An Autopsy Case of Diffuse Pleural Mesothelioma Associated with Pulmonary Asbestosis: Light and Electron Microscopic Study

Hidetaka MATSUZUKA, Hideo TSUCHIYAMA, Kioko KAWAI, Masamoto NAKANO, Hironobu ADACHI, Hayato SANETUJI, Kensuke BABA and Osamu KOIDE

1Department of Pathology and Surgical Pathology, School of Medicine, University of Occupational and Environmental Health, Japan. Kitakyushu 807, Japan
2Department of Pathology, School of Medicine, Nagasaki University. Nagasaki 852, Japan
3Department of Internal Medicine, Nagasaki City Hospital. Nagasaki 850, Japan
4Division of Pathology, School of Nursing and Medical Technology, University of Occupational and Environmental Health, Japan. Kitakyushu 807, Japan
5Unit of Anatomic Pathology, University of Occupational and Environmental Health, Japan. Kitakyushu 807, Japan

Abstract: Presented in this paper is an autopsy case of diffuse pleural mesothelioma with pulmonary asbestosis of a 65-year-old Japanese male who had been exposed to asbestos fiber for 27 years. The histological examination at autopsy showed a tubulo-papillary pattern with fibrosarcomatous components consisting mainly of spindle cells. An electron microscopic study of the spindle-shaped cells showed both epithelial differentiation in various degrees and fibroblastic cells. The findings support the concept of a mesothelial origin for diffuse mesothelioma and a bipotentiality of mesothelial cells. According to the autopsy series of the Japanese Pathological Society, the incidences of mesothelioma was 0.097% (124 cases) of which pleural mesothelioma were 0.066% (83 cases) of 126,535 autopsies in Japan from 1974 through 1978. None of them was associated with pulmonary asbestosis. The literature review reveals that a case of mesothelioma associated with asbestosis is rare in Japan and as far as we know only four cases were reported up until 1979.

Key words: diffuse pleural mesothelioma, light and electron microscopic features, asbestosis.

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An association of exposure to asbestos with subsequent development of malignant mesothelioma of the pleura has been found in a number of European epidemiologic studies. In Japan, mesothelioma associated with and without asbestosis is considered rare. Especially, the former, i.e. mesothelioma with asbestosis is so rare that it is worth reporting this additional case. We have studied an autopsy case of a typical diffuse pleural mesothelioma of a Japanese male who had distinct pulmonary asbestosis. The present paper describes light microscopic findings and the electron microscopic findings are discussed from a histogenetic point of view. In order to estimate the incidence of mesothelioma, we reviewed the Annuals of Pathological Autopsy Cases in Japan over a period of five years (1974–1978).
Case History

A 65-year-old Japanese male was admitted to Nagasaki City Hospital on July 26, 1978, complaining of dyspnea which had begun on his right side several weeks before the admission. The patient had been manufacturing boilers in a shipbuilding yard, handling asbestos fibers for 27 years since 1944, that is 34 years prior to the onset of the symptoms. He had been a nonsmoker.

Physical examination showed dullness on percussion and decreased breath sound on auscultation at the lower portion of the right lung. A chest roentgenogram revealed pleural effusion and pleural thickening of the right lung with a shift of the trachea to the left (Fig. 1). The abnormal laboratory findings were as follows: $a_1$-globulin, 19.2%; CRP, $2+$; blood sedimentation rate, 73 mm/hour. A pleuracentesis yielded straw-colored fluid with a total protein of 4.4 g/dl and LDH of 1,140 IU/l. Hyaluronic acid of the pleural fluid was not estimated. Cytological examinations were negative for malignant cells. A percutaneous needle biopsy was taken from the pleural growth and the specimens were diagnosed as a malignant tumor, mesothelioma or lung cancer by a light microscopy (LM), and diagnosed as mesothelioma by an electron microscopy (EM).

The patient initially received adriamycin intrapleurally and further combination chemotherapy (MFC) was done resulting in a symptomatic improvement. However, the patient began to have chest pains and a superior vena caval syndrome became manifest 2 months later. His condition deteriorated progressively despite chemotherapy and external radiotherapy and he suddenly died on June 2, 1979.

![Fig. 1.](image-url) (left) Chest roentgenogram 2 weeks after admission, showing density in right lower chest. (right) Chest roentgenogram 1 weeks before death, showing massive right pulmonary infiltration.
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Autopsy Findings

At autopsy the right and left lungs weighed 2,620 and 380 g, respectively. The right pleural cavity was obliterated by tumor. The right lung was completely covered by a tumor measuring up to 5.0 cm in thickness (Fig. 2). A boundary between the pulmonary parenchyma and the thickened pleura was sharply delineated. In the entire lung field, macroscopical examination failed to disclose any evidence suggesting a primary pulmonary origin of the tumor. There were invasions and metastases to both lungs, mediastinum, diaphragm, peritoneum, epicardium, both kidneys, right adrenal gland, mediastinal and perigastric lymph nodes. There was a loculated serous pleural effusion (500 ml) between the right lower lobe and diaphragm. Both lungs, which slightly increased in consistency and decreased in aeration, showed small anthracotic macules that measured several millimeters in diameter, and were distributed evenly throughout the organs. In the left lung, fibrous adhesion was not observed and 400 ml pleural effusion was present.

Light Microscopic (LM) Findings

The LM appearance of the tumor varied from one area to another, and was mainly composed of two types of histology, that is an epithelial pattern and a fibrosarcomatous pattern.

Fig. 2. (left) The external surface of the right lung covered by pleural tumor, appearing just like a suit of armor. (right) Half of bisected lung, demonstrating encasement of the lung by pleural tumor.
Fig. 3. Epithelial pattern of mesothelioma, showing tubulo-papillary fashion (H & E, ×100).

Fig. 4. Epithelial cells showing papillary infolding and solid nest in the cleft (Toluidine blue, ×100).

Fig. 5. Fibrosarcomatous pattern showing dense arrangement and interwoven bundles of spindle cells (H & E, ×100).

Fig. 6. Transforming features between epithelial and sarcomatous pattern (H & E, ×100).
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The epithelial pattern showed tubular, cystic and/or papillary formation (Fig. 3). In some places, cord-like patterns resembled a whirling loop. These epithelial-like cells possessed a tendency to line up around clefts. The cleft-lining cells were partially depolarized, transformed to a cord-like arrangement or solid nest and were infolded in the clefts in some areas (Fig. 4). The epithelial pattern was supported by collagenous stroma. The cytologic findings were characterized by uniform cuboidal cytoplasm and bland nuclei with distinct nucleoli. Alcian blue and colloidal iron stains demonstrated the positive materials in intracellular vacuoles, the inner surface of cleft-lining cells and extracellular collagenous stroma, which were digested by hyaluronidase. Both mucin stains and periodic acid-Schiff reaction were negative. These findings denoted that the reactive material was hyaluronic acid, which was thought to be produced by the tumor cells.

The fibrosarcomatous pattern was highly cellular, composed of interwoven bundles of spindle cells (Fig. 5). However, sarcoma-like cells varied in the cytological findings. There were plump cells with eosinophilic cytoplasm, vesicular nuclei and distinct nucleoli in some places, and extremely elongated fibrous cells with hyperchromatic spindle nuclei or stellate cells with hyperchromatic angular nuclei in other places. Mitotic figures were not frequently seen. Masson's trichrome stain showed only a few delicate collagen fibers between the spindle cells, and reticular fibers were also scanty. With alcian blue and colloidal iron stains, the stroma contained weak positive material.

Between the epithelial and sarcomatous patterns, various transforming features were observed (Fig. 6). Spindle cells focally formed alveoli and cord-like arrangements transforming into epithelial-like cords, or lined on small clefts especially in the collagenous stroma (Fig. 7). Sarcoma-like plump cells were gradually transformed to a solid nest of tumor cells (Fig. 8). Some slender cells had a vesicular round nuclei with distinct nucleoli which were the same as epithelial-like cells (Fig. 6). These histologic patterns and cytologic findings were considered to be a transformation between the epithelial and sarcomatous patterns.

Both lungs showed typical pulmonary asbestosis with marked interstitial fibrosis and destruction of alveolar septa with deposition of granular dusts enmeshed in dense fibrous tissues around the lymphatic vessels, bronchioles and small blood vessels. Air spaces were filled with fibrinous exudate containing alveolar macrophages (Fig. 9). In subpleural regions, septal thickening was more prominent, and fibrinous exudate was partially organized. Numerous asbestos bodies were dispersed in the alveoli and alveolar septa. They were golden-yellow in color with bulbous ends and a segmented or drumstick-like intervening part (Fig. 10).

Electron Microscopic (EM) Findings

The specimens for EM were obtained following a percutaneous needle biopsy and autopsy, and fixed in paraformaldehyde-glutaraldehyde according to Karnowsky (1965),
Fig. 7. Less cellular area with extensive hyalinization. Note the numerous narrow clefts (H & E, ×100).

Fig. 8. Plump cells having eosinophilic cytoplasm and a vesicular nuclei (H & E, ×100).

Fig. 9. Pulmonary asbestosis showing marked interstitial fibrosis and a large number of asbestos bodies (H & E, ×40).

Fig. 10. High-power photomicrograph to show in detail asbestos bodies (H & E, ×400):
rinsed in 0.1 M sodium cacodylate buffer (pH 7.2), post-fixed in 1.0% osmium tetroxide, dehydrated in a graded aceton (30–100%) and embedded in epon. The semithin sections stained with toluidine blue (Trump et al., 1961) were selected for the ultrathin sectioning. Ultrathin sections stained with uranyl acetate and lead citrate (Reynolds, 1963) were

Fig. 11. The tumor cells varies in size, shape and electron density of cytoplasms and nuclei (×2,070).

Fig. 12. Spindle-shaped cells with bush-like microvilli (×3,000).

Fig. 13. Intracytoplasmic lumen equipped with microvilli (×3,700).

Fig. 14. High magnification of spindle-shaped cells showing interdigitation of cytoplasmic membranes, desmosome and mitochondria with tendency to aggregate (×12,000).
examined under a JEM-7A and JEM-100 CX EM (JEOL, Tokyo).

The tumor cells varied in size, shape and electron density of cytoplasms and nuclei (Fig. 11). Spindle-shaped cells had elongated cytoplasmic processes, bush-like microvilli

Fig. 15. Tonofilaments showing many short fascicles with various directions (×23,200).

Fig. 16. Tonofilaments showing a perinuclear concentric circle of fascicles (×11,700).

Fig. 17. A basal lamina is formed beneath the cytoplasmic membrane of spindle-shaped cells (×7,700).

Fig. 18. Fibroblastic features of the tumor cells characterized by a prominent rough endoplasmic reticulum (×3,200).
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(Fig. 12) and interdigitations of cytoplasmic membranes (Fig. 14). Microvilli were sometimes presented in intracytoplasmic lumen (Fig. 13). These cells were frequently adjacent to each other by desmosomes. The basal lamina partially surrounded the spindle

Fig. 19. Ambiguous cells of which cytoplasm is low electron-dense and has numerous rough endoplasmic reticulum and a small quantity of microvilli (×2,400).

Fig. 20. Undifferentiated small cells show a small quantity of intracellular organelles (×2,000).

Fig. 21. Epithelial pattern showing intercellular gaps (×2,240).

Fig. 22. Narrow cleft lined with small spindle-shaped cells with basal lamina (×3,020).
cells (Fig. 17). Tonofilaments were found in most of the tumor cells in greater or lesser degree, and showed a perinuclear concentric circle in some cells (Fig. 16), or many short fascicles with a different direction in others (Fig. 15). Mitochondria varying in shape and size were moderate in number, and had a tendency of focal crowding and affinity for tonofilaments. Rough endoplasmic reticulum, polyribosomes and Golgi apparatus were prominent (Fig. 14). Large nuclei were elongated or oval, and had irregular indentation with prominent nucleoli (Fig. 15).

Extremely elongated fiber-like cells with dense cytoplasm were present among the spindle cells mentioned above or collagenous stroma, and were characterized by more conspicuously dilated rough endoplasmic reticulum, irregular nuclei and dense chromatin. Some of them had large mitochondria, microvilli and were associated with basal lamina (Fig. 17). However, the cytoplasms of others were mostly occupied by rough endoplasmic reticulum without basal lamina and microvilli. The latter were reminiscent of fibroblast (Fig. 18). Ambiguous cells or fibroblastic cells, the cytoplasm of which were low in electron-density and had numerous rough endoplasmic reticulum and polyribosomes in different quantities, were often observed. They were adjacent to the epithelial cells, and considered to be less-differentiated epithelial cells (Fig. 19). Undifferentiated small cells, which were scant of intracellular organelles, were also dispersed (Fig. 20).

Fig. 21 is an electron micrograph of cells, which is the epithelial pattern seen under a LM. Tumor cells were ovoid and formed tubular or solid nests showing intercellular gaps. Large mitochondria, tonofilaments, desmosomes, rough endoplasmic reticulum, microvilli and deeply indented nuclei with prominent nucleoli were observed as well as those seen in the spindle cells.

Narrow clefts were often observed (Fig. 22). Cleft-lining cells were small and spindle-shaped, and were poorly or less-differentiated. Basal lamina was formed beneath the cell membrane intermittently.

Regardless of variation in the shape of the tumor cells, they had ubiquitous characteristics in the ultrastructural findings, such as microvilli, desmosomes, tonofilaments and large mitochondria with epithelial-like cells.

Discussion

The histological diagnosis of diffuse mesothelioma is often extremely difficult because their histological appearances vary greatly from area to area and from case to case. Since the diffuse mesothelioma is usually biphasic, mesothelial and spindle cells, it is often mistaken for fibrosarcoma where spindle cells are predominant, or adenocarcinoma where the tumor cells appear to be arranged in a glandular fashion. No specific findings are present in diffuse mesothelioma under light microscope. However, some light microscopic features, which are helpful to distinguish mesothelioma from adenocarcinoma or other mesenchymal tumors, have been stated previously (Klima et al., 1976; Carter & Eggleston, 1980). Present in epithelial patterns are a loose coherence of cells forming glandular
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structures, free-lying cells or solid groups of cells in luminal spaces. Also, small cuboidal cytoplasm with vesicular ovoid nuclei and distinct nucleoli are found. In our case, a cord-like arrangement with winding and cleft-lining of cells was characteristic. The slit-like cavities lined with mesothelial cells have been previously emphasized (Kay & Silverberg, 1971). In the collagenous stroma, as shown in Fig. 7, careful examination discloses numerous narrow slits lined with spindle cells. In the sarcomatous areas of our case, spindle or plump cells had the same nuclei as epithelial-like cells. Also found as previously reported (Wanebo et al., 1976; Winslow & Taylor, 1960) were eosinophilic cytoplasm which seemed to represent a cytological and structural transformation from the epithelial pattern to the sarcomatous pattern.

EM studies of diffuse mesothelioma will be helpful to corroborate a diagnosis of a case suspected to be mesothelioma by clinical and LM findings (Suzuki et al., 1976; Wang, 1973; Kay & Silverberg, 1971; Klima et al., 1976; Benisch et al., 1981). Wang (1973) recapitulated the ultrastructural characteristics of diffuse mesothelioma. They included the presence of numerous microvilli, junctional structures, tonofilaments, glycogen granules, neolumina and basal lamina. All findings mentioned above are not specific individually. However, elongated and bushy microvilli are especially characteristic and are not detected in other adenocarcinoma of the lung and metastatic adenocarcinoma.

Our EM findings were almost in accord with those of previous findings regarding mesothelioma (Suzuki et al., 1976; Wang, 1973; Kay & Silverberg, 1971; Klima et al., 1976) and with those of mesothelial hyperplasia (Stoebner et al., 1970). The tumor cells, even when they were spindle-shaped, had the morphologic properties of epithelial-like cells with various differentiation. Fibroblastic, intermediate, ambiguous and epithelial-like cells were intermingled. The epithelial-like cells occupied the majority of them. In the sarcomatous pattern of the diffuse mesothelioma, some cells show the features without any specific differentiation (Klima et al., 1976) or a poor differentiation which might differentiate along the spectrum in accordance with as yet undetermined influences (Bolen & Thorning, 1980). Other cells have epithelial characteristics as mesothelial cells (Kay & Silverberg, 1971). Suzuki et al. (1976) showed the cells in transitional form between mesothelial cells and spindle cells, but these cells were not observed in our case. The cleft formation, illustrated in Fig. 22, lined by morphologically non-epithelial cells might account for the discrepancy between the cellular and structural differentiation in contradistinction to the epithelial pattern, illustrated in Fig. 21, which showed a glandular formation by epithelial-like cells. The histogenesis of the sarcomatous component in diffuse mesothelioma is controversial (Kay & Sliverberg, 1971; Klima & Gyorkey, 1977; Bolen & Thorning, 1980) as well as that of localized fibrous mesothelioma (Osamura, 1977; Hernandez & Fernandez, 1974; Scharifker et al., 1979). Klima et al. (1976) believed that the sarcomatous type of diffuse mesothelioma illustrates the best biphasic potential of the mesothelial cell, and they doubted whether this type of tumor represented the malignant counterpart of a benign solitary fibrous mesothelioma which had been generally considered to be a non-mesothelial origin (Hernandez & Fernandez, 1974;
Table 1. Mesotheliomas in the Annual of Pathological Autopsy Cases in Japan between 1974 and 1978

<table>
<thead>
<tr>
<th>Age</th>
<th>Pleura (M)</th>
<th>Pleura (F)</th>
<th>Peritoneum (M)</th>
<th>Peritoneum (F)</th>
<th>Pericardium (M)</th>
<th>Pericardium (F)</th>
<th>Others (M)</th>
<th>Others (F)</th>
<th>Total (%)</th>
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<tr>
<td>20–29</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>7 (5.6)</td>
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<tr>
<td>30–39</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 (4.0)</td>
</tr>
<tr>
<td>40–49</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21 (16.9)</td>
</tr>
<tr>
<td>50–59</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>3</td>
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<td>7</td>
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<tr>
<td>70–79</td>
<td>11</td>
<td>4</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>20 (16.1)</td>
</tr>
<tr>
<td>80–89</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Total (mean age)</td>
<td>83</td>
<td>(58.7)</td>
<td>27</td>
<td>(60.5)</td>
<td>12</td>
<td>(48.4)</td>
<td>2</td>
<td>(65.0)</td>
<td>124 (100)</td>
</tr>
</tbody>
</table>

Scharifker & Kaneko, 1979). In the present case, ultrastructures support the concept of a mesothelial origin for the diffuse mesothelioma and a bipotentiality of mesothelial cells in accordance to previous authors (Kay & Silverberg, 1971; Wang, 1973). The experimental studies (Davis, 1974; Raftery, 1973) suggested that the subendothelial mesenchymal cells were the precursor of mesothelial cells or subsurface fibroblasts. Bolen & Thorning (1980) emphasized a close histogenetic relationship between the surface mesothelial cells and subendothelial mesenchymal cells.

Mesothelioma is a fairly rare neoplasm. Of 126,535 autopsies recorded in the Annual of Pathological Autopsy Cases in Japan between 1974 and 1978 (The Japanese Pathological Society ed., 1975–1979), 124 cases (0.097%) of mesothelioma were found. They originated from pleura (83 cases, 0.066%), peritoneum (27 cases, 0.021%), pericardium (12 cases, 0.009%) and other locations (2 cases, 0.002%) (Table 1). Of 124 mesothelioma, 87 (70.2%) were males and 37 (29.8%) were females. The patients ranged from 20 to 80 years of age with a mean age of 58.2 years. In the pleural mesothelioma, the right is more common than the left (right 64.9%, left 35.1%), and there was a predominance of males (males 73.5%, females 26.5%). In the primary malignant tumor of the pleura, mesothelioma is the most common. Sixteen cases of malignant neoplasms other than mesothelioma of pleural origin are found among the consecutive autopsies done during the same period in Japan. They include fibrosarcoma (5 cases), liposarcoma (3 cases), unclassified sarcoma (2 cases), malignant fibrous histiocytoma (2 cases), leiomyosarcoma (1 case), rhabdomyosarcoma (1 case), malignant reticulosis (1 case) and squamous cell carcinoma (1 case). The diagnosis of fibrosarcoma of pleura are controversial regarding its histogenesis and classification of mesothelioma. The following two possibilities are considered as follows: the malignant counterpart of solitary benign fibrous mesothelioma showing the same histologic features as fibrosarcoma arising in other sites (Klima & Gyorkey, 1977; Wanebo et al., 1976), and the sarcomatous type of diffuse mesothelioma (Klima & Gyorkey, 1977; Suzuki et al., 1976). If the pure fibrosarcomatous form shows
epithelial features ultrastructurally, it should not be categorized under fibrosarcoma but under a malignant diffuse mesothelioma.

Wagner (1965) reported a marked increase in the incidence of primary pleural mesothelioma in the asbestos miners in Cape Province, South Africa. Since then numerous epidemiologic and experimental studies have supported the relationship of mesothelioma with exposure to asbestos (Elmes et al., 1965; Newhouse & Thompson, 1965; Wagner et al., 1974; McEwen et al., 1970). Newhouse & Thompson (1965) found 76 mesothelioma in London between 1917 and 1964. In these cases, 51 cases (67.1%) were correlated with an exposure to asbestos. However, in some reports (McDonald et al., 1973; Oels et al., 1971; Klima et al., 1976; Selikoff et al., 1965), it has been suggested that exposure may play a smaller part in the results. Oels et al. (1971) found 37 cases of diffuse mesothelioma of which 27 cases were not associated with asbestos exposure. None of the 124 cases of mesothelioma in Japan and 13 cases reported by Klima et al. (1976) were associated with asbestosis. Only four mesothelioma associated with asbestosis were reported in Japan until 1979 in literatures, as far as we know (Koizumi et al., 1973; Kang et al., 1974; Ogawa et al., 1978; Hisa et al., 1979). Other etiologic factors other than asbestos exposure have not been determined yet.

On the other hand, asbestos causes carcinoma of the lung as well. Merewether (1974) reported 31 (13.2%) cases of lung cancer among 235 patients with asbestosis. In Japan, 19 (23.2%) cases of lung cancer were reported among 82 patients of asbestosis (Yokoyama & Sera, 1978). In the Annuals of the Pathological Autopsy Cases in Japan between 1974 and 1978, 7 cases of asbestosis were found, of which 3 cases were associated with lung cancer and 1 case was associated with laryngeal carcinoma. The mean age of the patients with carcinoma was 59.5 years. As mentioned above, the epidemiological studies showed the difference between the ratio of mesothelioma and lung cancer associated with asbestosis. Mesothelioma has a longer latent period between exposure and manifestation than lung cancer in asbestosis (Oels et al., 1971; Mikami et al., 1981), and lung cancer with asbestosis is seen in a younger age group than those without asbestosis (Matsuda, 1978). Our patient's symptoms appeared after 34 years after the beginning of asbestos exposure.

Asbestos mines are few in Japan, however the importing of asbestos has increased rapidly in the postwar period and the range of its use has been expanding. Recently, asbestos pollution is not only occupational but also environmental (Matsuda, 1975; Aisner & Wiernik, 1978). Accordingly, the incidence of malignant neoplasms caused by asbestos is expected to increase in Japan (Mikami et al., 1981; Watanabe, 1976). The prevention of asbestosis and malignant neoplasms and histopathological studies of mesothelioma of individual cases are important for the future.

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References


Diffuse Pleural Mesothelioma


肺石綿症を合併した亜急性胸膜中皮腫の1剖検例

松尾 秀峻1・土山 秀夫2・河合 紀生子2・中野 正心3・安達 博信4・実藤 幹人5
馬場 謙介1・小出 紀1

1産業医科大学第二病理学教室  2長崎大学医学部病理解剖教室  3長崎市民病院内科
4産業医科大学医療技術短期大学病理  5産業医科大学病院病理

要旨： 運転所でボイラー製作に従事し、27年間、石綿粉塵を吸入していた65才男性に見られた肺石綿症合併の亜急性胸膜中皮腫剖検例を報告した。腫瘍は、右胸膜に原発し、両側腎臓、右副腎、傍胃リンパ節などに転移し、末期には、上大静脈症候群をきたして、他症13ヶ月後に、突然死した。組織学的には二相性で線維肉腫様の紡錘型細胞が混じり、またこれらと種々の程度に移行する腺管状、乳頭状増殖を示していた。電顕的には紡錘型細胞にも、上皮的性質を示すものが見られた。日本剖検報告によると過去5年間（1974－1978年）の全剖検例126,535例中、124例（0.97%）が中皮腫で死亡しており、部位別には、胸膜83例、腹膜27例、心臓12例、腸間膜1例、小腸膜1例であった。これらは、いずれも石綿症を伴っていなかった。一方、同期間中の肺石綿症剖検例は7例で、そのうち3例に肺癌、1例に喉頭癌を合併していた。文献的には本邦における肺石綿症合併の中皮腫は4例に過ぎなかった。