Value of in Situ Elemental Microanalysis in the Histologic Diagnosis of Silicosis

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Pulmonary specimens obtained from ten normal subjects and 53 patients with various pulmonary diseases were studied with energy-dispersive x-ray analysis. The amount of silicon in the pulmonary tissue was determined and expressed as a silicon/sulfur (Si/S) ratio. This Si/S ratio was below 0.2 in the ten normal subjects and in 14 patients who had various interstitial pulmonary diseases but had no previous history of exposure to silica or other dusts known to cause pulmonary fibrosis. The Si/S ratio was greater than 0.3 in 19 of 22 patients who had a history of exposure to silica dust and had clear-cut histologic evidence of silicosis. The Si/S ratio was less than 0.2 in 12 and between 0.2 and 0.3 in two of the 14 patients who had a history of exposure to silica dusts but no clinical or histologic evidence of silicosis. We conclude that the determination of the silicon content of tissue by energy-dispersive x-ray analysis is useful in separating the fibrosis due to silicosis from the other causes of pulmonary fibrosis.

The diagnosis of silicosis is usually based on a history of significant exposure to silica and a chest roentgenogram consistent with pneumoconiosis. When there is doubt, a histologic examination may become necessary, with the specimen usually obtained by open lung biopsy. The hallmark of the histologic diagnosis of silicosis is the presence of an amorphous whorled structure, often referred to as a silica nodule. The presence of doubly refractile crystals by polarized microscopy lends support to the diagnosis.

Histologic diagnosis becomes difficult, however, when the specimen fails to show a silica nodule. In such cases an elemental analysis may be helpful in establishing a definitive diagnosis. Although ashing a sample of pulmonary tissue followed by x-ray diffraction provides very useful information, this technique requires a large sample, and the amount of tissue obtained by an open lung biopsy is often not sufficient to perform this procedure. Recently, new methods of elemental analyses such as microprobe analysis or energy-dispersive x-ray analysis have been introduced into the study of biologic materials. Previously, we have studied pulmonary tissue from well-established silicosis by energy-dispersive x-ray analysis and have compared it with that of normal control subjects. We then established a level of silicon in the tissue at which fibrosis was associated with silicosis and which discriminated this group from normal control subjects. These data provided a method of establishing a histologic diagnosis in the absence of the classic silica nodule. This report describes further experience with energy-dispersive x-ray analysis in silicosis, specifically (1) the variability of the amount of silicon in the different lobes, and (2) the level of silicon in the tissue of patients who had a history of exposure but had no clinical or histologic evidence of silicosis.

Materials and Methods

Four groups of subjects were studied. Group A consisted of ten men aged 26 to 87 years (mean, 57 years) who had no previous exposure to silica or other inorganic dusts known to cause pneumoconiosis and who were without active pulmonary disease. The pulmonary specimens were obtained at autopsy from subjects who died of nonpulmonary causes. Group B consisted of 16 men and one woman, aged 23 to 67 years (mean, 50 years), who also had no history of exposure to silica or other inorganic dusts but had bilateral interstitial pulmonary disease. In this group, all pulmonary specimens were obtained by open lung biopsy performed for diagnostic purposes. Group C consisted of 14 men aged 26 to 82 years (mean, 56 years) who had a history of exposure to silica for a minimum of five years but had no clinical or histologic evidence of silicosis. This included ten foundry workers, one sandblaster, one subject who worked in a die shop where he was exposed to silica dust, and one coal miner. In one case the detail of exposure was not recorded except that the subject had an exposure to silica-containing dust. In this group the specimens were obtained at autopsy in six, biopsy in seven, and at the time of surgery for bronchogenic carcinoma in one. Group D consisted of 22 men aged 44 to 85 years (mean, 63 years) who had well-established silicosis. The criteria for the diagnosis of silicosis were as follows: (1) history of exposure to silica; (2) bilateral nodular densities on chest roentgenograms, when available; and (3) the presence of clear-cut silica nodule(s) in the histologic specimen(s). In this group, there were 14 foundry workers, four sandblasters, two stone cutters, one tunnel digger (this person was a prisoner of war during World War II and engaged in tunnel digging without any protective measures for over three years), and one coal miner. The pulmonary specimens were obtained by autopsy in 11 and biopsy in 11.

Preparation of Specimens

Specimens of pulmonary tissue stained with hematoxylin-eosin

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were examined under the light and polarized microscopes in the usual manner, and the presence or absence of classic nodules was carefully recorded. Details of the examination by energy-dispersive x-ray analysis have been published previously. Briefly, sections of 30µ thickness were cut from the paraffin tissue blocks and were then treated with xylene to remove the paraffin. The sections were hydrated by passing them through decreasing concentrations of alcohol into distilled water and were floated from the distilled water onto the surface of spectroscopically pure carbon planchets.

Analysis

The planchets containing the sections were examined in a scanning electron microscope (JSMU 25). Areas of thickened septa were randomly selected for examination while vessels, bronchioles, and nodules were avoided. These areas were photographed at a magnification of 100×, and then energy-dispersive x-ray analyses were performed with a nuclear semiconductor 150-eV resolution Se (Li) detector and a computer base data-handling system (Tracor Northern NS890). A total of five areas were analyzed routinely from each specimen. In a few selected cases, higher magnifications of 1,000×, 3,000×, and 9,000× were used. When there was an obvious pathologic process such as a malignant neoplasm, this area was avoided for elemental analysis.

Results

Specimens from the control group A (ten subjects) showed no pulmonary pathologic findings other than mild edema and patchy atelectasis. Group B consisted of 17 subjects who had no exposure to silica or other dusts. Of those, 15 had usual interstitial pneumonia with fibrosis, one had pulmonary fibrosis due to progressive systemic sclerosis, and one had sarcoidosis. Among the 14 patients in group C who had a history of exposure to silica dust, four had usual interstitial pneumonia with fibrosis, three had primary bronchogenic carcinoma, and the others consisted of single cases of pulmonary hemangiosarcoma, vasculitis, progressive systemic sclerosis, Mycobacterium avium intracellular disease, sarcoidosis, acute histoplasmosis, and chronic congestive heart failure. All 22 patients with silicosis, group D, had clear-cut silica nodules and various degrees of septal fibrosis but no other abnormalities.

Elemental Analysis

In order to overcome the difference of tissue mass in each area of analysis, the amount of silicon is expressed as a silicon/sulfur (Si/S) ratio. When more than two samples were available for study, the mean Si/S ratio was used as a final value for that particular subject. As shown in Figure 1, all ten control subjects (group A) and 17 subjects who had interstitial pulmonary disease without a history of exposure to silica (group B) showed a Si/S ratio below 0.2. In 14 patients who had a history of exposure to silica and had various pulmonary diseases (group C), two showed a Si/S ratio of between 0.2 and 0.3, but none had a ratio above 0.3. By contrast, only two of 22 patients with silicosis (group D) had Si/S ratios below 0.2. In one of these two patients who had Si/S ratio below 0.2, the specimen was very small and showed only an atypical nodule, the patient subsequently died, and the specimen obtained at autopsy showed clear-cut silica nodules, and the Si/S ratio was 0.6. His chest x-ray film revealed diffuse bilateral nodular densities consistent with simple silicosis. The second case also showed bilateral diffuse nodular densities on the chest roentgenogram. The histologic specimen showed very little interstitial fibrosis, although it contained a small silica nodule.

In order to determine the sample variation between the different lobes, one specimen from each of five lobes was examined in five normal control subjects, five of the subjects with silica exposure, and four cases of silicosis. As shown in Figure 2, all of the specimens in control group A showed a Si/S ratio of below 0.2, while most of the specimens with silicosis (group D) showed a Si/S ratio of 0.3 or higher. Five specimens in group D showed an Si/S ratio of 0.3 or lower. Specimens from five subjects who had a history of exposure to silica but no silicosis, group C, showed wider scatter; however, only two specimens showed a Si/S ratio of greater than 0.3.

Effect of Magnification

In order to evaluate the effect of the level of magnification on the element/sulfur ratio, three silicotic lungs were studied at three different magnifications. As shown in Table I, the higher magnifications of 1,000×, 3,000×, and 9,000× showed quite different Al/S and Si/S ratios than the magnification of 100×.

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21406/ on 06/27/2017)
DISCUSSION

A histologic diagnosis of silicosis can be made confidently if a classic silica nodule is present in the specimen; without it, the diagnosis is less secure. Examination of the specimen under polarized light is not only nonspecific but also lacks an objective quantification of the materials observed.\(^7\)\(^8\) For this reason the use of \textit{in situ} analysis of the elements by electron probe or energy-dispersive x-ray analysis has found increasing application.\(^7\)\(^8\) Although both methods detect silicon as an element, they do not define the chemical structure of the silicon compound; however, careful correlation with the subject's occupational history is usually helpful in resolving this shortcoming.

The major problem with these analyses is determining the etiologic significance of the silicon found in the specimen with the tissue pathology. Furthermore, the distribution of silica in the lung is relatively diffuse but not uniform; therefore, the finding of silica in a given sample may not be representative of a fibrotic process in the lung as a whole.

In a previous study, we determined the amount of silicon in fibrotic nonnodular areas of silicotic lungs. We found that a level of silicon in the fibrotic tissue expressed as a Si/S ratio of 0.3 could be used to reliably separate silicotic lungs from normal controls.\(^8\) The use of the Si/S ratio was necessary to overcome the differences in tissue mass between the various areas of analysis. This semiquantitative elemental analysis of the tissue provides a basis for the histologic diagnosis of silicosis in the absence of a classic silica nodule; however, there were several unanswered questions: (1) the presence or absence of a positive correlation between the amount of silicon determined by energy-dispersive x-ray analysis and the degree of tissue fibrosis; (2) the discriminatory power of this ratio between the fibrosis due to silicosis and other causes of pulmonary fibrosis, particularly in patients who had a previous history of exposure to silica; and (3) the degree of variability of this Si/S ratio among the different lobes of the lung from the same patient.

Recently, we conducted a study to answer the first question by using morphometric measurements combined with energy-dispersive x-ray analysis and demonstrated that a positive correlation did exist between the amount of silicon in the tissue and the degree of fibrosis.\(^12\) The present study deals with the latter two questions. As shown in this study (Fig 1), a Si/S ratio of 0.3 discriminated tissue fibrosis due to silicosis from that of nonsilicotic processes in the majority of cases. There were only two silicotic subjects who showed a ratio below 0.2. In one patient the initial biopsy was a poor specimen, while a subsequent specimen obtained at autopsy showed silica nodules and a high Si/S ratio of 0.6, indicating a sampling problem. In the other patient the specimen showed very little or no fibrosis, although it did contain a small silica nodule, again suggesting a sampling problem. The majority of cases in group C showed the ratio to be lower than 0.2, although there were three cases with values between 0.2 and 0.3. This means that when a ratio of 0.3 is used as a cutoff level, the fibrosis occurring in a background of exposure to silica can be reliably separated from that associated with silicosis.

Table 1—Effect of Magnification on Element/Sulfur Ratio

<table>
<thead>
<tr>
<th>Case</th>
<th>Magnification</th>
<th>Al/S</th>
<th>Si/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>100×</td>
<td>0.06</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>1,000×</td>
<td>0.54</td>
<td>2.62</td>
</tr>
<tr>
<td>Case 2</td>
<td>100×</td>
<td>0.11</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>3,000×</td>
<td>0.72</td>
<td>1.65</td>
</tr>
<tr>
<td>Case 3</td>
<td>100×</td>
<td>0.15</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>9,000×</td>
<td>2.40</td>
<td>0.37</td>
</tr>
</tbody>
</table>

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When multiple samples from different lobes were compared (Fig 2), all specimens in group A clustered in a narrow range below 0.2, while groups C and D showed a wider scatter. In spite of this wider scatter, the majority of the samples from group C showed a ratio below 0.3, while most of the specimens in group D had values greater than 0.3. This indicates that when the analysis reveals a ratio below 0.3, the tissue fibrosis is unlikely to be due to silica in the tissue. The discrepancy between Figures 1 and 2 in terms of number of specimens falling in the range between 0.2 and 0.3 in groups C and D is due to the fact that the mean value of multiple samples was used in Figure 1 to represent individual cases where more than one sample was available for study.

This study confirms our previous observation that the pathologic diagnosis of silicosis can be made reliably by measuring the Si/S ratio in the fibrotic tissue even when a classic silica nodule is not present. As expected, no interstitial pulmonary disease per se affected the significance of this ratio. Specimens from the subjects who had a history of exposure to silica dust with no histologic evidence of silicosis often showed an increased amount of silicon in the tissue; however, the quantitative analysis by energy-dispersive x-ray analysis reliably separated the tissue fibrosis associated with silicosis from that of nonsilicosis. This suggests the importance of quantitative analysis of silicon in the lung rather than only qualitative analysis. It should be emphasized that the Si/S ratio of 0.3 used in this study is valid only at the magnitude of 100×. As shown in Table 1, when different magnifications were used, the element/sulfur ratio was different in the same area of analysis due to uneven distribution of the foreign elements. We used a low magnification of 100× to obtain a relatively large area for analysis. When higher magnifications were used, the Si/S ratio tends to be higher. Therefore, identification of silicon or any foreign elements at very high magnifications makes it very difficult, if not impossible, to evaluate the significance of that element in terms of tissue pathology.

We conclude, therefore, that the elemental analysis by energy-dispersive x-ray analysis is useful in the histologic diagnosis of silicosis in the absence of classic silica nodules. The Si/S ratio of 0.3 can be used reliably when magnification of 100× is used. The use of higher magnification should be discouraged because of the ubiquitous nature of silica in the lung. The occasional presence of small silica particles in the normal lung may lead to erroneously high Si/S ratios in some areas and make it difficult to interpret the significance in terms of tissue pathology.

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