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A Case of Malignant Pleural Mesothelioma With Osseous and Cartilaginous Differentiation

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Abstract: A 69-year-old man with a history of exposure to asbestos was admitted because of a chest radiographic abnormality. Subsequent findings from computed tomography and a thoracoscopic biopsy suggested malignant mesothelioma. Punctate calcification was observed in the pleural tumor on computed tomography scanning. The patient underwent pleuropneumonectomy, and the tumor was pathologically diagnosed as malignant mesothelioma, sarcomatoid type with osseous and cartilaginous differentiation. Malignant mesothelioma with osseous and cartilaginous differentiation is a rare condition. Punctate calcification in the pleural mass as a lesion distinct from the pleural plaque may indicate osseous or osteosarcomatous differentiation in malignant mesothelioma.

Key Words: malignant pleural mesothelioma, osseous differentiation, cartilaginous differentiation, computed tomography, calcification

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Malignant pleural mesothelioma is a rare primary tumor of the pleura. It is macroscopically classified as localized or diffuse type, and histologically divided into epithelioid, sarcomatoid, desmoplastic, and biphasic types according to the World Health Organization Classification of Tumours, 2004.[1]

Osseous and/or cartilaginous differentiation is an extremely rare presentation in malignant mesothelioma. Osteosarcomatous lesions that appear as dense, punctate calcified foci on computed tomography (CT) scans are rarer still, and only a few cases have been reported.[2–5] Here, we report a case of malignant pleural mesothelioma with osseous and cartilaginous differentiation, in which dense, punctate calcifications were observed on CT scanning.

CASE REPORT

A 69-year-old man who had no significant past medical history was admitted to the department of thoracic surgery. Five months before admission, the patient was asymptomatic but had an abnormal chest radiograph. Results from a subsequent CT scan and thoracoscopic biopsy suggested the diagnosis of malignant mesothelioma. The patient was a building contractor and had been exposed to asbestos for 48 years. There were no significant findings on physical examination. Findings from laboratory tests and tumor markers, including carcinoembryonic antigen, cytokeratin fragment, cancer antigen 19-9, and pro-gastrin-releasing peptide, were within normal range; however, levels of neuron-specific enolase and squamous cell carcinoma antigen were slightly elevated.

Chest x-ray revealed an approximately 10-cm mass with clear margins in the right middle hemithorax and a smaller caudal mass (Fig. 1). In addition, right-sided pleural thickening was observed. CT scanning revealed masses contiguous with the right pleura, and dense, calcified foci were detected in the main tumor (Fig. 2). The calcifications were punctate and uniform (largest diameter, 5 mm) and were diffusely scattered throughout the tumor. Linear calcification also appeared in the pleural plaque. In the lung window setting, the right lung parenchyma was compressed by the pleural tumors, but no tumors were observed within the right lung parenchyma or the left hemithorax. There was no evidence of pulmonary fibrosis.

Right pleuropneumonectomy was performed with chest wall resection. Macroscopic examination revealed multiple nodules and tumors, which arose from the parietal pleura. The largest tumor, which was yellowish white and 9 cm in diameter with clear margins, compressed the right lung adjacent to the tumor (Fig. 3A). Calcifications could be palpated in the tumor and pleura.

Histologic examination revealed a solid growth pattern with oval-to-elongated spindle cells (Fig. 3B). Osteosarcomatous components were scattered in the tumor nests (Fig. 3C), and focal chondrosarcomatous components were observed. Although the tumor invaded the lung parenchyma, most of the tumor grew in the parietal and visceral pleurae. Immunohistochemical examination revealed atypical spindle cells that expressed positive mesothelioma

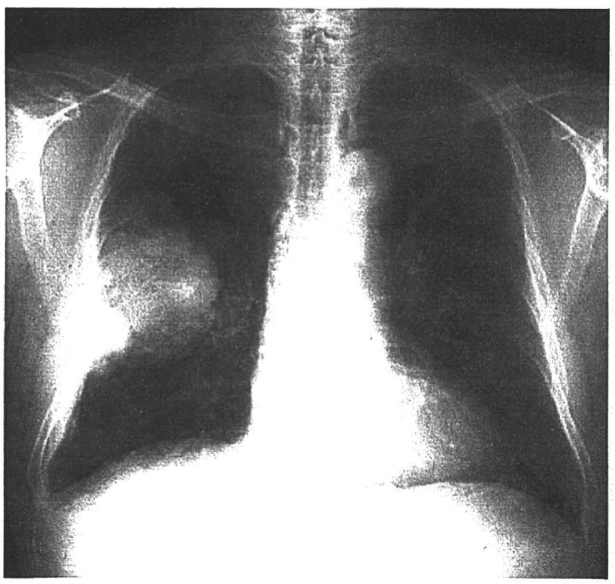

FIGURE 1. Chest radiograph showing well-defined tumor masses in the right hemithorax.

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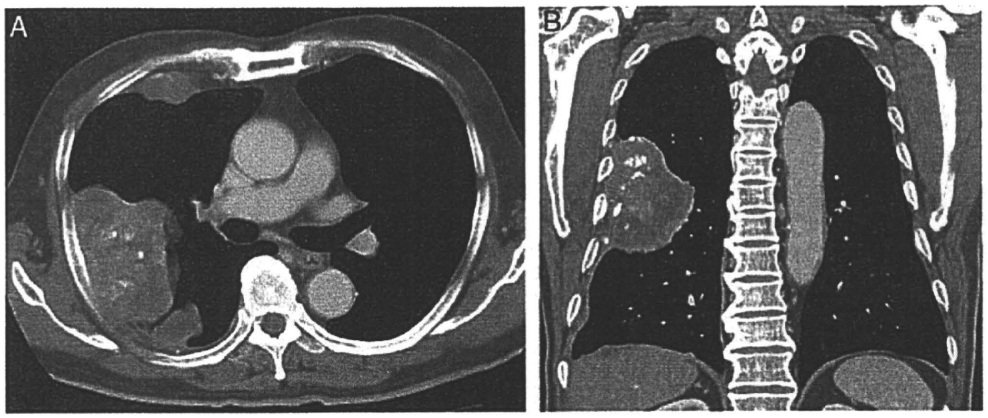

FIGURE 2. A, Axial contrast-enhanced CT scan of the thorax showing masses in the right pleura. Punctate calcifications were detected in the main tumor. B, Coronal reformatted image clearly shows that the tumor arises from the pleura.

markers (calretinin, podoplanin, and Wilms tumor-1), but did not express negative mesothelioma markers (carcinoembryonic antigen, thyroid transcription factor-1, and Ber-Ep4). Asbestos bodies were detected in the lung parenchyma. On the basis of these findings, we diagnosed malignant pleural sarcomatoid mesothelioma with osseous and cartilaginous differentiation.

The patient developed both local recurrence and metastasis and died 19 months after surgery.

DISCUSSION

Malignant pleural mesothelioma is a rarely encountered, high-grade malignant primary tumor. Cases among men have declined in the United States[6]; however, the incidence is increasing in Japan.[7] Development of osseous or cartilaginous differentiation in malignant mesothelioma is very rare, and Goldstein first reported 2 cases in 1979.[8] He suggested that the pluripotentiality of coelomic mesothelium may be the cause of its differentiation toward bone and cartilage, and also proposed the following alternative hypotheses: (1) the cartilage and bone, developed separately from the neoplasm, could be caused by previous tuberculous pleurisy; (2) the mesothelioma might have produced a substance that promoted cartilage and bone formation, directly or by stimulating the parathyroid glands; (3) the cartilage and bone might be integral components of the neoplasm and in parts the spindle cells might be merging or transforming into the cartilage; (4) 2 separate neoplasms may have been present, a mesothelioma with classical tubular formation and a fibrochondrosarcoma; and (5) asbestotic pleural plaques often undergo calcification.

Bolen et al[9] demonstrated the process by which subserous connective tissue cells obtained epithelial characteristics. They suggested that the pathogenesis is caused by the multipotency of mesothelial cells, using the term multipotential subserosal cells, which supports the hypothesis of pluripotent coelomic mesothelium proposed by Goldstein.[8] Yousem and Hochholzer[10] also favored this hypothesis. Our case supports this hypothesis, as there was no evidence of tuberculosis infection or other primary

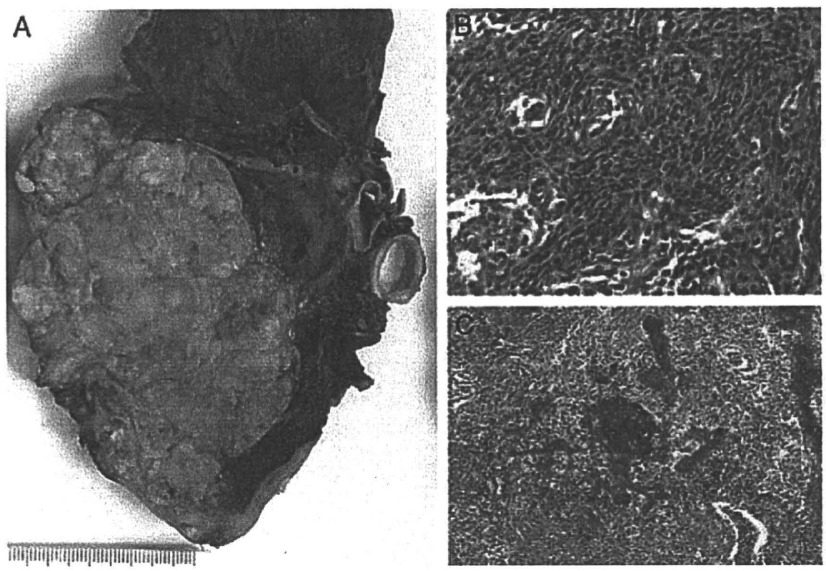

FIGURE 3. A, The cut surface of the largest tumor. The tumor has a diameter of 9 cm, is composed of yellowish white nodules with focal ossification, and is compressing the right lower lobe of the lung. B, Sarcomatoid mesothelioma shows oval-to-elongated spindle cells. C, Irregular-shaped osteoid components with calcium deposition are observed in the nests.

tumors and the osseous lesion was not colocalized with asbestos plaque. However, the possibility of parathyroid hormone influence cannot be excluded.

Of the 2 cases reported by Goldstein,[8] one case showed osteosarcomatous differentiation, and the other showed bone and cartilage differentiation. Sonja et al[11] summarized 27 cases of malignant mesothelioma with heterologous elements. In their report, they suggested that the term "heterologous" should be reserved for tumors that show malignant heterologous elements, such as osteosarcomatous, chondrosarcomatous, or rhabdomyoblastic elements. Pathologically, the differential diagnosis of these cases includes a primary or secondary pleural sarcoma. They concluded that mesothelioma cannot be excluded if cytokeratin staining is negative and should be diagnosed by anatomic distribution. The prognosis after diagnosis of mesothelioma with heterologous elements is similar to that associated with pleural mesothelioma of the sarcomatoid type; survival is approximately 6 months. Our case included heterologous elements such as osteosarcomatous and chondrosarcomatous differentiation.

Several reports have described imaging findings of pleural mesothelioma, but only 3 reports mentioned tumor calcifications detected by CT scanning.[2–5] Arnold et al[2] reported 2 cases of diffuse malignant mesothelioma that presented with large and dense calcified pleural masses, which were visualized on CT scan. In this report, it was described that the diagnosis of osteocartilaginous differentiation in diffuse malignant mesothelioma was based on the past history of asbestosis exposure, the typical radiographic appearance of encasing pleural tumor, the histopathologic features of malignant mesothelioma, and the absence of any osteogenic sarcoma or chondrosarcoma elsewhere. In this case, large calcification inside the main tumor was not seen, but punctate calcification was evident on CT scanning. Calcification of benign pleural plaque and osseous differentiation in mesothelioma could be distinguished by their shape and location. Calcification of benign pleural plaque is linear and is located on thickened pleural plaque, whereas osseous differentiation in mesothelioma is punctate or large and is located inside the tumor. The radiologic differential diagnoses of malignant pleural tumor with calcification include lung cancer with pleural dissemination, sarcoma derived from pleura, and metastatic lung or pleural tumor, such as colorectal cancer, osteosarcoma, and chondrosarcoma.

In conclusion, we report a case of malignant mesothelioma with osseous and cartilaginous differentiation. The punctate calcifications in the pleural tumor, distinct from the pleural plaque, may indicate osseous or osteosarcomatous differentiation in malignant mesothelioma.

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Soluble mesothelin-related protein in pleural effusion from patients with malignant pleural mesothelioma

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Abstract. Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm primarily arising from surface serosal cells of the pleura and is strongly associated with asbestos exposure. Patients with MPM often develop pleural fluid as initial presentation. However, cytological diagnosis using pleural fluid is usually difficult and has limited utility. A useful molecular marker for differential diagnosis particularly with lung cancer (LC) is urgently needed. The aim of the present study was to investigate the diagnostic value of soluble mesothelin-related protein (SMRP) in pleural fluid. Pleural fluids were collected from 23 patients with MPM, 38 with LC, 26 with benign asbestos pleurisy (BAP), 5 with tuberculosis pleurisy (TP) and 4 with chronic heart failure (CHF), and the SMRP concentration was determined. All data were analyzed by using non-parametric two-sided statistical tests. The median concentration of SMRP in MPM, LC, BAP, TP and CHF were 11.5 (range 0.90-82.80), 5.20 (0.05-36.40), 6.65 (1.45-11.25), 3.20 (1.65-6.50) and 2.03 (1.35-2.80) nmol/l, respectively. The SMRP concentration was significantly higher in MPM than in the other diseases (P=0.001). The area under the ROC curve (AUC) values of the MPM diagnosis was 0.75 for the differential diagnosis from the other groups. Based on the cut-off value of 8 nmol/l, the sensitivity and specificity for diagnosis of MPM were 70.0 and 68.4%, respectively. These results indicate that the SMRP concentration in pleural fluid is a useful marker for the diagnosis of MPM.

Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with a poor survival rate that arises from the surface cells of the pleura. It is a rare tumor; however, MPM has become a very serious public health concern in Japan. A newspaper article, published in June 2005, reported that five residents who had lived near a now-closed asbestos cement pipe plant in Amagasaki, Japan, developed pleural mesothelioma (1). The industrial use of asbestos has been banned in Japan since 2006, but the incidence of MPM is expected to continue increasing for the next few decades due to the past usage of asbestos (2).

MPM has therapeutic and diagnostic challenges. Surgical resection, often combined with radiotherapy or adjuvant chemotherapy, is indicated for the treatment of MPM in the earlier stage. There is a small population of patients who achieve prolonged disease-free survival. Yet the majority of cases are already progressive at the time of diagnosis, and these patients exhibit an extremely poor prognosis (3). Systemic chemotherapy or radiotherapy to date has not had an impact on patient survival for advanced cases. Thus, it is quite important to diagnosis MPM at an early stage. Most MPM cases demonstrate pleural effusion at the time of diagnosis, but cytological diagnosis with pleural effusion is usually difficult and has limited utility. To obtain a definite diagnosis, a thoracoscopic or percutaneous biopsy should be performed to obtain adequate specimens for pathological and immunohistochemical analyses. Yet, even with these procedures, it is sometimes difficult to differentiate MPM from other pleural diseases including benign asbestos pleurisy (BAP), tuberculosis pleurisy (TP), or pleural metastasis of lung cancer (LC). Several investigators have sought to improve the differential diagnosis of pleural effusion by measuring tumor markers. Shi *et al* reported the usefulness of measuring the pleural carcinoembryonic antigen for the diagnosis of malignant pleural effusion (4). Similar findings were reported regarding cytokeratin 19 fragment 21-1 and carbohydrate antigen (CA) 125, CA15-3 and CA19-9 (5). Aoe *et al* previously reported that the concentration of receptor-binding cancer antigen expressed on Siso cells (RCAS1) was higher in malignant pleural effusion than in non-malignant effusion (6), but the usefulness of these markers has not yet been fully established in clinical practice. A useful molecular marker for the differential diagnosis of these diseases is therefore urgently needed.

Mesothelin is a 40-kDa cell surface glycosylated phosphatidylinositol (GPI)-anchored glycoprotein which has putative functions in cell-to-cell adhesion (7). Mesothelin

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Key words: mesothelin, mesothelioma, asbestos

Table I. Patient characteristics.

	MPM	PMLC	BAP	TP	CHF
No.	23	38	26	5	4
Age (years)					
Median (range)	64 (47-89)	70 (48-90)	75.5 (58-88)	82 (68-88)	74 (68-82)
Gender					
Male/Female	21/2	28/10	26/0	5/0	3/1
Asbestos exposure period (years)					
Median (range)	33 (5-51)	-	30 (3-46)	-	
Histology					
Epithelioid	15	-	-	-	
Biphasic	2	-	-	-	
Sarcomatoid	4	-	-	-	
Unknown	2	-	-	-	
Adenocarcinoma		24			
Squamous cell carcinoma		3			
Small-cell carcinoma		4			
Not determined		7			
Stage					
I	3	-	-	-	
II	2	-	-	-	
III	9	-	-	-	
IV	6	-	-	-	
Unknown	3	-	-	-	

MPM, malignant pleural mesothelioma; PMLC, pleural metastasis of lung cancer, BAP, benign asbestos pleurisy; TP, tuberculosis pleurisy; CHF, chronic heart failure.

is expressed on normal mesothelial cells (8); however, it is highly overexpressed in cancers such as MPM (9,10), pulmonary carcinomas (11-14) and other neoplasms (15,16). Soluble mesothelin-related protein (SMRP) is recognized as a cleaved fragment of membrane-bound mesothelin (17). Robinson and colleagues reported that serum SMRP levels were elevated in MPM when compared with healthy asbestos-exposed and non-exposed subjects, and with other pulmonary diseases including LC (18). Similar results were reported by Cristaudo *et al* (19) and Schneider *et al* (20) who demonstrated that SMRP blood concentrations were significantly higher in MPM than in LC cases. These findings suggest the usefulness of serum SMRP as a diagnostic or screening marker of MPM.

The SMRP value in pleural fluid was evaluated by Scherpereel *et al* (21) and Pass *et al* (22). Both research groups reported that the pleural SMRP value was higher than that in serum, and the level was higher in MPM than in other pulmonary diseases. Therefore, the aim of the present study was to investigate the SMRP level in pleural fluid in Japanese patients with MPM. For this purpose, SMRP concentrations in pleural fluid from Japanese patients with MPM were examined and compared with those of patients with BAP, TP or LC. Correlations between SMRP and asbestos exposure were also examined.

Materials and methods

Materials. Pleural fluid was collected from patients with MPM. For these cases, pathological diagnosis of MPM was confirmed based on standard H&E staining and positive immunohistochemical reactivity to mesothelial markers such as calretinin, Wilms' tumor 1, or thrombomodulin, and negative reactivity to carcinoembryonic antigen. The clinical stage of MPM was determined according to the International Mesothelioma Interest Group (IMIG) criteria (23) and was based on staging procedures including computed tomographic (CT) scans of the chest and abdomen, magnetic resonance images of the brain and Technetium-99m hydroxymethylene diphosphonate bone scans. Survival data of the patients with MPM were determined from the day of diagnosis to the day of death or last follow-up. Pleural fluid was also collected from patients with LC, BAP, TP and with chronic heart failure (CHF) as controls. LC was diagnosed in cases where lung cancer cells were detected in the pleural effuion. Histological subtypes of LC were based on the World Health Organization (WHO) classification (24). The clinical stage of the disease was assessed using the International Staging System (25). TP was diagnosed in cases in which *Mycobacterium tuberculosis* was detected in the pleural fluid. TP was also diagnosed in cases with higher concentrations of adenosine deaminase

(>50 IU/l) and when lymphocyte dominancy was shown in the fluid. CHF was diagnosed in cases which demonstrated transudate fluid with known cardiac diseases. The diagnosis of BAP was determined by exclusion of other specific causes in patients with past asbestos exposure, in which malignant diseases were ruled out with thoracoscopy. Informed consent was provided by all patients, and the study was conducted with approval of the appropriate institutional review boards.

SMRP measurement. SMRP was measured using the chemiluminescent enzyme immunoassay (CLEIA) (Fujirebio Diagnostics, Malvern, PA, USA) based on the 2-step sandwich method. In brief, 20 μl of sample was mixed with 180 μl of sample diluents, then 20 μl of the diluted sample was incubated with 250 μl of anti-SMRP antibody-coated ferrite particles at 37°C for 10 min. After washing, 250 μl of anti-SMRP antibodies coupled with alkaline phosphate was added and incubated at 37°C for 10 min. After a washing step, 200 μl of substrate [3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1,2-dioxetane disodium salt; AMPPD] solution was added, followed by incubation at 37°C for 5 min. Luminescence at a wavelength of 477 nm was measured, and the SMRP concentration of each sample was calculated with the standard curve method.

Asbestos body burden. Quantification of asbestos bodies was performed using the protocol modified by Kohyama and Suzuki (26). In brief, portions of paraffin-embedded normal lung tissue (1-2 g) obtained from surgery or autopsy were deparaffinized with xylene, then microcut. These were digested with solution containing 5-20% sodium hypochlorite and KOH for 6 h at 60°C. Following digestion, samples were pelleted and resuspended in distilled water. Samples were then mixed well and filtered through a cellulose ester membranous filter which was dehydrated and cut in half. Pieces of the filter were mounted on microscope slides and dried with acetone vapor. Asbestos bodies were then counted, and the asbestos bodies per (wet weight) gram of lung were calculated.

Statistical analyses. Comparisons between groups were performed using the Kruskal-Wallis test and non-parametric analysis using the Mann-Whitney U test. Areas under receiver operating curves (ROC) were calculated using standard techniques. Survival data were determined from the day of diagnosis to the day of death or last follow-up and analyzed based on the Kaplan-Meyer method. Correlations between pleural SMRP values and asbestos body or patient survival were calculated based on Pearson's correlation coefficient (PCI). Statistical calculations were performed with SPSS Statistical Package version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics. Between January 2004 and July 2007, pleural fluids were collected from 23 patients with MPM, 38 with LC, 26 with BAP, 5 with TP and 4 with CHF at the Okayama Rosai Hospital. Of the 23 cases (median age 64 years; range 47-89; male/female 21/2) diagnosed with MPM, there were 15 epithelioid, 2 biphasic, 4 sarcomatoid and 2 unknown pathological subtypes. According to the IMIG staging system, there were 3 cases in stage I, 2 in stage II, 9 in stage III, 6 in stage IV and 3 unknown. Of the 38 cases (median age 69.5 years; range 46-91; male/female 29/9) diagnosed with LC, there were 24 patients with adenocarcinoma, 4 with small-cell carcinoma, 3 with squamous cell carcinoma and 7 undetermined pathological subtypes. The characteristics of the patients are summarized in Table I.

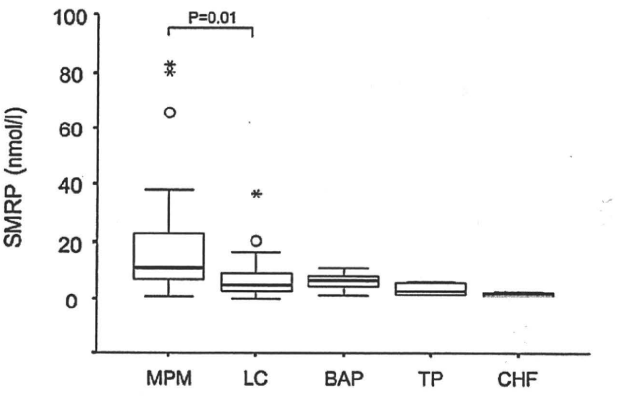

Figure 1. SMRP concentrations in pleural fluid. MPM, malignant pleural mesothelioma; LC, lung cancer; BAP, benign asbestos pleurisy; TP, tuberculosis pleurisy; CHF, chronic heart failure.

SMRP value in MPM. According to the clinical stage and pathological subtypes of MPM, a trend was noted in which the SMRP value was higher in advanced stages (III and IV, n=16; median 13.8, range 2.85-82.8 nmol/l) compared with the value in early stages (I and II, n=5; median 7.9, range 2.5-33.9 nmol/l), and higher in epithelioid type (n=13; median 15.4, range 2.2-82.8 nmol/l) than in sarcomatoid (n=4; median 13.8, range 2.85-10.45 nmol/l), though there were no significant differences (P=0.158 and 0.389, respectively).

SMRP and asbestos exposure. Occupational asbestos exposure was revealed in 21 patients with MPM. We examined the duration of asbestos exposure and the SMRP value in the pleural fluid, but no correlation was shown (PCI, -0.069). Quantification of asbestos bodies was performed in 17 cases of MPM. The median number of bodies was 2,180 (239-526,000) per gram of dried lung. We examined the correlation between the SMRP value in pleural fluid and the number of asbestos bodies, but no correlation was found (PCI, -0.156). Survival data was available in 22 cases. No correlation was found between the SMRP value and survival (PCI, -0.179). We compared the survival of two groups, those with a lower concentration of SMRP (≤8.0 nmol/l) and those with a higher concentration, but no statistical difference was demonstrated (data not shown).

SMRP value for differential diagnosis. The median concentration of SMRP in MPM, LC, BAP, TP and CHF were 11.5 (range 0.9-82.8), 5.2 (0.05-36.4), 6.65 (1.45-11.25), 3.20 (1.65-6.5) and 2.03 (1.35-2.8) nmol/l, respectively. The SMRP concentration was significantly higher in MPM than in the other diseases (P=0.001, Kruskal-Wallis test, Fig. 1). The area under the ROC curve (AUC) values of the MPM diagnosis

was 0.75 [95% confidence interval (CI), 0.615-0.884] for the differential diagnosis from the other groups. Based on the cut-off value of 8 nmol/l, the sensitivity and specificity for diagnosis of MPM were 70.0 and 68.4%, respectively. The SMRP concentration in MPM was significantly higher than that in LC (P=0.004, Mann-Whitney U test). The AUC for the differential diagnosis of MPM and LC was 0.724 (95% CI, 0.583-0.866). Based on the cut-off value of 8 nmol/l, the sensitivity and specificity for diagnosis of MPM were 69.6 and 68.4%, respectively. The SMRP concentration in MPM was significantly higher than in BAP (P=0.004, Mann-Whitney U test). The AUC value for the differential diagnosis of MPM and BAP was 0.74 (95% CI, 0.586-0.894). Based on the cut-off value of 8 nmol/l, the sensitivity and specificity for diagnosis of MPM were 69.6 and 69.2%, respectively.

Discussion

In this study, we first examined the SMRP value in pleural fluid from patients with MPM. SMRP was higher in the epithelioid subtype than in the sarcomatoid, and higher in advanced stages (III and IV) than in early stages (I and II), though the differences were not statistically significant. These findings collaborate a previous study by Scherpereel *et al* (21). They examined the SMRP values, both in serum and pleural fluid, and reported that SMRP both in serum and pleural fluid was higher in the epithelioid subtype and in advanced diseases of MPM. The differences in our study were not statistically significant, probably due to the small number of samples, but our results reflect a similar trend in MPM in Japan. In addition, we examined the correlation between pleural SMRP and overall survival of patients with MPM, but no correlation was found. The role of serum SMRP as a prognostic marker was examined by Cristaudo *et al*. In their study, a high SMRP level in serum was an independent negative prognostic factor in patients with MPM (19). The present study is the first report to examine the role of pleural SMRP as a prognostic factor, but these results should be interpreted carefully because of the small number of cases. Further studies are warranted to clarify the role of pleural SMRP as a prognosis predictive marker.

We next examined the usefulness of pleural SMRP as a diagnostic marker of MPM. We compared the SMRP value in the pleural fluid of MPM to that of LC, BAP, TP and CHF. The SMRP value in MPM was significantly higher than in the other diseases. Similar findings were also reported by Scherpereel *et al* (21). They reported that the serum or pleural fluid SMRP level was significantly higher in patients with MPM than in subjects with benign pleural lesions related to asbestos exposure (BPLAE) or in LC. In their report, BPLAE was defined based on the definition by the American Thoracic Society (27), which corresponds with BPE in our study. In our study, subjects with TP and CHF were also included as controls. TP is the single most frequent cause of death by an infectious agent and is also a major cause of pleural effusion (28). Several molecular markers in pleural effusion have been examined as diagnostic markers of TP (29), but the differential diagnosis is still often problematic in clinical practice. Our results revealed, for the first time, the usefulness of pleural SMRP to distinguish MPM and TP.

We also analyzed the correlations between the SMRP concentration and asbestos exposure. We determined the number of asbestos bodies in the lungs of patients with MPM. The duration of occupational asbestos exposure was determined through patient interview. As a result, no correlation was revealed between SMRP values and the duration of asbestos exposure or asbestos bodies in the lung. These findings indicate that elevation of SMRP in the pleural effusion of MPM is not influenced by asbestos, but is one of the cancer-specific events. The mechanisms of accumulation of SMRP in pleural fluid have not as yet been established. SMRP is reported as a proteolytically cleaved fragment of membrane-bound mesothelin (17). The release of SMRP could also be due to a frameshift mutation of the protein (21). Further studies are warranted to examine the mechanisms involved in the elevation of SMRP in MPM.

In conclusion, we examined the SMRP concentration in pleural fluid from patients with MPM, LC, BAP, TP and CHF and demonstrated that the SMRP value in MPM was significantly higher than that in the other diseases. These results indicate the usefulness of pleural SMRP as a diagnostic marker of MPM.

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Clinical study of asbestos-related lung cancer in Japan with special reference to occupational history

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A total of 152 patients with asbestos-related lung cancer recognized by the criteria of Japanese compensation law for asbestos-related diseases were examined and compared with 431 patients with non-asbestos-related lung cancer. Male comprised 96% of patients. Ages ranged from 50 to 91 years with a median of 72 years. Eighty-nine percent were smokers or ex-smokers. Almost all patients had occupational histories of asbestos exposure. The median duration of asbestos exposure was 31 years and the median latency period was 47 years. Thirty-four percent of patients exhibited asbestosis and 81% exhibited pleural plaques by radiography. Regarding asbestos particles in the lung for 73 operated or autopsied patients, 62% had more than 5,000 particles per gram. On the other hand, 100% of non-asbestos-related lung cancer patients had <5000 particles per gram with a median of 554 particles. The number of asbestos bodies in the lung, male gender, absence of symptoms, smoking index, and early stage of cancer were significantly much more than those of non-asbestos-related lung cancer. In this study, a diagnosis of asbestos-related lung cancer was made in 34% of patients by asbestosis, in 62% by presence of both pleural plaques and more than 10 years' occupational asbestos exposure, and in 4% by more than 5000 asbestos particles per gram of lung tissue. Occupational histories, duration of asbestos exposure, and pleural plaques are common categories for the recognition of asbestos-related lung cancer in Japan. (*Cancer Sci* 2010; 101: 1194–1198)

The disaster of asbestos exposure has been a serious social problem in Japan since 2005,[1] with neighborhood exposure to asbestos inducing mesothelioma in more than 100 patients in the Amagasaki area. Furthermore, the number of patients with mesothelioma and asbestos-related lung cancer (Fig. 1) has recently increased. In this study, clinical features and occupational histories for asbestos-related lung cancer patients in Japan were investigated and compared with those of non-asbestos-related lung cancer patients.

Materials and Methods

In this study, the definition of asbestos-related lung cancer was primary lung cancer with the following: (i) asbestosis on chest radiography; (ii) pleural plaques with more than 10 years' occupational asbestos exposure; (iii) asbestos particles or fibers on the lung tissues with more than 10 years' occupational asbestos exposure; and (iv) more than 5000 asbestos particles per gram of dry lung tissue with occupational asbestos exposure. These criteria fulfill the Japanese compensation law of asbestos-related lung cancer.

Retrospective study of asbestos-related lung cancer patients from 2000 to 2008 treated in 18 Rosai hospitals throughout Japan was performed. Gender, age, diagnostic motive, smoking history, histological type of lung cancer, clinical stage, therapeutic procedures and prognosis, occupational history, and radiological findings of asbestos-related changes were examined.

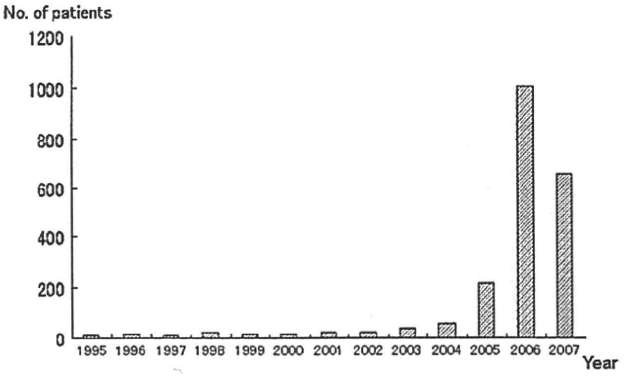

Fig. 1. Number of asbestos-related lung cancer in Japan from 1995 to 2007 was shown in this figure. The number of patients with asbestos-related lung cancer has drastically increased after the 2005 Kubota Shock. (Data from statistics published by the Ministry of Health, Labor, and Welfare of Japan.)

Non-asbestos-related lung cancer patients treated in Okayama Rosai hospital from 1997 to 2007[2] were also examined for gender, age, smoking history, histological type of lung cancer, clinical stage, and therapeutic procedures and prognosis. Non-asbestos-related lung cancer does not fulfill the criteria for the Japanese compensation law of asbestos-related lung cancer. The findings of asbestos-related changes such as pleural plaques were examined by chest X-ray and chest computed tomography (CT) (including high resolution computed tomography (HRCT)) for all patients with asbestos-related lung cancer and non-asbestos-related lung cancer. Prognosis of asbestos-related lung cancer was calculated by the complication of asbestosis. Prognostic factors in both asbestos-related and non-asbestos-related lung cancers were calculated by multivariate analysis.

The number of asbestos particles was counted for the operated or autopsied patients (73 patients with asbestos-related and 23 with non-asbestos-related lung cancers). The number of asbestos particles in the lung was counted by the method of Kohyama.[3] One to 2*g* of lung tissue without cancer invasion was dissolved in sodium hypochlolite and 5% potassium hydroxide (KOH) for 12 h, and complete dissolution of the lung tissue was confirmed. The supernatant was discarded, the sediment was dissolved with 10 mL of chloroform and 50% ethanol, and the solution was centrifuged at 18G for 5 min. The supernatant was discarded, the sediment was dissolved with 95% ethanol, the solution was passed through a Millipore filter, and the asbestos particles on the filter were counted under phase-contrasted microscope at

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×200 magnification. The number of asbestos particles per gram of dry lung tissue was then calculated. The data were statistically analyzed, using Student's *t*-test and $P < 0.05$ was regarded as statistically significant.

Results

A total of 152 patients with asbestos-related lung cancer were examined in this study. Regarding gender, 146 were male and six were female. Ages ranged from 50 to 91 years with a median of 72 years. Sixty-four patients were diagnosed by the chief complaints of dyspnea, cough, etc. Fifty-nine patients were diagnosed by regular health check-up and another 20 patients were accidentally diagnosed during the following of other diseases. For nine patients there was no information. Only 15 patients (10%) were non-smokers and another 134 were smokers or ex-smokers. The smoking index for 149 patients ranged from 0 to 2550 with a median of 900. The smoking index for 71 patients exceeded 1000. The smoking history of three patients was unknown (Table 1).

Table 1. Characteristics of asbestos-related and non-asbestos-related lung cancers

	Asbestos-related lung cancers (n = 152)	Non-asbestos-related lung cancers (n = 431)	P-values
Gender	(%)	(%)	<0.01
Male	146 (96.1)	311 (72.2)	
Female	6 (3.9)	120 (27.8)	
Age (years)			
<50	0	25 (5.8)	0.09
50–59	16 (10.5)	70 (16.2)	
60–69	41 (27.0)	110 (25.5)	
70–79	62 (40.8)	153 (35.5)	
≥80	33 (21.7)	73 (16.9)	
Symptom			
Absent	79 (55.2)	172 (39.9)	<0.01
Present	64 (44.8)	259 (60.1)	
Smoking habit			
Never smoker	15 (9.9)	88 (20.4)	<0.01
Smoker	134 (90.1)	343 (79.6)	
BI ≥ 1000	71 (47.0)	133 (30.9)	
Pathology			
Adenocarcinoma	85 (55.9)	238 (55.2)	
Squamous cell carcinoma	39 (25.7)	86 (20.0)	
Small cell carcinoma	18 (11.8)	72 (16.7)	0.07
Others	10 (6.6)	35 (8.1)	
Stage			
IA	33 (22.4)	68 (15.8)	<0.01
IB	17 (11.6)	30 (7.0)	
IIA	3 (2.0)	3 (0.7)	
IIB	5 (3.4)	20 (4.6)	
IIIA	9 (6.1)	33 (7.7)	
IIIB	35 (23.8)	123 (28.5)	
IV	45 (30.6)	154 (35.7)	
Therapeutic procedure			
Operation	53 (34.9)	100 (23.2)	0.06
Chemotherapy	56 (36.8)	187 (43.4)	
Chemo. + radiotherapy	12 (7.9)	67 (15.5)	
Others	8 (5.3)	25 (5.8)	
Best supportive care	23 (15.1)	52 (12.1)	

BI, Brinkmann index.

Regarding the histology of 143 patients with asbestos-related lung cancer, 85 exhibited adenocarcinoma, 39 had squamous-cell type, 18 had small-cell type, and one had large-cell type. The histological types of nine patients were not determined. The features of non-asbestos-related lung cancer are described in Table 1. Rates of male gender, being symptom free, smoking, and early stage disease for asbestos-related lung cancer are significantly ($P > 0.01$) higher than those of non-asbestos-related lung cancer.

The survival term was overall 17.4 months with 57.0% having 1 year-survival and 25% having 5 year-survival. On the other hand, the survival term for 431 patients with non-asbestos-related lung cancer was 19.2 months with 70.1% having 1 year-survival and 24.5% of having year-survival. The difference in survival term between the two groups was not statistically significant (Fig. 2). Three patients with asbestos-related lung cancer died within 3 months of surgery and another four patients died from respiratory failure due to advanced asbestosis. Two of three patients with asbestosis were did not have good survival after surgery because of acute exacerbation of asbestosis. However, the survival of 51 patients with asbestosis was 17.2 months and that of 101 patients without asbestosis was 18.1 months; there was no statistical significance in either group. Prognostic factors calculated by multivariate analysis in both groups, included age, gender, and stages, but not asbestos exposure or pathology (Table 2).

Regarding therapy, 53 patients underwent surgery; 56 received chemotherapy, with nine receiving a combination of surgery and chemotherapy; 16 received radiation, with 12 receiving a combination of chemotherapy and radiation; and 23 received the best available supportive care. These numbers resemble those for non-asbestos-related lung cancer. Survival for patients who received surgery was 55.1 months with 45% having 5-year survival; and for radiation, chemotherapy, and best supportive care, survival was 9.3 months, 10.3 months, and 7.0 months, respectively.

One hundred and fifty (98%) of 152 patients whose occupational histories were ascertained had occupational exposure to asbestos. For another two patients with more than 5000 asbestos particles in the lung, occupational histories were not confirmed. Thirty-four patients had occupational histories of shipyard work, 29 had construction work, 15 had exposure due to making asbestos products, 15 had piping works, and 14 had insulation work, with the remainder also having been employed in asbestos-related work (Table 3).

Age at exposure to asbestos for the first time ranged from 14 to 50 years with a median of 21 years. The duration of asbestos exposure for 146 patients ranged from 1 to 60 years with a median of 31 years, and the latency period from first exposure to the appearance of lung cancer ranged from 5 to 71 years with a median of 47 years.

Regarding the radiographical findings of asbestos-related changes, only 51 patients (34%) exhibited asbestosis; 122 (81%) exhibited pleural plaques and 100 (66%) showed calcified plaques. Seven patients exhibited rounded atelectasis and four diffuse pleural thickening. Only 33 patients (22%) had complicated pleural effusion (Table 4). Thirty-two patients with asbestosis were exposed to asbestos due to work in asbestos product making, insulation, and asbestos spraying. And other 19 patients were exposed to asbestos due to work in shipyards and construction work and work with piping. Ninety-four patients (62%) with asbestos-related lung cancer were diagnosed by the presence of pleural plaques and had more than 10 years' occupational asbestos exposure (Fig 4). On the other hand, 10 patients with non-asbestos-related lung cancer showed pleural plaques, but no other findings such as asbestosis or diffuse pleural thickening.

As for the number of asbestos particles in the lung in 73 patients, 45 (62%) had more than 5000 asbestos bodies per gram

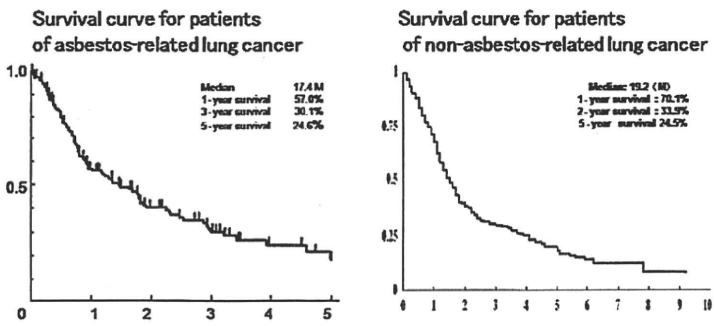

Fig. 2. Survival curves for asbestos-related lung cancer and non-asbestos-related lung cancer show almost the same pattern which indicates almost the same rates of survival between these two types of lung cancer.

of dry lung tissue which meant they had an occupational history of asbestos exposure. Furthermore, 21 (29%) exceeded 50 000 particles which meant heavy exposure. Seven (4%) were diagnosed with more than 5000 asbestos particles per gram of lung tissue (Fig. 4). However, 14 (19%) had <1000 asbestos particles which indicated the non-exposed citizen level. These 14 patients had pleural plaques with more than 10 years' occupational asbestos exposure and were diagnosed with asbestos-related lung cancer.

Among 18 asbestosis patients, 10 (56%) exceeded 50 000 particles, but two patients had <5000 particles. On the other hand, for 55 patients without asbestosis, 14 had <1000 particles and 11 (20%) exceeded 50 000 particles (Fig. 3). Twenty-three patients with non-asbestos-related lung cancer had 0 to 3751 asbestos particles per gram of lung tissue with a median of 554 particles.

Discussion

Asbestos is known to be carcinogenic for malignant mesothelioma and lung cancer. It has been established that exposure to asbestos can induce malignant mesothelioma. Regarding the onset of primary lung cancer, the involvement of smoking has been emphasized.[4] Asbestos enhances the mutagenicity of tobacco carcinogen and it acts independently to tissue damage responsible for fibrosis, that is asbestosis.[5] High incidences of lung cancer among individuals exposed to asbestos have been demonstrated by various cohort studies.[3] The present study was undertaken to characterize primary lung cancer observed in asbestos-exposed individuals in Japan and to examine these patients for the presence or absence of concomitant lung lesions such as asbestosis and pleural plaques. While no definition of asbestos-related lung cancer has been established, Helsinki criteria[6] indicates that having 25 asbestos fiber-years doubles the risk of lung cancer.

The present study adopted the criteria of asbestos-related lung cancer defined by the Japanese compensation law of asbestos-related diseases in 2006. A total of 152 patients with asbestos-related lung cancer were examined and the median age was 72 years which was 7 years older than that of a group with Japanese malignant pleural mesothelioma described in 2004.[7] Ninety-six percent of them were males who had occupational histories of asbestos exposure, and the median duration of asbestos exposure was 31 years. Forty-two percent were diagnosed by subjective complaints, but another 68% were diagnosed during regular check-ups or accidentally diagnosed due to abnormal chest shadows without subjective complaints. This data seems to be due to the Japanese system of having regular check-ups for lung cancer, because 60% of patients with non-asbestos-related lung cancer were diagnosed by subjective complaints.

Regarding smoking, only 15 patients (10%) were non-smokers and 134 were smokers or ex-smokers. Seventy-one patients (47%) exceeded the smoking index of 1000 which meant they

Table 2. Univariate and multivariate analysis for the prognosis of asbestos and non-asbestos-related lung cancer

Univariate analysis				
Factors		*n*	MST (95% CI)	Log-rank test
Asbestos	Related	152	16.2 months (8.3–24.1)	$P = 0.673$
	Non-related	431	17.2 months (15.1–19.3)	
Age (years)	≤70	278	20.8 months (15.9–25.7)	$P < 0.001$
	71+	305	14.1 months (11.7–16.5)	
Gender	Male	457	15.4 months (13.7–17.1)	$P < 0.001$
	Female	126	24.5 months (17.8–31.2)	
Pathology	NSCLC	493	18.1 months (15.4–20.8)	$P = 0.001$
	SCLC	90	13.4 months (11.1–15.7)	
Stage	I–II	179	21.5 months (17.7–25.3)	$P < 0.001$
	III–IV	399	13.4 months (11.1–15.7)	

Multivariate analysis			
Factors	Exp (β)	95% CI	*P*-values
Asbestos	1.051	0.816–1.353	0.699
Age	1.625	1.312–2.013	<0.001
Gender	1.686	1.255–2.273	0.001
Pathology	1.290	0.973–1.710	0.077
Stage	1.945	1.548–2.443	<0.001

CI, confidence interval; MST, median survival term; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

Table 3. Occupational histories of asbestos-related lung cancer patients

Area of occupation	*n*
Shipbuilding	34
Construction	29
Asbestos product making	15
Piping	15
Insulation	14
Electrician	8
Chemicals	6
Arc welding	5
Transportation	4
Steel company	4
Asbestos spraying	3
Fire bricklaying	3
Automobile making	3
Metal making	2
Furnace making	2
Warehousing	1
Casting	1
Other	1

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Table 4. Radiological findings in asbestos-related diseases

Findings	*n* (%)
Asbestosis	51 (34.0)
Pleural plaques	122 (81.3)
Calcified PQ	81 (73.6)
Rounded atelectasis	7 (4.7)
Diffuse pleural thickening	4 (2.7)
Pleural effusion	33 (22.3)

PQ, pleural plaque.

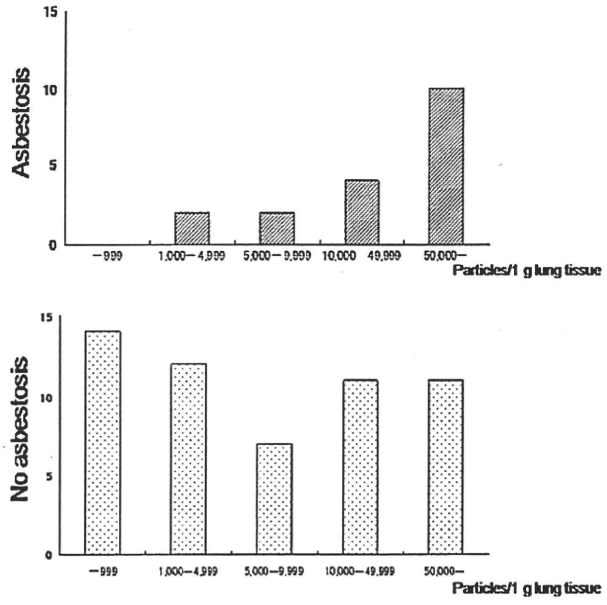

Fig. 3. Number of asbestos particles per gram of lung tissue for asbestosis and non-asbestosis by chest X-ray. For asbestosis, two patients (11.1%) had <5000 asbestos particles; 14 (77.8%) had more than 10 000 particles; while 10 had more than 50 000 particles (55.6%) which means heavy asbestos exposure. On the other hand, for non-asbestosis, 31 patients had more than 5000 particles and 14 had <1000 particles. For the patients with non-asbestosis, no particular pattern in number of asbestos particles could be observed.

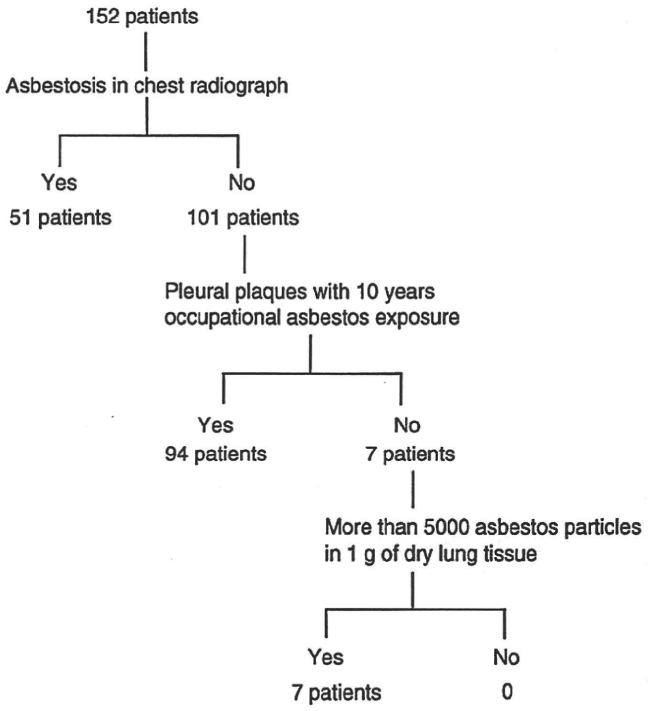

Fig. 4. Flow chart of asbestos-related lung cancer diagnoses.

were heavy smokers. Lung cancer can occur in nonsmokers exposed to asbestos; however, the risk is magnified several-fold by smoking,[8] and increased risk for lung cancer remains up to 20 years after cessation of smoking.[9] Forty-seven percent of our patients were heavy smokers whose lung cancer was suggested to be related not only to asbestos exposure but also to smoking. Ex-smokers stopped smoking within 20 years of the appearance of lung cancer.

Histological classification of asbestos-related lung cancer indicated that 59% had adenocarcinoma and 27% had squamous cell type, which is a similar pattern to that of non-asbestos-related lung cancer (control group) in Japan.

Overall survival of 152 patients with asbestos-related lung cancer was 17.4 months with 25% having 5 year-survival. This data is similar that of the control group which had an overall survival of 19.2 months with 25% having 5-year survival. As for therapeutic procedures, the survival for patients who underwent surgery was 55.1 months with a 5-year survival rate of 45% and that of chemotherapy was 10.3 months. Lung cancer which shows limited small areas of ground glass opacity by CT scanning is typically early stage; therefore, the survival of this type is better after surgery. These data showed that therapeutic procedures for asbestos-related lung cancer and survival were also similar to controls. Some patients with early stage experienced the exacerbation of asbestosis after surgery. But the presence of asbestosis did not affect the survival of those with asbestos-related lung cancer. Prognostic factors of survival for asbestos-related and non-asbestos-related lung cancers by multivariate analysis proved not to be asbestos exposure or pathology, but rather age, gender, and stage.

As for occupational history, shipyard workers, asbestos product makers, piping workers, and insulation workers mainly comprised asbestos-related lung cancer patients, which was similar to that for malignant mesothelioma as described previously[3]. Thirty-four patients who were shipyard workers with more than 10-year occupational histories were considered as proof of moderate density of exposure to asbestos. Insulation workers, asbestos product makers, and piping workers were considered to have had heavy exposure to asbestos. The term of exposure to asbestos ranged from 1 to 60 years with a median of 31 years. Workers who were suggested to have had heavy exposure to asbestos through insulation or asbestos spraying tended to have had short-term exposure, and construction workers thought to have had lower levels of exposure tended to have had longer histories of asbestos exposure. The latency period had a median of 47 years which is longer than that of malignant mesothelioma (37 years)[6] and that of asbestos-related-lung cancer patients (43 years) who were treated in several Rosai Hospitals from 1995 to 2000.[10]

Regarding the radiological findings for asbestos-related changes, 34% of patients had complicated asbestosis, but 81% had pleural plaques which occurred even due to low density of asbestos exposure, which was about same percent as Bianchi's data.[11] Sixty-two percent of patients were diagnosed as having asbestos-related lung cancer on the evidence of both pleural plaques and 10 years' occupational asbestos exposure. Ten patients with non-asbestos-related lung cancer also exhibited pleural plaques, but no evidence of enough asbestos particles or

occupational asbestos exposure. Therefore, these patients were not diagnosed as having asbestos-related lung cancer.

Regarding asbestos particles in the lung tissues of asbestos-related lung cancer patients, 61% exceeded 5000 particles per gram of dry lung tissue, but 19% had <1000 particles which corresponded to the citizen's level of exposure. These patients with pleural plaques on chest X-ray were exposed to low-density asbestos for more than 10 years. More than 5000 asbestos particles per gram of lung tissue corresponds with 25 fiber-years, and is consistent with a doubling of the lung cancer risk.[6,12] Bianchi *et al.* [11] reported that 31% of 414 necropsy cases of lung cancer exceeded 5000 particles per gram of dry lung cancer. Our data is double this data which suggests denser exposure to asbestos.

However, 11 patients without asbestosis had more than 50 000 particles and two who were construction workers with asbestosis had <5000 particles. Construction workers who ordinarily had treated chrysotile asbestos tended to have less asbestos particles in the lung than insulation or piping workers. We should examine asbestos fibers for these two patients by electron microscopy. On the other hand, the carcinogenicity and fibrogenicity of asbestos has been described as not always being correlated. Fischer suggested that 42% of asbestosis on chest radiograph had fewer asbestos particles than 25 fiber-year occupational histories.[13] Our result of asbestos particles in the lung and radiographic asbestosis corresponds with his data. Lung cancer risk was elevated in the presence of radiographic asbestosis, but occurred as a result of asbestos exposure in the absence of asbestosis. The incidence of non-small-cell lung cancer for patients with asbestosis increased, compared with asbestos-exposed patients without asbestosis.[14] Lung cancer risk increased almost linearly with cumulative dose of asbestos.[15] It is still controversial that lung cancer in the absence of asbestosis can be attributed to asbestos exposure. We should extend the number of the patients for asbestos-related lung cancer and clarify the criteria for the diagnosis of asbestos-related lung cancer without asbestosis.

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Disclosure Statement

The authors have no conflict of interest.

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Clinical Study on Mesothelioma in Japan: Relevance to Occupational Asbestos Exposure

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Background *In 2003, the number of deaths due to malignant mesothelioma in Japan was 878; however, only 85 cases of mesothelioma due to asbestos exposure were authorized for compensation. The reasons for this discrepancy require evaluation.*

Method *We examined medical records, X-rays, and pathology results to evaluate mesothelioma cases in Japan between 2003 and 2005; used a questionnaire to identify occupational and environmental histories, and determined the concentration of asbestos fibers in pathology specimens.*

Results *We identified 442 definite cases of malignant mesothelioma with a median age of 68 years. There were 316 malignant mesothelioma cases with occupational asbestos exposure, 12 cases with neighborhood exposure and 5 cases with likely domestic exposure. Most (78%) of the 87 cases exceeded 1,000 asbestos particles per gram of dry lung tissue.*

Conclusion *We conclude that 79.2% of cases of mesothelioma in Japan in recent years were caused by asbestos exposure.* Am. J. Ind. Med. 53:1081–1087, 2010.

***KEY WORDS:** mesothelioma; pleural plaques; exposure; asbestos particles; Helsinki criteria*

BACKGROUND

Until 1994, the International Classification of Diagnosis (ICD)-9 classified death due to mesothelioma and other causes of death together, and, therefore, statistics on only mesothelioma could not be obtained. After 1995, when ICD-10 was implemented and deaths due to mesothelioma were reclassified, statistics regarding incidents of death due to mesothelioma could be obtained in Japan, permitting a better understanding of this type of tumor. In 1995, the number of deaths was 500, increasing to 878 cases in 2003 and 1,050 in 2006. In Europe and America, 80% of the cases of mesothelioma are attributed to asbestos exposure; however, in Japan, only 85 cases of mesothelioma due to asbestos exposure were authorized to receive worker's compensation insurance during the 2003 fiscal year. We sought to clarify the cause of this disparity between the number of deaths and the number of compensation-authorized cases of malignant mesothelioma. There are reports [Kishimoto, 1992; Kishimoto et al., 2004] on mesothelioma and asbestos exposure from specific regions in Japan; however, there has not yet been any large-scale investigation targeting the whole nation. Accordingly, from 2003 to 2005 we conducted a 3-year nationwide study targeting 2,742 incidences of death due to mesothelioma. In addition to the relationship between asbestos exposure and mesothelioma, we investigated the diagnosis of mesothelioma in Japan.

METHODS

We reviewed all the cases in which the cause of death was diagnosed as mesothelioma based on "ICD CD46" in the demographic statistics from 2003 to 2005 and obtained

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detailed information on the clinical diagnosis, and occupational asbestos exposure for those cases.

Families that provided a letter of consent were given a questionnaire to obtain the occupational and residential histories. We also re-examined the diagnosis of mesothelioma itself based on review of medical records, radiology films, and pathology reports. We obtained cellular and pathological tissue samples and tumor tissues from the medical institutions that issued the death certificates. One radiologist and two pulmonologists re-examined the data, looking for the presence or absence of asbestos exposure based on chest images or based on the classification of pleural mesothelioma by the International Mesothelioma Interest Group (IMIG). Two pathologists reviewed the tissue and cell samples and tried to provide a definitive diagnosis.

We determined the presence or absence of asbestos exposure based on entries in the clinical records and also the family questionnaire investigation results (asbestos question sheet regarding occupational history). We investigated if the attending physician made entries regarding the occupational history in the clinical records for the incidents of death in 2004 and 2005. We define the lifetime as the time at which diagnosis was determined until the time of death.

For the cases in which excised lungs or autopsied lungs were provided by the medical institutions, we measured the number of asbestos particles in the tumor-free portion of the pulmonary tissue using the method by Kohyama [2008] at the Okayama Rosai Hospital. More specifically, the lung tissue was dehydrated at 100°C, and after accurately determining the dry weight, the tissue was dissected into small pieces and dissolved in sodium hypochlorite solution. After centrifugation at 10,000 rpm for 10 min, the supernatant was removed and the pellet was re-suspended in a new solution to total 50 ml in volume. The asbestos particles were collected on a 0.45-μm Millipore filter membrane using vacuum suction filtration and fixed with acetone on the filter membrane. The asbestos particles were counted under a phase contrast microscope and expressed as the number per gram of dry weight of lung tissue.

We used the student's test to determine the difference in the average value, and the χ^2 test to compare between two groups. Furthermore, we used the Kaplan–Meier method to compute the lifetime using the date of diagnosis as the starting point, and used the Logrank test to compare lifetimes.

RESULTS

Among the targeted 2,742 cases (878 cases in 2003, 953 in 2004, and 911 in 2005), we obtained familial consent from 956 cases (454 cases in 2003, 260 in 2004, and 242 in 2005). In the investigation of deaths in 2003, which was conducted immediately following the so-called Kubota Shock, during which the neighborhood exposure to asbestos induced more than 100 cases of mesothelioma in 2005 and public attention was focused on workplace asbestos exposure, familial consent was obtained in 51.7% of deaths. However, in 2004 and 2005, the percent decreased to 27.3% and 26.6%, respectively. From among the 956 cases in which consent was received, we obtained clinical records, medical treatment information, etc., from the medical institutions that issued the death certificates for 541 cases (56.6%), including 235 cases in 2003, 145 in 2004, and 161 in 2006 as indicated in Table I. From the information for the 541 cases provided by the medical institutions, there were 442 cases (81.7%) in which definitive diagnosis was obtained based on tissue samples. There were 49 cases (9.1%) in which only speculative clinical diagnosis was made based on data such as imaging and the concentration of hyaluronic acid in the pleural fluid, or definitive diagnosis could not be made pathologically or histologically, which were labeled as "suspected" as in Table I.

Regarding the site of mesothelioma, there were 418 cases of pleural mesothelioma (372 confirmed diagnoses and 46 suspected cases); 68 cases of peritoneal mesothelioma (65 confirmed diagnoses and three suspected cases); 3 confirmed diagnoses of pericardial mesothelioma; and 2 confirmed diagnoses of mesothelioma of the tunica vaginalis. However, 50 cases (9.2%) were determined to be diseases other than mesothelioma. In 20 of the 50 cases, lung cancer was diagnosed based on the tissue and cell samples from the autopsies carried out at the medical institutions. Furthermore, we made a comprehensive judgment considering the results from the imaging viewpoint, tissue pathology viewpoint, tumor markers, etc., and found 18 cases that were more likely lung cancer than mesothelioma and were labeled as "suspected lung cancer." Among the other 12 cases, there were 6 cases of ovarian cancer, 1 case of malignant lymphoma, 1 case of renal cancer, and other cases that were thought to be from malignant tumors such as 1 case of a solitary fibrous tumor, and 3 cases of benign asbestos pleurisy (fibrous pleurisy) that were diagnosed as mesothelioma.

TABLE I. Number of Japanese That Died of Malignant Mesothelioma From 2003 to 2005

	2003	2004	2005	Total
Population vital statistics	878	953	911	2,742
Consent from bereaved family	454	260	242	956
Information provided by hospitals	235	145	161	541
Mesothelioma	182	125	135	442
Suspected mesothelioma	26	8	15	49
Other diseases	27	12	11	50

TABLE II. Background of Patients With Mesothelioma and s/o Mesothelioma

	Confirmed mesothelioma		Suspected cases	
	Pleura	Peritoneum	Pleura	Peritoneum
No. of cases	372	65	46	3
Median age (range)	68 (38–94)	63 (16–89)	80 (54–97)	78 (59–86)
Gender				
Male	320	46	32	2
Female	52	19	14	1

Age and Gender

When comparing the background factors for the cases of mesothelioma and suspected mesothelioma, the median age for confirmed diagnosis of pleural mesothelioma was 68, and the median age for suspected mesothelioma was 80. Those cases with suspected mesothelioma were at a significantly advanced age as shown in Table II. Also, for peritoneal mesothelioma, the median age for confirmed diagnosis was 63, and 78 for suspected cases. Furthermore, there were 320 male and 52 female (6.2:1 males/females) cases of confirmed pleural mesothelioma and 32 male and 14 female (2.3:1 males/females) cases of suspected mesothelioma. On the other hand, there were 46 male and 19 female (2.2:1 males/females) confirmed cases of peritoneal mesothelioma, two male and one female case of suspected mesothelioma with there was no discernable difference in the gender groups.

Diagnostic Method

Among the 361 of 442 cases (81.7%) where the basis of diagnosis was clear, definitive diagnosis was made based on tissue analysis as indicated in Table III. The method for gathering tissue samples for the diagnosis of pleural mesothelioma cases was video-assisted thoracoscopic biopsy. This method was used for 116 cases. Cases were diagnosed based on not only video assisted thoracoscopic surgery (VATS) under general anesthesia but also with thoracoscopic surgery under local anesthesia. Subsequently, there were 106 cases of needle biopsy based diagnosis, 71 cases of thoracotomy-based diagnosis, and 11 cases where the autopsy was the first pathological diagnosis obtained.

Most cases (n = 37) of peritoneal mesothelioma were diagnosed based on laparotomy; nine cases diagnosed following laparoscopic biopsy, and four cases diagnosed based on needle biopsy. Furthermore, there were 45 cases of pleural mesothelioma and 11 cases peritoneal mesothelioma diagnosed only based on pleural fluid and ascites cell analysis. In the diagnoses based on histological analysis, there were 329 of 353 cases (93.2%) in which the presence or absence of immunostaining confirmed the diagnosis, whereas among the 56 cases of cytological examination based diagnosis less than half of the cases, 23 cases, were confirmed diagnoses (41.1%).

Tissue Type

Among the 442 cases of definitively diagnosed mesothelioma, only 305 cases (69.0%) had the cell type identified in the clinical records. There were 163 epitheliod cases (53.4%), 70 biphasic cases (23.0%), and 62 sarcomatoid cases (20.3%) [Inai, 2005].

History of Asbestos Exposure in the Workplace

There were 421 (95.2%) cases in which the presence or absence of the occupational history could be investigated based on the clinical records and the family questionnaires. Among those cases, 316 cases (75.1%) were suspected to

TABLE III. Diagnostic Procedures for Mesothelioma

	Pleura	Peritoneum	Total[a]	Immunohistochemical[b] staining
Cases	372	65	442	352/409 (86.1%)
Histological diagnosis	304	52	361	329/353 (93.2%)
Open lung and peritoneum	71	37	113	102/106 (96.2%)
Video assisted thoracoscopical biopsy	116	9	125	112/125 (89.6%)
Needle biopsy	106	4	110	105/110 (95.5%)
Autopsy	11	2	13	10/12 (83.3%)
Cytological examination	45	11	56	23/56 (41.1%)
Unknown	23	2	25	

[a]Includes a total of five cases of peritoneal and tunica vaginal mesothelioma.
[b]Denominator represents cases in which immunostaining method was employed.

have had exposure to asbestos including indirect or direct exposure as stipulated in their occupational histories. Furthermore, based on the questionnaire responses from the families, there were eight cases in which patients resided in the vicinity of the old Kubota Kanzaki factory in Amagasaki city in Japan. There were four additional cases of patients who resided in the neighborhood of an asbestos product manufacturing plant or a shipyard, totaling 12 cases of suspected neighborhood asbestos exposure. There were also five cases of occupational history in which family members were exposed to asbestos, which implied likely domestic asbestos exposure. As a result, we conclude that there were 333 cases (79.1%) of suspected asbestos exposure.

From the 188 cases of suspected occupational asbestos exposure, we identified the occupation histories of 165 cases (87.8%) based on the family questionnaires, and we concluded that the occupation histories of no more than 51 cases (27.1%) were recorded into the clinical records by the attending physician. In other words, despite the diagnosis of mesothelioma, we found that those clinicians did not obtain detailed occupational histories in many cases.

The occupational histories of the 316 cases of suspected occupational asbestos exposure are shown in Table IV. For cases in which there was the possibility of asbestos exposure in pursuing multiple occupations, the investigation selected the occupation in which the patient worked the longest. There were 69 construction workers, which makes up the largest group, 45 shipyard workers, 30 electricians, 28 steel and other manufacturing workers, 22 auto manufacturers or maintenance workers, 21 plumbers, 20 asbestos product manufacturers, and 16 wrecking crew workers and concrete product workers. There were 9 cases (27.2%) of asbestos product manufacturing workers, who were exposed to high concentrations of asbestos, among the 33 cases of peritoneal mesothelioma indicating a feature that denotes high frequency of occurrence in this occupation.

Exposure Period and Incubation Period

We investigated the exposure period, date, age, and latency period of the 316 cases of suspected occupational asbestos exposure. We examined the exposure period and incubation time for only the cases that had clinical record entries or responses by the families. The median asbestos exposure period for peritoneal mesothelioma is 20 years and the mean value is 21.7 years. For pleural mesothelioma, the median is 29 years and the mean value is 26.4 years. The latency period, which is considered to be from the first exposure to asbestos to the onset of mesothelioma, for pleural mesothelioma is a median of 41 years and an average value of 42.5 years. For peritoneum mesothelioma, the median is 41 years and the average value is 43.0 years. The median for all types of mesothelioma is 41 years, and the average value is 42.4 years. We confirmed that mesothelioma expressed itself after 40 years or more from the first exposure.

Pleura Plaque

We investigated 353 cases of the 442 cases of definitively diagnosed mesothelioma based on chest X-rays or chest CT scans, The scans were provided by the medical institutions targeting the presence of pleural plaque that was considered to be specific to asbestos exposure. We found 144 cases (40.8%) of pleural plaque. In 64 of the 144 cases (44.4%), there was calcification accompanying the pleural plaque. However, there was no statistically significant correlation found between the location of the mesothelioma and the frequency at which the pleural plaque occurred. Furthermore,

TABLE IV. Frequency of Cases Regarding Occupational Histories of Asbestos Exposure

	Pleura	Peritoneum	Pericardium	Tunica vaginalis	Total
Construction worker	65	3		1	69
Shipyard worker	40	4	1		45
Electrician	27	3			30
Steel industrial worker	25	2		1	28
Automobile manufacturer	21	1			22
Plumber	18	2	1		21
Asbestos products manufacturer	11	9			20
Wrecking crew	16				16
Cement product worker	10	1			11
Machinist	7	2			9
Warehouse worker	5	3			8
Chemical industrial worker	6		1		7
Glass maker	4				4
Others	23	3			26
Total	278	33	3	2	316

there were 316 suspected cases from the 442 cases of occupational exposure to asbestos, and among the 270 cases of the 316 cases where chest imaging was provided, 129 cases (47.8%) of pleural plaque were confirmed. In 14 of 86 cases (16.3%) in which occupational exposure to asbestos could not be confirmed, pleural plaque was confirmed. Among the 17 suspected cases of non-occupational exposure to asbestos (exposure to the neighborhood or in the home), 3 cases of pleural plaque were confirmed in which the patient was in the vicinity of the asbestos plant, a family member was working in a shipyard or engaged in plumbing as indicated in the residential history.

Asbestos Particles

We were able to measure the asbestos particles in the lungs of 40 of the pleural mesothelioma cases and 47 of the peritoneal mesothelioma cases based on the excised or autopsied lungs provided by the medical institutions. Table V shows an analysis of the number of asbestos particles and where they were found. We were able to confirm based on the Helsinki Criteria [Consensus Report, 1997], the standard for occupational exposure to asbestos, that there were 37 cases (78.7%) in which there were 1,000 particles or more of asbestos/1 g of dry lung tissue detected and 21 cases (44.7%) in which 5,000 particles or more were detected. There were a total of three unclear cases of asbestos exposure, two cases of pleural mesothelioma, and one case of peritoneal mesothelioma. Despite that pleural plaque could not be identified based on the images, the presence of more than 1,000 particles of asbestos was confirmed but these cases are thought to be mesothelioma due to asbestos exposure. Furthermore, although pleural plaque could not be confirmed, there were six cases where over 5,000 particles of asbestos were detected. We believe that we cannot make a determination on asbestos exposure based solely on the presence or absence of pleural plaque.

Asbestos Exposure and Mesothelioma

From the 442 cases in which mesothelioma was diagnosed based on pathology out of the 541 cases in this investigation, we found that there are 316 cases (71.5%) who had suspected asbestos exposure based on the occupational histories. There were 12 other cases of suspected exposure due to the neighborhood environment, and 5 cases of exposure in the home. Furthermore, another 14 cases had pleural plaques in the radiography while asbestos exposure could not be positively determined from the clinical history and the 3 other cases in which more than 1,000 asbestos particles/1 g of dry lung tissue were detected. While asbestos exposure could not be confirmed from the clinical history or pleural plaque. We determined these 17 cases also as positive asbestos exposure. Accordingly, we concluded based on these examinations that the above 350 cases (79.2%) out of the 442 cases of pathologically diagnosed mesothelioma were caused by asbestos exposure.

TABLE V. Number of Asbestos Particles

No. of asbestos particles[a]	Pleura	Peritoneum	Total
<999	10	0	10
1,000–4,999	15	1	16
>5,000	15	6	21
Total	40	7	47

[a]Per 1 g of dry lung tissue.

DISCUSSION

Among the 2,742 deaths from malignant mesothelioma based on vital statistics recorded over the 3-year period from 2003 to 2005, we targeted 956 cases in which family consent was obtained for a retrospective investigation and clarified the exposure histories of these mesothelioma cases. Among the 541 cases in which data gathering such as clinical records was possible, we confirmed the pathological diagnosis of 81.7%. We found that 372 cases originated from the pleura, 65 cases from the peritoneum, 3 cases from the pericardium, and 2 cases from the tunica vaginalis. In over 80% of the cases a definitive diagnosis was made based on histological diagnosis including immunostaining. On the other hand, in 56 cases where diagnosis was made based on cytological examination, immunocytochemical staining was positive only in 41.1% of the cases, and this brings to light the problem of diagnostic accuracy.

Currently in Japan, if mesothelioma is diagnosed the patient can receive aid through workman's compensation insurance or the asbestos health damage relief law. Although there is recognition of the improvement in diagnosis accuracy, it is clear that in the 3-year period from 2003 to 2005 the immunostaining method was not always reliable in the diagnosis of mesothelioma. In other words, in a case where diagnosis is made based only on cell examination, there may be a problem in discriminating between fibrous pleurisy (reactive mesothelial cells) [Kradin and Mark, 2006; Lyons-Boudreax et al., 2008] and lung cancer. For that reason, we found 9.2% in our examination to be diagnosed as other than mesothelioma such as lung cancer or ovarian cancer, as a result of comprehensive judgment on reviewing autopsy results, clinical records, images, etc. Because HE staining only or cell examination only was used for diagnosis in many cases, when we performed immunostaining, we were able to diagnose definitively not only lung cancer and ovarian cancer but also fibrous pleurisy (benign asbestos pleurisy). It was reported [Ordonez, 2003, 2006, 2007; Kushitani et al., 2008] that immunostaining is indispensable in a pathological

diagnosis of mesothelioma in order to distinguish pleural mesothelioma from lung cancer accompanying cancerous pleurisy, etc. Or in order to distinguish peritoneal mesothelioma from ovarian cancer accompanying cancerous pleurisy, etc. Furthermore, a definitive diagnosis could not be made based on the pathology in 9.1%. The reasons that a definitive diagnosis could not be reached were that the disease advanced rapidly and a detailed examination could not be performed, or although the attending physician recommended tests to diagnose suspected cases of mesothelioma, because the patient was of advanced age either the patient or the family requested not to have invasive tests done. Taking these conditions into consideration, pressing for improvement in the diagnostic accuracy in the diagnosis of mesothelioma is a paramount problem.

Among the 541 cases in this investigation, 442 cases were diagnosed with mesothelioma based on pathology and, among those cases, 71.5% were suspected to be exposed to asbestos based on the occupational history. The types of occupation that were common were construction work, working in a shipyard, electricians, steel products, and other manufacturing work. From 1950 and later, we understand that asbestos was used in these types of occupations, and asbestos was imported into Japan in large quantities for these types of work. We identified that these occupations frequently appear in high-risk groups. Seventeen other cases of non-occupational exposure to asbestos were suspected (12 cases suspected based on residential histories, and 5 cases were thought to be exposure in the home). On the other hand, 40.8% of cases with pleural plaque were confirmed from the 353 cases where the medical institutions provided chest images. Furthermore, among the 47 cases in which the asbestos particles were found in the lungs, 78.7% were found to have more than 1,000 particles/1 g of dry weight lung tissue. There were a total of 79.2% that had occupational or residential histories indicating asbestos exposure, images indicating the existence of pleural plaque, or measurements of the asbestos particles in the lungs and any of these would imply asbestos exposure. Based on the analysis done on these various types of data, 79.2% of the 442 cases were found to have asbestos exposure as the cause of mesothelioma. Furthermore, by examining the origin of the mesothelioma based on the occupations, the cases in which the occupational histories indicated asbestos product manufacturing work, where the patient would be exposed to high concentrations of asbestos, had high levels of asbestos particles in the lungs and were characteristic of the peritoneal mesothelioma cases, which comprise a large number of the cases.

Since 1950 the amount of asbestos used in Japan increased and reached its peak in 1974 at 350,000 tons. After that a trend appeared that showed a decrease in asbestos use until its ban in September 2006. For that reason, compared to Australia, England, and Belgium the amount used and the period of usage are high [Kohyama and Hoshino, 2008]. However, the frequency of occurrence of mesothelioma in the three countries was 30/1,000,000 people, and in Japan the occurrence rate was 7/1,000,000 people [Bianchi and Bianchi, 2007] but currently it is 9/1,000,000 people. Based on the current investigation, if we consider that in Japan the incubation period from the first time the patient was exposed to asbestos to the occurrence of mesothelioma is 43 years, based on the report by Murayama et al. [2006], we must expect two- to threefold the number of new patients in Japan. However, despite having a history of asbestos exposure, in no more than 27.1% of the cases was the occupational history entered in the clinical records. The importance and the repercussions of obtaining and recording the occupational history must be instilled in the clinicians who examine patients of asbestos-related diseases.

CONCLUSIONS

Among 442 cases of definite malignant mesothelioma, between 2003 and 2005, in Japan, 316 cases were exposed to occupational asbestos exposure; 12 cases had neighborhood exposure; and 5 cases had domestic exposure. We conclude that 79% of Japanese mesothelioma cases have been caused by asbestos exposure in recent years.

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