a background population in 13% of cases. Approximately 87% of pleural mesotheliomas that we have analyzed have elevated fiber content and thus appear to be related to prior asbestos exposure.

Malignant Peritoneal Mesothelioma. The median asbestos body count is higher for patients with peritoneal as compared to pleural mesotheliomas. For cases that also had asbestosis, the median values were 132,000 AB/gm for peritoneal as compared to 12,400 per gram for pleural cases. For PPP only, the values were 350 vs. 845 AB/gm. For peritoneal cases with neither PPP nor asbestosis, the median asbestos body count was only 3.9 AB/gm. These findings are consistent with the observation that, on average, greater exposure to asbestos is necessary for the development of peritoneal mesothelioma than is needed to develop pleural mesothelioma.

Malignant Mesothelioma in Women. The median asbestos body count for 48 women with mesothelioma was 15.5 AB/gm. Twenty-seven cases had asbestos body counts within background range, and eight of these had an elevated fiber count by SEM. Hence, about 40% of mesotheliomas in women had an asbestos content indistinguishable from background. Approximately 64% of pleural mesotheliomas in women that we have studied appear to be

asbestos related. Most of these are secondary to exposure as a household contact of an asbestos worker. The majority of peritoneal mesotheliomas in women are not related to asbestos.

Mesothelioma and Fiber Type. The predominant fiber type identified in patients with mesothelioma is commercial amphibole (amosite or crocidolite). In a study of 94 cases from the United States, Roggli et al.² found that 58% of more than 1500 fibers analyzed were amosite, whereas only 3% were crocidolite. When cases were grouped by exposure category, more than 94% of 1445 cases fit into one or more of 12 different industrial, six different occupational, or one non-occupational categories.³ The one non-occupational category, that of a household contact of an asbestos worker, accounted for 6% of all cases and more than half of mesotheliomas among women. Most cases with a normal-range asbestos body count and elevated fiber content by SEM had predominantly non-commercial amphiboles (mostly tremolite). These fibers typically are in the size range between 5 and 20 μ m. Asbestos bodies usually form on fibers that are greater than 20 μ m in length. Chrysotile is a much less potent inducer of mesothelioma in humans, and there is no convincing evidence that chrysotile causes or contributes to the development of peritoneal mesothelioma.⁴

Mesothelioma and Fiber Size. In view of the experimental observations that fibers 8.0 μ m or greater in length and 0.25 μ m or less in diameter are the most efficient at producing mesotheliomas, it is of interest to examine fiber dimension data in studies of human cases of malignant mesothelioma. In a study of amphibole asbestos-induced mesotheliomas, Churg and Wiggs⁵ reported that 39% of amosite fibers and 23% of crocidolite fibers exceeded 5 μ m in length. In contrast, a study of chrysotile-related mesotheliomas showed that only 11% of chrysotile fibers and 13% of tremolite fibers were 5 μ m or greater in length.⁶ The vast majority of fibers in both studies were less than 0.25 μ m in diameter. McDonald et al. found that amphibole fibers 8 μ m or greater in length and 0.25 μ m or less in diameter accounted for essentially all of the risk of mesothelioma, with no additional information provided by short fibers, chrysotile fibers, or non-asbestos mineral fibers.⁷ The bio-persistence of relatively long amphibole fibers in lung tissues is the likely reason for the greater potency of amosite and crocidolite fibers in the production of mesothelioma as compared to chrysotile. The latter tends to fragment into shorter fibers and has a much shorter half-life within the lung.

Analysis of Pleural Samples. It should be noted that most of the studies of fiber burdens in mesothelioma patients have examined lung parenchyma. It is reasonable to assume that fibers actually reaching the pleura are the ones responsible for pleural disease, and the dimensions and types of fibers accumulating in the pleura are of interest in this regard. Sebastien et al. reported that in individuals exposed to mixtures of fibers, short chrysotile fibers (<5 μ m) tended to accumulate in the pleura whereas longer amphibole fibers accumulated in the lung parenchyma.⁸ Suzuki and Yuen⁹ also reported primarily short chrysotile fibers in the pleura and in mesothelial tissues. Dodson et al.¹⁰ found some long commercial amphibole fibers in samples of pleural plaque from asbestos workers, and Gibbs et al.¹¹ also identified similar fibers in pleural samples of patients with diffuse visceral pleural thickening. Boutin et al.¹² found a preferential concentration of long commercial amphibole fibers in black spots on the parietal pleura. Clearly, fibers of the type and size known to be associated with the greatest risk of mesothelioma do in fact migrate to pleural tissues. The identification of short chrysotile fibers in these tissues is of questionable relevance, since there is no convincing data that these fibers are pathogenic.

References:

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TABLE 1. ASBESTOS CONTENT OF LUNG IN 368 PLEURAL MESOTHELIMA CASES

	<u>N</u>	<u>AB/gm</u>	<u>N</u>	<u>UF/gm</u>
Asbestosis	44	12,400	44	77,800
Plaques	113	845	106	21,800
Other ^a	207	105	201	15,200

a. Neither plaques nor asbestosis

TABLE 2. ASBESTOS CONTENT OF LUNG IN 28 PERITONEAL MESOTHELIMA CASES

	<u>N</u>	<u>AB/gm</u>	<u>N</u>	<u>UF/gm</u>
Asbestosis	12	132,000	12	328,000
Plaques	5	350	5	23,300
Other ^a	11	3.9	11	6120

a. Neither plaques nor asbestosis