

Usefulness of malignant pleural effusion for early cytological diagnosis of mesothelioma *in situ*: A case report

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Abstract. Mesothelioma *in situ* (MIS) is defined as a preinvasive mesothelioma that forms a single layer of mild atypical mesothelial cells lining on the serosa surface of pleura. The atypical mesothelial cells present loss of BRCA-1 associated protein-1 (BAP-1) and/or methylthioadenosine phosphorylase as examined by immunohistochemistry (IHC) and/or homozygous deletion of cyclin-dependent kinase inhibitor 2A/p16 as examined by fluorescence *in situ* hybridization. It is difficult to diagnose because of the unremarkable clinical findings except for pleural effusion. The present report describes a case in which MIS was diagnosed at the time of sampling due to the presence of clearly malignant mesothelial cells in the pleural fluid. In 2016, a 74-year-old man with a history of past exposure to asbestos was admitted to Ibaraki Higashi National Hospital (Tokai-mura, Japan) with dyspnea. Chest CT indicated only

right pleural effusion. Malignant mesothelial cells were suspected in a cell block made using pleural effusion; therefore, right pleural biopsy was performed. Pathologically, there was proliferation of mesothelial cells with mild atypia that formed a single-flat layer on the pleural surface; however, there was no invasion. Furthermore, IHC revealed loss of BAP-1 in cells from the biopsied pleura and pleural effusion. MIS was suspected at the time; however, the patient arbitrarily quit his medical check-ups. After 44 months, the patient was readmitted to our hospital complaining of dyspnea. CT indicated a large right pleural mass. A specimen of the mass obtained via CT-guided needle biopsy revealed malignant mesothelioma. The patient continued to deteriorate and eventually died. This case indicated that pleural effusion could be used to demonstrate overtly malignant mesothelial cells and diagnose MIS at the time of sampling. To the best of our knowledge, this is first report of MIS with overtly malignant mesothelial cells in pleural effusion. Pleural effusion may serve an important role in MIS diagnosis.

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Abbreviations: BAP-1, BRCA-1 associated protein-1; Ber-EP4, epithelial specific antigen; CDKN2A/p16, cyclin-dependent kinase inhibitor 2A/p16; CEA, carcinoembryonic antigen; CXR, chest X-ray; CYFRA, cytokeratin 19 fragment; D2-40, podoplanin; EDTA, Tris-ethylenediaminetetraacetic acid; EMA, epithelial membrane antigen; FISH, fluorescence *in situ* hybridization; H&E, hematoxylin and eosin; HEG-1, sialylated protein HEG homolog 1; IHC, immunohistochemistry; MIS, mesothelioma *in situ*; MTAP, methylthioadenosine phosphorylase; TTF-1, thyroid transcription factor-1; WHO, World Health Organization

Key words: BAP-1, cell block, malignant mesothelial cells, atypical cells, first obtained pleural fluid

Introduction

Mesothelioma is a tumor with a poor prognosis that occurs mostly from mesothelial cells in the pleura or peritoneum (1). Mesothelioma is associated with exposure to asbestos and has a poor prognosis; the median survival is 9-12 months and the 5-year survival rate is 5% (1,2). Mesothelioma is classified into three morphologic subtypes, namely epithelioid, biphasic, and sarcomatoid; the latter two subtypes have even shorter survival times (1,3). Clinically, there are poor or non-specific symptoms, and the latent period from asbestos exposure to onset is long. Therefore, in many cases, mesothelioma tends to be diagnosed at a later stage of the disease (1-3). Recently, there have been reports that mesothelioma has an early phase, known as mesothelioma *in situ* (MIS) (1-4). MIS is defined as a single layer of atypical mesothelial cells proliferating along the pleural surface (2,4); it may be cured with appropriate therapies (1-3). However, it is difficult to distinguish

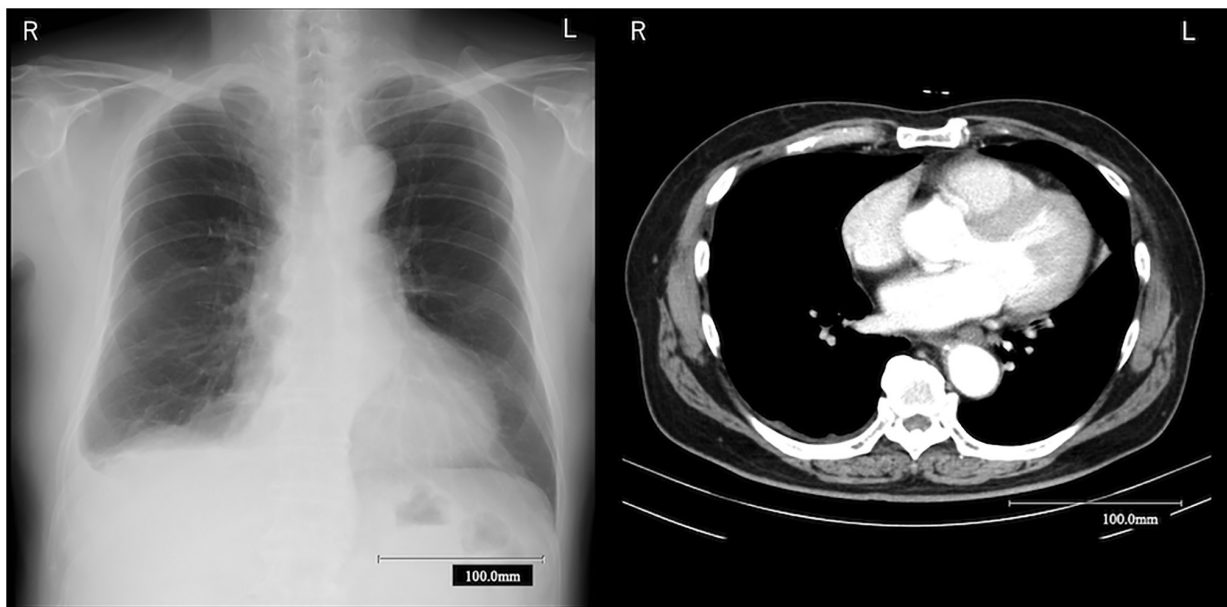


Figure 1. Chest X ray and CT from the patient's first visit to our hospital showing only right pleural effusion. R, right side; L, left side.

MIS from reactive surface mesothelial proliferation based on routine morphology (5). Thus, the 2021 World Health Organization (WHO) classification of tumors of the pleura included the following criteria of MIS (4): 1) pleural effusion (non-resolving), 2) no thoracoscopic or imaging evidence of tumor, 3) a single layer of mesothelial cells (with or without atypia) on the pleural surface, 4) no histological features of invasive growth, 5) loss of BRCA-1 associated protein-1 (BAP-1) and/or methylthioadenosine phosphorylase (MTAP) based on immunohistochemistry (IHC) and/or cyclin-dependent kinase inhibitor 2A/p16 (CDKN2A/p16) homozygous deletion based on fluorescence *in situ* hybridization (FISH), and 6) multidisciplinary discussion of the diagnosis.

There have been some cases of MIS published. Due to the difficulty of detecting MIS, for many of these cases, the diagnosis was made retrospectively, using previously collected specimens, after the patient had progressed to mesothelioma. Here, we present a case of MIS that was diagnosed at the initial presentation based on cytology of pleural effusion. As far as we know, this is the first report of MIS with overtly malignant mesothelial cells in the first pleural effusion cytology.

Case report

The patient was a 74-year-old man, an ex-smoker. He had been a mason from 23 to 60 years old of age, with exposure to particles of cement containing asbestos and hexavalent chromium without a dust respirator. He had no remarkable past medical history. Until 2015, there had been no abnormality in his medical checkups; however, a year later he went to a local hospital with a complaint of dyspnea. Because chest X-ray (CXR) showed right pleural effusion (Fig. 1), he was referred and admitted to Ibaraki Higashi National Hospital (Tokai-mura, Japan). On presentation, there were no abnormal physical findings. Blood examinations revealed normal laboratory data and negative serum tumor markers. Chest contrast-enhanced computed tomography (CT) presented only

minimal right pleural effusion (Fig. 1). Right pleural effusion revealed by thoracentesis was exudative based on Light's criteria, and the value of hyaluronic acid was normal. On the other hand, cytology of pleural effusion was classified as class V (overtly malignant) according to the Papanicolaou classification. Note that the Papanicolaou smears had been borrowed from a previous hospital and have already been returned, so we could not show the image here. Immunohisto/immunocytochemical staining was performed on 4- μ m-thick sections mounted on glass slides. Endogenous peroxidase activity was then blocked for 5 min at room temperature using blocking reagents, and epitopes were activated by protease at 37°C or Tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.5) at 95°C for different times for each antibody and incubated with MTAP clone 2G4 (Abnova) (EDTA, 64 min), BAP-1 clone C-4 (Nichirei) (EDTA, 32 min), sialylated protein HEG homolog 1 (HEG-1) Clone SKM9-2 (Nichirei) (EDTA, 64 min), thyroid transcription factor-1 (TTF1) clone SP141 (Roche Diagnostics) (EDTA, 64 min), podoplanin (D2-40) clone D2-40 (Roche Diagnostics) (EDTA, 64 min), epithelial membrane antigen (EMA) Clone E29 (Agilent Technologies Japan) (EDTA, 64 min), Desmin Clone D33 (Agilent Technologies) (EDTA, 36 min), carcinoembryonic antigen (CEA) Clone COL-1 (Nichirei) (EDTA, 36 min), p53 Clone DO7 (Roche Diagnostics) (EDTA, 32 min), Calretinin clone SP65 (Roche Diagnostics) (EDTA, 64 min) and epithelial specific antigen (Ber-EP4) Clone Ber-EP4 (Protease, 4 min). OptiView DAB IHC Detection Kit (Roche Diagnostics) or ultraView Universal DAB Detection Kit (Roche Diagnostics) were used according to the manufacturer's recommendations for the visualization of each primary antibodies. In a cell block made using the pleural effusion at our hospital, staining hematoxylin and eosin (H&E), the mesothelioma cells had nuclear enlargement, irregular nuclear membranes, frequent binucleation or multinucleation, humps, and cellular pleomorphism. These features indicated overtly malignant mesothelial cells (Fig. 2A and B). Based on IHC, the overtly malignant mesothelial cells were positive for

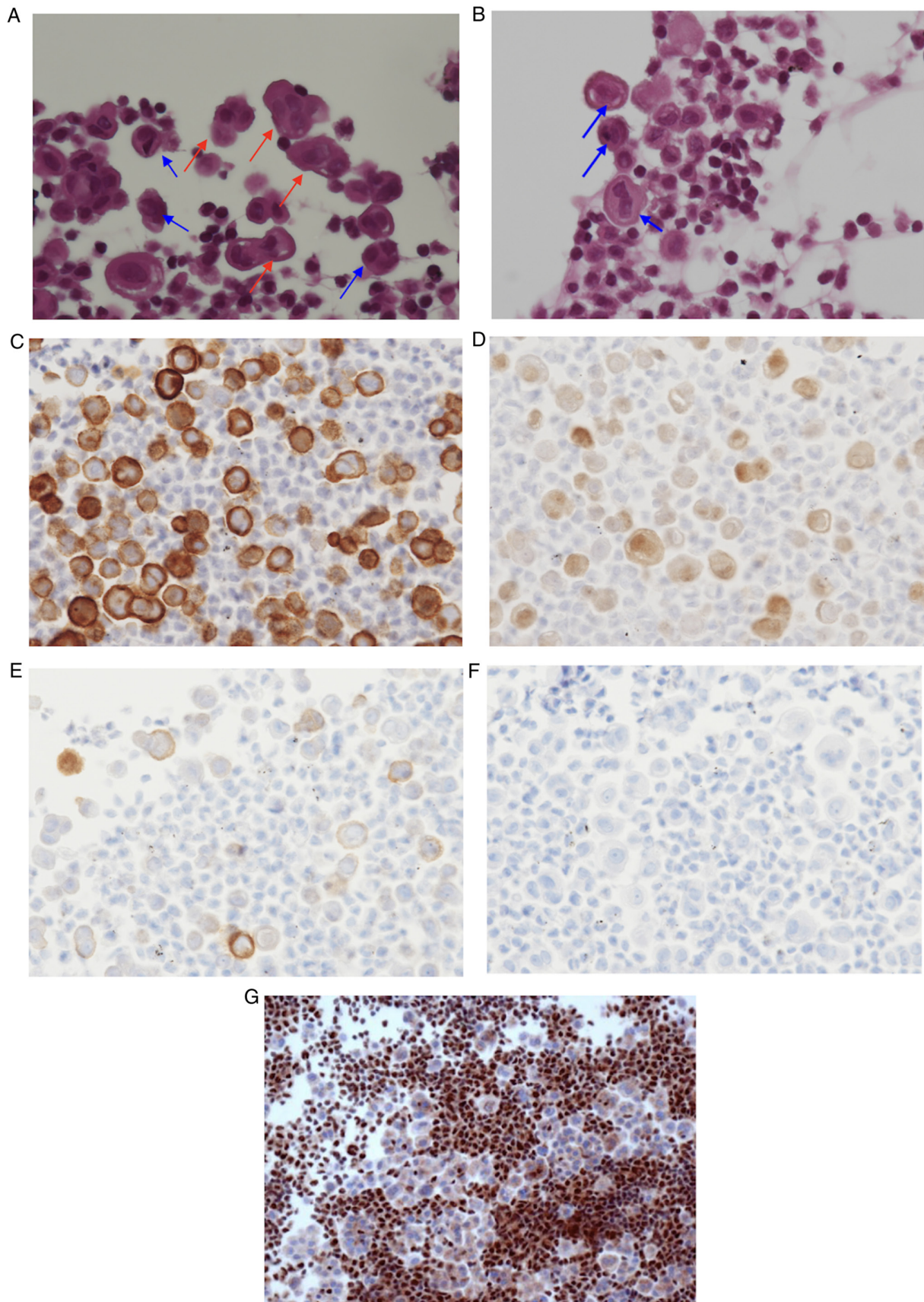


Figure 2. Cytological specimen of right pleural effusion showing malignant cells. Based on the cell block made using pleural effusion, (A) H&E staining showed malignant cells that formed glomerular or papillary clusters (magnification, x400). In detail, the cells presented nuclear enlargement, irregular nuclear membranes, frequent binucleation or multinucleation indicated by the blue arrows, humps indicated by the red arrows and cellular pleomorphism. (B) H&E staining also showed nuclear enlargement, binucleation or multinucleation indicated by blue arrows (magnification, x400). Immunohistochemically, the cells were positive for (C) podoplanin (D2-40) in the cytoplasmic membrane (magnification, x400), (D) calretinin in the cytoplasm and nucleus (magnification, x400) and (E) EMA in the cytoplasm and membrane (magnification, x400), while they were negative for (F) desmin (magnification, x400) and (G) BAP-1 (magnification, x400). BAP-1, BRCA-1 associated protein-1; EMA, epithelial membrane antigen.

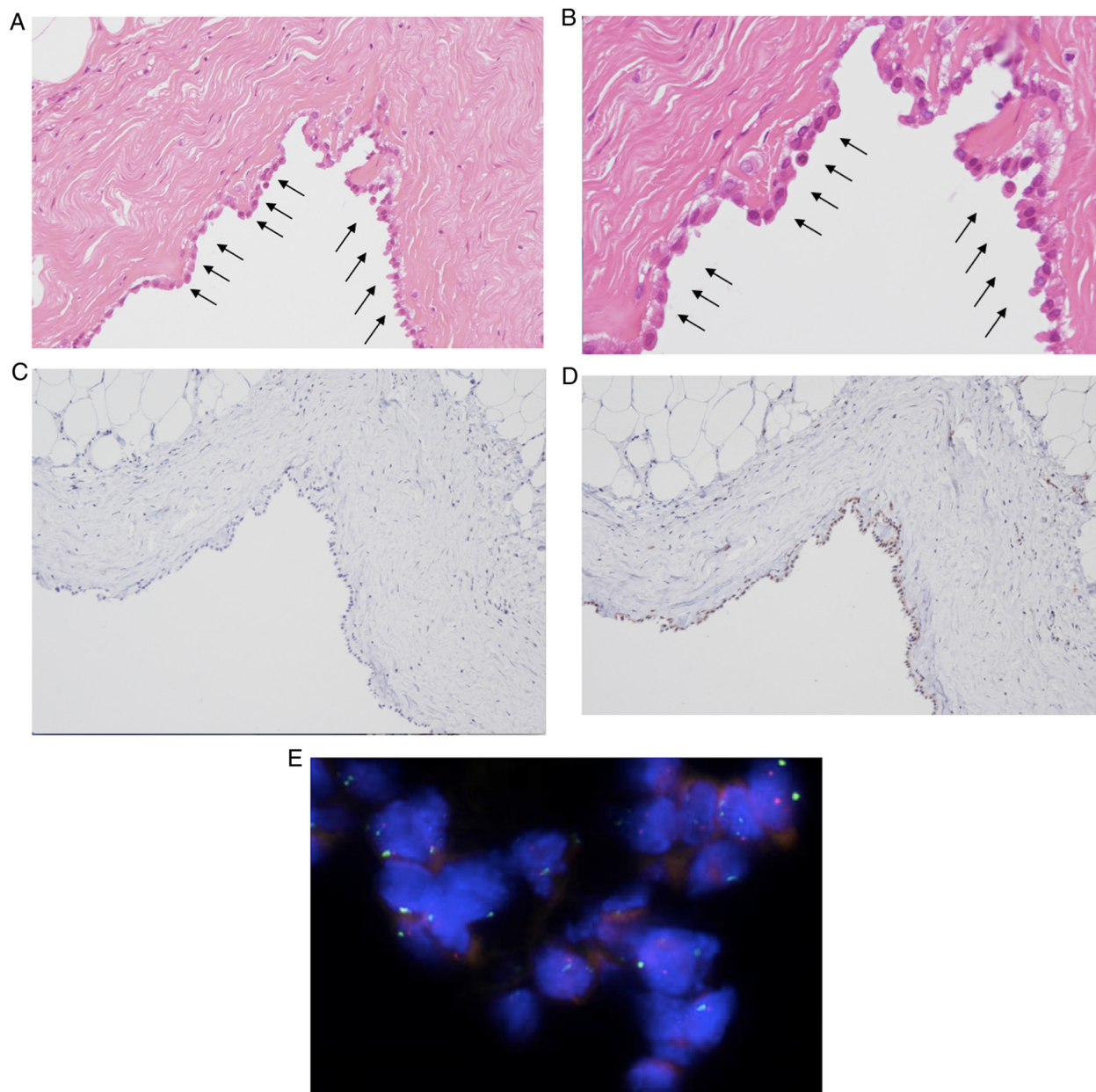


Figure 3. Pathological finding in the first right pleural biopsy showing mildly atypical mesothelial cells forming a single layer indicated by black arrows based H&E staining. (A) Magnification, x100. (B) Magnification, x200. (C) Loss of BAP-1 (magnification, x100) and (D) presence of MTAP (magnification, x100) based on immunohistochemistry, and (E) presence of CDKN2A based on FISH (original magnification, x63). BAP-1, BRCA-1 associated protein-1; CDKN2A, cyclin-dependent kinase inhibitor 2A; FISH, fluorescence *in situ* hybridization; MTAP, methylthioadenosine phosphorylase.

three markers, namely D2-40 in the cytoplasmic membrane (Fig. 2C), calretinin in cytoplasm and nucleus (Fig. 2D), and EMA in the cytoplasm and membrane (Fig. 2E), while they were negative for TTF-1, CEA, and desmin (Fig. 2F). Furthermore, although CDKN2A/p16 homozygous deletion was not confirmed by FISH, there was loss of BAP-1 based on IHC (Fig. 2G). A right pleural biopsy was performed for precise diagnosis. The surgical findings did not show an obvious nodule in the thoracic cavity and no thickening of the pleura. The sample was taken from all layers of the right dorsal parietal pleura. The pathological findings included mild cellular atypia with proliferation of mildly atypical cuboidal or columnar cells derived from mesothelial cells that formed a single layer in places (Fig. 3A and B). IHC for atypical cells was positive for D2-40, calretinin, and EMA, and negative for

desmin, TTF-1, CEA, and p53. These findings were consistent with the pleural effusion cytology. Furthermore, loss of BAP-1 was confirmed (Fig. 3C), while MTAP was retained with IHC and CDKN2A/p16 homozygous deletion was not identified with FISH (Fig. 3D and E). Due to mild cellular atypia, MIS rather than mesothelioma was suspected at the time. The case retrospectively met the 2021 WHO criteria of MIS. Unfortunately, the patient did not agree to undergo an operation and quit attending his medical check-ups 4 months after the MIS diagnosis.

Forty-four months later, he was re-referred to our hospital due to a complaint for dyspnea and worsening right pleural effusion. Cytokeratin 19 fragment (CYFRA), a serum tumor marker, was increased to 19.4 ng/ml. Chest CT revealed a large mass that originated from the right pleura, diffuse pleural

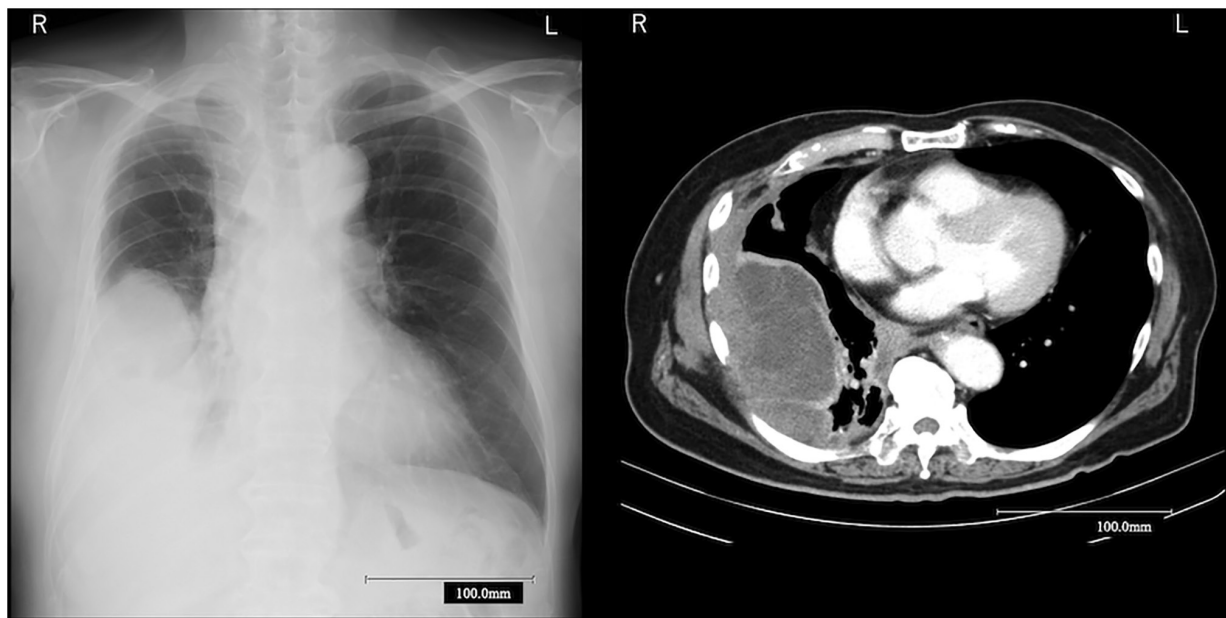


Figure 4. Chest X-ray and CT 44 months after the patient had first visited our hospital showing the appearance of a large mass infiltrating some ribs and thoracic wall in the right lower lung and another mass in the mediastinum. R, right side; L, left side.

thickening, and a mediastinal mass (Fig. 4). Because progression to mesothelioma was suspected, CT-guided needle biopsy from the right large mass was performed. Based on cytology of needle lavage fluid, there were atypical cells with nuclear enlargement in an isolated or accumulated state, suspected to be malignant mesothelial cells (Fig. 5A). The pathological findings of biopsied specimen included tumor cells (Fig. 5B) with IHC positive for D2-40, calretinin, EMA, pankeratin, and HEG-1 (Fig. 5C), and negative for TTF-1, CEA, desmin, and Ber-EP4. These findings fulfilled the diagnosis of mesothelioma. Although there was no loss of MTAP based on IHC and homozygous deletion of CDKN2A/p16 based on FISH (Fig. 5E and F), there was loss of BAP-1 based on IHC (Fig. 5D). After diagnosis, the patient was started on chemotherapy with carboplatin with pemetrexed; this continued for five courses. Subsequently, the tumor progressed, and the patient was switched to nivolumab, but he did not respond to treatment. His disease then worsened and he died 52 months after the initial diagnosis of MIS.

Discussion

We have presented a case of MIS that showed obviously malignant mesothelial cells based on cytology of pleural effusion. Based on our search of the literature, 17 cases of pleural MIS have been reported (Table I) (2,5-12). According to available data from previous reports, MIS was confirmed only in 8 cases before progression to mesothelioma (2,5,6,8,10,11). It is difficult to suspect MIS at the time of sampling due to unremarkable clinical findings including symptoms, serum tumor markers, radiology, and even pathology. On the other hand, all cases had pleural effusion from the first examinations (Table I). Among them, with the available information, in six cases cytology of pleural fluid had been performed; there was mild or no cellular atypia (Table II) (2,5-10,12). Moreover, two cases were confirmed to be malignant based on the loss

of BAP-1 expression (Table II) (10,12). In our case, although biochemical examinations including hyaluronic acid were normal, the initially obtained pleural fluid cytology showed overtly malignant class V mesothelial cells (based on the Papanicolaou classification), a factor that played a key role in suspicion of MIS. Mesothelioma can be diagnosed without ancillary tests such as loss of BAP-1 expression and/or homozygous deletion of CDKN2A by FISH when overtly malignant features are identified (13). As far as we know, this is the first report of MIS with overtly malignant mesothelial cells in the initially obtained pleural fluid. It may be useful to perform cytology of pleural effusion when considering the diagnosis of MIS.

BAP-1 is a tumor suppressor gene located at 3p21.1 (3,6); it acts as a nuclear deubiquitinating agent, regulating especially chromatin remodeling to suppress cell proliferation and apoptosis (3,4). Loss of BAP-1 based on IHC has been reported in 60% of mesothelioma cases and has a specificity of 100% to distinguish malignant from benign mesothelial proliferation (2,4). CDKN2A/p16 located at 9p21.3 is a tumor suppressor gene whose product arrests the cell cycle in G1 (14). Homozygous deletion of CDKN2A/p16 results in uncontrolled cell proliferation, which is commonly detected in mesothelioma (14). This mutation has 100% specificity to differentiate between a benign and a malignant tumor (14). On the other hand, MTAP located at 9p21.3 encodes an enzyme used for the salvage pathway of adenine and methionine (14). Because MTAP and CDKN2A/p16 are located on the same chromosome, it has been reported that loss of MTAP based on IHC could be a surrogate marker for CDKN2A homozygous deletion (15).

According to Table II, three cases (including our case) demonstrated BAP-1 loss based on IHC of pleural fluid, which could be a key factor to diagnose MIS based on the initially obtained samples. According to WHO, ancillary analyses such as IHC for BAP-1 and MTAP are necessary for the diagnosis of mesothelioma and are expected to

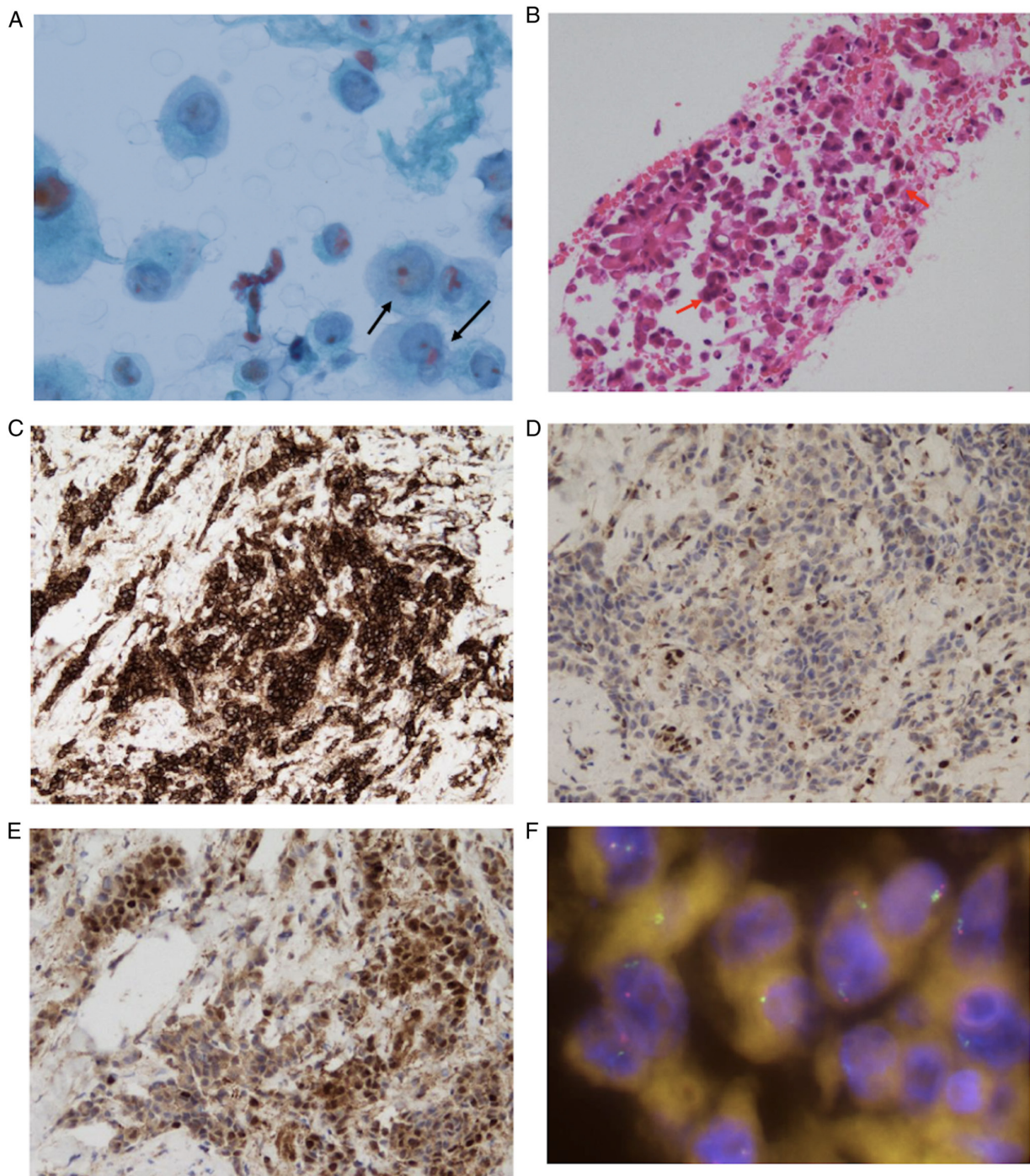


Figure 5. CT-guided needle biopsy of the right large mass was performed. (A) Cytology of needle lavage fluid showing atypical cells with nuclear enlargement indicated by black arrows (magnification, x600). (B) The pathological findings based on H&E staining included tumor cells with humps on the edge indicated by red arrows (magnification, x200) and (C) positive for HEG-1 based on IHC (magnification, x200). Also shown are the results of right pleural biopsy, with (D) loss of BAP-1 (magnification, x200) and (E) presence of MTAP (magnification, x200) based on IHC, and (F) presence of CDKN2A based on FISH (original magnification, x63). BAP-1, BRCA-1 associated protein-1; CDKN2A, cyclin-dependent kinase inhibitor 2A; FISH, fluorescence *in situ* hybridization; HEG-1, sialylated protein HEG homolog 1; IHC, immunohistochemistry; MTAP, methylthioadenosine phosphorylase.

become more widespread. Additionally, BAP-1 and MTAP may be used as prognostic markers in mesothelioma (16). Nishikubo *et al* (11) reported that, among 13 patients with MIS, the median progression-free survival for patients with CDKN2A homozygous deletion or MTAP loss was 18 months; in patients who had lost BAP-1 but retained CDKN2A and MTAP, the median progress-free survival was 60 months. Although the authors did not elucidate the

mechanism, they hypothesized that BAP-1 loss occurs during the early phase of the disease and MTAP/CDKN2A deletion occurs at a later phase (6,17). In our patient, we confirmed progression to mesothelioma 44 months later. This relatively slow progression might have been because he had lost BAP-1 but retained CDKN2A and MTAP.

In conclusion, we have presented a case of MIS with malignant mesothelioma cells in pleural effusion. We suggest

Table I. Clinical characteristics of 17 previously reported MIS cases and the present case.

First author/s, year	Age, years	Sex	CT findings	BAP1/MTAP/CDKN2A	Periods from MIS to mesothelioma	(Refs.)
Churg <i>et al</i> , 2018; Churg <i>et al</i> , 2020	70	F	Right PE	Loss/loss/loss	36 months	(5,6)
Churg <i>et al</i> , 2020	71	F	PE, smooth PT	Loss/retain/NA	64 months	(6)
Churg <i>et al</i> , 2020	72	F	PE, smooth PT	Loss/retain/retain	92 months	(6)
Churg <i>et al</i> , 2020	68	M	PE	Loss/retain/retain	58 months	(6)
Churg <i>et al</i> , 2020	69	M	PE	Loss/NA/retain	69 months	(6)
Churg <i>et al</i> , 2020	79	M	PE	Loss/NA/NA	60 months	(6)
Churg <i>et al</i> , 2020	67	M	Po resection, no PE	Loss/loss/retain	Stable for 12 months	(6)
Churg <i>et al</i> , 2020	68	M	PE	Loss/retain/retain	Stable for 120 months	(6)
Churg <i>et al</i> , 2020	76	M	Po resection, no PE	Loss/retain/retain	Stable for 57 months	(6)
Churg <i>et al</i> , 2020	53	F	Ascites	Loss/loss/retain	Stable for 12 months	(6)
Haefliger <i>et al</i> , 2021	57	M	PE	Loss/NA/NA	NA	(7)
Minami <i>et al</i> , 2020; Nishikubo <i>et al</i> , 2022	73	M	Right PE, Slightly PT	Retain/loss/loss	25 months	(8,11)
Hidaka <i>et al</i> , 2020	50s	F	Right PE	Loss/retain/retain	168 months	(9)
Pulford <i>et al</i> , 2020; Klebe, 2022	74	F	Right PE	Loss/retain/NA	Stable for 36 months	(2,10)
Pulford <i>et al</i> , 2017	89	M	PE	Loss/NA/NA	Died 24 months later	(3)
Pulford <i>et al</i> , 2017	79	M	PE	Loss/NA/NA	Stable for 9 months	(3)
Churg <i>et al</i> , 2022	70	NA	Right PE	Loss/NA/NA	NA	(12)
Present study	74	M	Right PE	Loss/retain/retain	44 months	-

BAP-1, BRCA-1 associated protein-1; CDKN2A, cyclin-dependent kinase inhibitor 2A; F, female; M, male; MIS, mesothelioma *in situ*; MTAP, methylthioadenosine phosphorylase; NA, not available; PE, pleural effusion; Po, post; PT, pleural thickening.

Table II. Cytological features of 6 previously reported mesothelioma *in situ* cases and the present case.

First author/s, year	Age, years	Sex	Findings of initial pleural fluid cytology	BAP1 in initial pleural fluid cytology	(Refs.)
Churg <i>et al</i> , 2018; Churg <i>et al</i> , 2020	70	F	No atypical cells	NA	(5,6)
Haefliger <i>et al</i> , 2021	57	M	Mild atypical mesothelial cells satellited by lymphocytes	NA	(7)
Minami <i>et al</i> , 2020; Nishikubo <i>et al</i> , 2022	73	M	Atypical epithelioid cells	NA	(8,11)
Hidaka <i>et al</i> , 2020	50s	F	No atypical cells	NA	(9)
Pulford <i>et al</i> , 2020; Klebe, 2022	74	F	Mild atypical cells	Loss	(2,10)
Churg <i>et al</i> , 2022	70	NA	Multiple balls of slightly atypical mesothelial cells	Loss	(12)
Present study	74	M	Malignant mesothelial cells	Loss	-

BAP-1, BRCA-1 associated protein-1; F, female; M, male; NA, not available.

that cytology of pleural fluid may play an important role in diagnosis of MIS at the time of presentation. When atypical

cells are detected in pleural fluid, if available, IHC of BAP-1 and MTAP should be performed.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YY, KHi, TS, NH and YM designed this case report. HO, JK, KHa, SOI and TS acquired the data. TN, MS, KHa, SU and SOI performed analysis of the data. YY, KHi, NH and YM drafted and revised the manuscript. YY and YM submitted the final manuscript. YM and KHi confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ibaraki Higashi National Hospital ethical committee (Tokai-mura, Japan).

Patient consent for publication

Written informed consent was obtained from the patient's family for publication of this case report and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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