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Definitive Diagnosis of Pleural Mesothelioma by Pleural Effusion Cytology: The MesoCyto Study

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Simple Summary: Recent advancements in diagnostic ability of pleural effusion cytology including the cell block method could provide high diagnostic accuracy in pleural mesothelioma (PM). We conducted a prospective study to validate the efficacy of diagnosing PM using pleural effusion cytology. Total of 50 patients were enrolled. Both pleural effusion cytology including cell block and pleural biopsy were independently performed and compared to assess the diagnostic reliability of pleural effusion cytology. Of the 50 enrolled patients, histological examination confirmed PM in 42 in which 29 met the diagnostic criteria of PM in pleural effusion cytology and the remaining 13 displayed atypical cells suggestive of PM in pleural effusion cytology but did not meet the diagnostic criteria. No atypical cells suspicious of PM was seen in remaining 8. Thus, pleural effusion cytology specificity was 100%. This study revealed PM could be diagnosed through only pleural effusion cytology including the cell block method.

Abstract: Objective: We evaluated the possibility of diagnosing pleural mesothelioma (PM) through pleural effusion cytology in a multicentric prospective clinical trial (MesoCyto study). **Methods**: We included patients with pleural effusion and suspected PM scheduled for thoracentesis and pleural biopsy. Both pleural effusion cytology including cell block and pleural biopsy were independently performed and compared to assess the diagnostic reliability of pleural effusion cytology. The primary endpoint aimed to demonstrate that the specificity of PM diagnosis by pleural effusion cytology reached 100%. Secondary endpoints included the frequency of adverse events during thoracentesis and pleural biopsy and assessment of diagnostic accuracy of pleural effusion cytology for each histological type. **Results**: Of the 50 enrolled patients, histological examination confirmed PM in 42 patients; 29 patients met the diagnostic criteria of PM in pleural

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effusion cytology and received a cytological diagnosis. Of the remaining 21 patients, 13 displayed atypical cells suggestive of PM based on pleural effusion cytology but did not meet the diagnostic criteria, and in 8, atypical cells were not identified and PM diagnostic criteria were not met. Thus, pleural effusion cytology specificity was 100%. The diagnostic concordance rate by pleural effusion cytology for each histological type was 72.2% for epithelioid, 75% for biphasic, and 0% for sarcomatoid. No adverse events were associated with thoracentesis, but the incidence of adverse events during pleural biopsy was 14%. **Conclusions**: PM can potentially be diagnosed through only pleural effusion cytology including the cell block method using immunohistochemical staining.

Keywords: pleural mesothelioma; pleural effusion; cell block; pleural biopsy

Introduction

Pleural mesothelioma (PM) is a malignancy affecting the pleura and is characterized by a poor prognosis. Frequently associated with pleural effusion, it is often identified through initial symptoms such as cough and dyspnea on exertion [1]. Distinguishing PM from other cancers, such as lung adenocarcinoma, and discerning between benign and malignant tumors poses challenges. Consequently, histopathological diagnosis via pleural biopsy is recommended as the gold standard and adhered to in diagnostic guidelines for PM [2–6]. Further, pleural biopsy is a relatively minimally invasive procedure and carries a low incidence of reported adverse events [7]. PM is known for its high invasiveness, frequently infiltrating subcutaneous tissue at biopsy and drainage sites [8,9]. If the diagnosis of PM could be achieved solely through pleural effusion cytology, it would not only expedite the diagnostic timeline but also mitigate the risks associated with pleural biopsy and the potential for tumor invasion at the biopsy site.

Originally, the diagnostic yield for PM through pleural effusion cytology exhibited considerable variability, ranging from 16% to 73%, and was reported to depend on the diagnostician's experience [2]. Recent advancements in diagnostic assays focusing on genetic mutations related to mesothelioma have introduced the potential for differentiating neoplastic proliferating mesothelial cells in 70%–90% of cases in both pleural effusion cytology and histological diagnosis, particularly through the loss of BRCA1-associated protein-1 (BAP1) or Methylthioadenosine Phosphorylase (MTAP) expression in immunohistochemical staining [10–14]. Girolami et al. reported in a meta-analysis that diagnostic sensitivity could be enhanced by concurrently performing immunostaining for BAP1 and MTAP [15]. Despite these advancements, the World Health Organization classification acknowledges that stromal invasion cannot be reliably evaluated through pleural effusion cytology alone. It is suggested that auxiliary assays, such as immunohistochemical staining for BAP1 and MTAP or fluorescence in situ hybridization for CDKA2A, can aid in distinguishing MPM from benign diseases [4]. Building on this context, our hypothesis posited that a reliable diagnosis could be achieved through pleural effusion cytology in cases where PM is strongly suspected clinically. To verify this hypothesis, we designed a prospective clinical trial to validate the efficacy of diagnosing PM using pleural effusion cytology. The ultimate objective of this study was to attain 100% diagnostic specificity for PM through pleural effusion cytology.

Materials and Methods

Study Information

This prospective clinical study took place at three prominent centers specializing in PM in Japan, namely, Hyogo University of Medicine, Fukuoka University, and the University of Occupational and Environmental Health. The study's primary endpoint was to validate the efficacy of diagnosing PM based solely on pleural effusion testing. The study adhered to the principles outlined in the Declaration of Helsinki and the Japanese Clinical Trials Act and is registered with UMIN (UMIN000038709). The research protocol received approval from the ethics committees of each participating facility (Hyogo University of Medicine: #3387, Fukuoka University: H20-03-009, University of Occupational and Occupational Health: UOEHCRB20-140; Date of approval: 28

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November 2019). All participating patients provided informed consent prior to their involvement in the study.

Eligibility/Exclusion Criteria

The eligibility criteria were as follows:

i. Non-receipt of treatment
ii. Inability to rule out PM on imaging tests (pleural effusion + pleural thickening or pleural tumor)
iii. Necessity of thoracentesis for diagnosis and treatment
iv. Ability to safely undergo thoracentesis
v. Ability to undergo a pleural biopsy under general anesthesia
vi. Age 20 years or older and provision of consent
vii. No prerequisite for a history of past thoracentesis.

The exclusion criteria were as follows:

i. Cases in which biopsy other than pleural effusion cytology was performed
ii. Presence of other malignant tumors
iii. Presence of other intrathoracic diseases (such as empyema, pleurisy)

Study Flow

This was a prospective, open-label, single-arm study aimed at evaluating the diagnostic accuracy of pleural effusion cytology in patients with pleural effusion and suspected PM. Eligible patients who provided written informed consent underwent thoracentesis as the initial procedure, with subsequent pleural effusion cytology analysis. Pleural biopsy was conducted within one month of the thoracentesis. The diagnoses based on pleural effusion cytology and histology was determined independently.

Pleural Effusion Cytology

Under local anesthesia, thoracentesis was performed to collect a minimum of 20 mL of pleural fluid. A smear of pleural effusion was prepared from a portion of the collected sample, followed by Papanicolaou and Giemsa staining. Additionally, a cell block was generated from the remaining specimen. Specimens derived from cell blocks were subjected to hematoxylin/eosin (HE) staining and immunohistochemical analysis to assess the presence or absence of cell atypia, determine the cell type origin, and ascertain whether the cells exhibited benign or malignant characteristics. The procedure for preparing the pleural effusion cell block in this study was: 1) Centrifugation of the collected pleural effusion at 1500 rpm for 10 minutes; 2) Mixing of the precipitate with a small amount of supernatant in an Eppendorf tube; 3) Recentrifugation, removal of the supernatant, covering with 10% neutral buffered formalin, and fixation overnight at 20°C; and 4) Cutting of the tube with a scalpel, removal of the precipitate (cells), embedding in paraffin, and slicing to prepare a specimen.

PM was diagnosed when the smear and cell block analyses collectively met all the following diagnostic criteria (i) to (v). Instances where atypical cells suspected of PM were identified but did not align with the full diagnostic criteria were labeled as suspicious (atypical cells). If no atypical cells were detected, a negative diagnosis was assigned. If a disease other than PM could be definitively diagnosed, the specific disease name was employed.

i. Cytomorphological features indicative of malignancy are supported by smear specimens subjected to Papanicolaou staining and Giemsa staining.
ii. Positive immunohistochemical staining for cytokeratin is observed in the cell block analysis.
iii. Immunohistochemical staining of the cell block is positive for calretinin and one or more other mesothelial cell markers (WT-1, D2-40, HEG-1).
iv. Immunohistochemical staining of the cell block is negative for two or more mesothelial cell negative markers (CEA, TTF-1, Napsin-A, claudin-4), with CEA being among the negative markers.
v. Immunohistochemical staining of cell blocks demonstrates the loss of expression in at least one of BAP1 and MTAP.

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Histological Diagnosis

Histopathological diagnosis involved thoracoscopic pleural biopsy performed under general anesthesia. Following the method described by Hashimoto et al., the pleural biopsy typically included the simultaneous insertion of a thoracoscope and forceps through a single wound. The procedure aimed to collect all layers of the parietal pleura from multiple sites [16]. Subsequently, paraffin blocks were prepared from the obtained parietal pleura, followed by HE and immunohistochemical staining for histological examination. The diagnostic criteria for PM based on histological diagnosis in this study are given below. Further, if a disease other than PM can be definitively diagnosed, the specific disease name was used for the diagnosis.

I The presence of atypical cell proliferation accompanied by deep fat tissue infiltration is observed in HE-stained specimens. (i–iii must all be met)

i. Positive immunohistochemical staining for cytokeratin is observed.
ii. Immunohistochemical staining is positive for calretinin and one or more other mesothelial cell markers (WT-1, D2-40, HEG-1).
iii. Immunohistochemical staining is negative for two or more mesothelial cell-negative markers (CEA, TTF-1, Napsin-A, claudin-4), with CEA being among the negative markers.

II In cases where atypical cells are identified in HE-stained specimens, but stromal invasion is not observed (criteria i–iv must be fulfilled).

i. Positive immunohistochemical staining for cytokeratin is observed.
ii. Immunohistochemical staining is positive for calretinin and one or more other mesothelial cell markers (WT-1, D2-40, HEG-1).
iii. Immunohistochemical staining is negative for two or more mesothelial cell-negative markers (CEA, TTF-1, Napsin-A, claudin-4), with CEA being among the negative markers.
iv. If one or more of the following items are met

A) Loss of BAP1 expression by immunohistochemical staining
B) Loss of MTAP expression by immunohistochemical staining
C) More than 10% homozygous deletion of p16 in fluorescence in situ hybridization

Endpoints

Clinical information, cytology and histology results, and adverse events related to the tests were gathered for all cases in this study. Cytology and histology procedures were carried out at individual institutions, and expert pathologists (T.T and K.N) conducted central diagnosis to validate the cytology and histology diagnoses.

The primary endpoint of this study aimed for 100% specificity determined through pleural effusion cytology. Secondary endpoints included assessing the diagnostic accuracy of pleural effusion cytology for each histological type and determining the frequency of adverse events linked to diagnostic procedures (thoracentesis and pleural biopsy). Adverse events related to diagnostic techniques and their severity were evaluated using the Clavien–Dindo classification [17].

Results

A total of 50 cases were enrolled between December 1, 2019, and October 20, 2021, distributed across Hyogo Medical School (42 cases), Fukuoka University (7 cases), and the University of Occupational Medicine (1 case). The characteristics of the 50 cases are summarized in Table 1. Among the 50 patients, 46 were men (92%), with a median age of 71.5 years (ranging from 53 to 93 years), and 31 patients (62%) presented symptoms on the right side. Asbestos exposure history was noted in 38 patients, and a family history of mesothelioma was observed in 2 patients. Common symptoms at the time of consultation included dyspnea in 26 cases, chest pain in 10 cases, and cough in 9 cases. Thoracentesis was followed by thoracoscopic pleural biopsy under general anesthesia in all 50 patients. No adverse events related to thoracentesis were observed in any of the cases. However, adverse events associated with pleural biopsy were noted in 7 cases, as detailed in Table 2. Among these, four cases were classified as G3a or higher according to the Clavien–Dindo classification (G4: 1 case of bleeding, G3a: 2 cases of surgical site infection, 1 case of empyema). The surgery-related mortality rate for pleural biopsy was 0%.

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Table 1. Patient characteristics.

Patient characteristic		N
Age (years)	Median (range) 71.5 (53-93)	
Sex	male	46
	female	4
Laterality	right	31
	left	19
Asbestos exposure	occupational	27
	environmental	9
	both	2
	possible	4
	none	8
Family history of MPM	present	2
	none	48
Symptoms	cough	9
	dyspnea	26
	chest pain	10
	fever	2
	other	2
	none	9

MPM: malignant pleural mesothelioma.

Table 2. Distribution of adverse events related to pleural biopsy.

Clavien–Dindo classification	G1-2	G3a	G3b	G4a	G4b	G5
Surgical site of infection		2				
Bleeding				1		
Empyema		1				
Dehydration	1					
Subcutaneous emphysema	1					
Delirium	1					

Diagnosis results from pleural effusion cytology and histology are presented in Table 3. Pleural effusion cytology led to the diagnosis of PM in 29 cases. Other diagnoses included 1 case with lung adenocarcinoma, 7 cases with atypical cells, and 13 cases with no atypical cells. Histological examination revealed that 42 cases were PM, with 36 cases of the epithelioid type, 4 cases of the biphasic type, and 2 cases of the sarcomatoid type. Atypical mesothelial cells were observed only in the pleural surface layer in 1 case; fibrous pleuritis was seen in 5 cases, lung adenocarcinoma in 1 case, and granulomatous pleurisy in 1 case.

Table 3. Results of pleural effusion examination and pleural biopsy.

Cytological examination		
PM		29
Lung cancer (adenocarcinoma)		1
Atypical mesothelial cell		5
Atypical cell		2
No atypical mesothelial cell		13
Histological examination		
Pleural mesothelioma		42
	Epithelioid	(36)
	Biphasic	(4)
	Sarcomatoid	(2)

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Atypical mesothelial cell ln the surface	1
Lung cancer (adenocarcinoma)	1
Fibrous pleuritis	5
Granulomatous pleuritis	1

PM: pleural mesothelioma.

Table 4 shows a comparison of the results between pleural effusion cytology and histology. All 29 cases diagnosed as PM by pleural effusion cytology were also diagnosed with PM by histology. Furthermore, in all eight cases where PM was not diagnosed by histological examination, PM was not diagnosed by pleural effusion cytology. Consequently, the specificity of this test was 100% (8/8). All seven cases diagnosed with atypical cells by pleural effusion cytology were confirmed as PM by histology. On the other hand, among the 13 patients diagnosed with no atypical cells by pleural effusion cytology, 6 were diagnosed with PM by histology, and 1 was diagnosed with atypical mesothelial cells on the surface. Five patients were identified as having fibrous pleuritis, and the remaining one had granulomatous pleurisy. Thus, the sensitivity of pleural effusion cytology in diagnosing PM was 69.0% (29/42). When examining the diagnostic concordance rate by pleural effusion cytology for each histological type, it was 72.2% (26/36) for the epithelioid type, 75% (3/4) for the biphasic type, and 0% (0/2) for the sarcomatoid type.

Table 4. a comparison of the results between pleural effusion cytology and histology.

Pleural effusion cytology	Pleural biopsy	Number
PM	PM	29
Atypical cell	PM	7
No atypical cell	PM	6
No atypical cell	fibrous pleuritis	6
No atypical cell	atypical cells on the surface	1
Aadenocarcinoma	adenocarcinoma	1

PM: malignant pleural mesothelioma.

Table 5 provides details on the 13 cases where PM was diagnosed by histological examination but diagnosing PM via pleural effusion cytology was challenging. Among the seven cases with morphologically atypical cells in pleural effusion cytology, two retained expressions of MTAP and BAP1. In four cases, evaluation was inconclusive due to poor staining of MTAP and BAP1. The remaining case could not be evaluated adequately due to the small number of atypical cells. Conversely, in the six cases where no atypical cells were identified in pleural effusion cytology, assessment was not possible because there were no cells available for evaluation via immunohistochemical staining. Of these six cases, three had bloody pleural effusion.

Table 5. Reasons for false-negative results in cytological examination.

	Cytological diagnosis	Subtype	Reason
1	Atypical mesothelial cells	E	BAP1(+) and MTAP(+)
2	Atypical mesothelial cells	E	BAP1(+) and MTAP(+)
3	Atypical cells	S	unable to evaluate the staining of BAP1 and MTAP
4	Atypical cells	E	unable to evaluate the staining of BAP1 and MTAP
5	Atypical mesothelial cells	E	unable to evaluate the staining of BAP1 and MTAP
6	Atypical mesothelial cells	E	unable to evaluate the staining of BAP1 and MTAP
7	Atypical mesothelial cells	E	unable to evaluate the immunohistochemical staining due to few cells
8	No mesothelial cells	E	unable to evaluate the immunohistochemical staining due to few cells
9	No mesothelial cells	E	unable to evaluate the immunohistochemical staining due to few cells

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10	No mesothelial cells	S	unable to evaluate the immunohistochemical staining due to few cells
11	No mesothelial cells	E	unable to evaluate the immunohistochemical staining due to few cells
12	No mesothelial cells	B	unable to evaluate the immunohistochemical staining due to few cells
13	No mesothelial cells	E	unable to evaluate the immunohistochemical staining due to few cells

E: epithelioid mesothelioma, B: biphasic mesothelioma, S: sarcomatoid mesothelioma.

Discussion

In this study involving 50 patients with suspected PM, the sensitivity of pleural effusion cytology for diagnosing PM was 69% and the specificity was 100%. The primary endpoint of achieving 100% specificity was met, indicating that the diagnostic criteria for PM in pleural effusion cytology used in this study are highly reliable. These findings suggest that, with detailed pleural effusion cytology, a definitive diagnosis of PM may be possible without the need for histological examination.

In this study, despite achieving 100% specificity, the sensitivity was relatively low at 69%. Two potential reasons for the false-negative pleural effusion cytology results are as follows. First, the diagnostic criteria for PM in this study included the presence of at least one of the markers indicating loss of MTAP and loss of BAP1 expression. While these markers are known to enhance diagnostic accuracy [10–14,18–20], there are reported cases of PM where both MTAP and BAP1 are retained [19,21]. Cases meeting this criterion may not have been captured by the diagnostic criteria, leading to false negatives in pleural effusion cytology. Second, the nature of the pleural effusion itself could contribute to false negatives. Cases with few atypical cells or the presence of a major amount of blood components may limit the effectiveness of immunohistochemical staining on the cell block, resulting in a negative outcome. In this study, 7 of 13 false-negative cases had these characteristics. The properties of the specimen influence the reliability of pleural effusion cytology, and in cases where pleural effusion cytology faces challenges in providing a conclusive diagnosis, pleural biopsy is recommended without hesitation, especially when MPM is clinically suspected.

In this study, the diagnostic accuracy of pleural effusion cytology was investigated for each histological type of PM. The incidence was 67% (26/36) for epithelioid type and 75% (3/4) for biphasic type, but 0% (0/2) for sarcomatoid type. This is speculated to be because sarcomatoid mesothelioma is rarely accompanied by pleural effusion and because the absolute number of atypical cells in the pleural effusion is small. While three of four cases of biphasic mesothelioma in this study were diagnosed as PM by pleural effusion examination, all were presumed to be epithelioid mesothelioma. Determining the histological type based on pleural effusion cytology alone, especially in identifying sarcomatoid components, is challenging. If a detailed histological subtype is necessary to determine the treatment approach, pleural biopsy should be pursued immediately.

In this prospective clinical trial, although there were no deaths, 7 patients (14%) experienced adverse events associated with pleural biopsy, and 1 patient (2%) had serious complications. On the other hand, no adverse events were reported during thoracentesis, and obtaining a definitive diagnosis from pleural effusion cytology alone could potentially markedly reduce complications associated with pleural biopsy. PM is known for its propensity for tumor invasion at biopsy and drainage sites due to its strongly invasive nature, and this risk is correlated with the size of the wound [9]. If it becomes possible to diagnose PM using pleural effusion cytology alone, the risk of tumor invasion at the biopsy site is expected to be predominantly reduced. Furthermore, obtaining a definitive diagnosis through pleural effusion cytology alone allows for prompt initiation of treatment, as pleural biopsy is not required. These potential advantages are beneficial for patients.

This research has some limitations. While the study was prospectively designed, it was conducted only at three institutions with extensive experience in diagnosing and treating MPM. Therefore, it is crucial to verify whether the diagnostic criteria used in this study are reproducible at other facilities and are appropriate. A nationwide prospective clinical trial, called MesoCyto 2, is being planned to address this. The aim is to assess the universality of the diagnostic method and the

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uniformity of diagnostic accuracy and demonstrate that MPM diagnosis is possible with pleural effusion cytology alone across different settings.

Conclusions

Based on this prospective multicentric study revealed that PM can potentially be diagnosed through only pleural effusion cytology including the cell block method using immunohistochemical staining.

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Institutional Review Board Statement: The study adhered to the principles outlined in the Declaration of Helsinki and the Japanese Clinical Trials Act and is registered with UMIN (UMIN000038709). The research protocol received approval from the ethics committees of each participating facility (Hyogo University of Medicine: #3387, Fukuoka University: H20-03-009, University of Occupational and Occupational Health: UOEHCRB20-140; Date of approval: 28 November 2019).

Informed Consent Statement: All participating patients provided informed consent prior to their involvement in the study.

Data Availability Statement: The datasets used or analyzed in the current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: None.

Abbreviations

BAP1	**BRCA1-associated protein-1**
MTAP	**Methylthioadenosine Phosphorylase**
HE	**hematoxylin/eosin**

References

1. Hasegawa S, Tanaka F. Malignant mesothelioma: current status and perspective in Japan and the world. *Gen Thorac Cardiovasc Surg*. 2008;56:317–323.
2. Woolhouse I, Bishop L, Darlison L, De Fonseka D, Edey A, Edwards J, Faivre-Finn C, Fennell DA, Holmes S, Kerr KM, Nakas A, Peel T, Rahman NM, Slade M, Steele J, Tsim S, Maskell NA. British Thoracic Society Guideline for the investigation and management of malignant pleural mesothelioma. Thorax. 2018 Mar;73(Suppl 1):i1-i30.
3. Hjerpe A, Ascoli V, Bedrossian C, Boon M, Creaney J, Davidson B, Dejmek A, Dobra K, Fassina A, Field A, Firat P, Kamei T, Kobayashi T, Michael CW, Önder S, Segal A, Vielh P. Guidelines for cytopathologic diagnosis of epithelioid and mixed type malignant mesothelioma. Complementary statement from the International Mesothelioma Interest Group, also endorsed by the International Academy of Cytology and the Papanicolaou Society of Cytopathology. Cytojournal. 2015 Nov 30;12:26.
4. Husain AN, Colby TV, Ordóñez NG, Allen TC, Attanoos RL, Beasley MB, Butnor KJ, Chirieac LR, Churg AM, Dacic S, Galateau-Sallé F, Gibbs A, Gown AM, Krausz T, Litzky LA, Marchevsky A, Nicholson AG, Roggli VL, Sharma AK, Travis WD, Walts AE, Wick MR. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. Arch Pathol Lab Med. 2018 Jan;142(1):89-108.
5. Hjerpe A, Abd-Own S, Dobra K. Cytopathologic diagnosis of epithelioid and mixed-type malignant mesothelioma: ten years of clinical experience in relation to international guidelines. *Arch Pathol Lab Med*. 2018;142:893–901.
6. Opitz I, Scherpereel A, Berghmans T, Psallidas I, Glatzer M, Rigau D, Astoul P, Bölükbas S, Boyd J, Coolen J, De Bondt C, De Ruysscher D, Durieux V, Faivre-Finn C, Fennell DA, Galateau-Salle F, Greillier L, Hoda MA, Klepetko W, Lacourt A, McElnay P, Maskell NA, Mutti L, Pairon JC, Van Schil P, van Meerbeeck JP,

Waller D, Weder W, Putora PM, Cardillo G. ERS/ESTS/EACTS/ESTRO guidelines for the management of malignant pleural mesothelioma. Eur J Cardiothorac Surg. 2020 Jul 1;58(1):1-24.
7. Iaffaldano A, Charrier T, Lococo F, Damotte D, Bobbio A, Alifano M, Fournel L. Surgical Diagnosis of Malignant Pleural Mesothelioma: 20 Years' Experience at a High-Volume Referral Center. J Clin Med. 2021 May 4;10(9):1973.
8. Agarwal PP, Seely JM, Matzinger FR, MacRae RM, Peterson RA, Maziak DE, Dennie CJ. Pleural mesothelioma: sensitivity and incidence of needle track seeding after image-guided biopsy versus surgical biopsy. Radiology. 2006 Nov;241(2):589-94.
9. Hashimoto M, Yuki M, Kitajima K, Fukuda A, Nakamichi T, Nakamura A, Kuroda A, Matsumoto S, Kondo N, Sato A, Yamakado K, Tsujimura T, Hasegawa S. Incidence and Risk Factors of Chest Wall Metastasis at Biopsy Sites in Patients with Malignant Pleural Mesothelioma. Cancers (Basel). 2022 Sep 7;14(18):4356.
10. Hiroshima K, Wu D, Hamakawa S, Tsuruoka S, Ozaki D, Orikasa H, Hasegawa M, Koh E, Sekine Y, Yonemori Y, Nabeshima K, Tsuji S, Miyagi Y, Imai K. HEG1, BAP1, and MTAP are useful in cytologic diagnosis of malignant mesothelioma with effusion. Diagn Cytopathol. 2021 May;49(5):622-632.
11. Kinoshita Y, Hida T, Hamasaki M, Matsumoto S, Sato A, Tsujimura T, Kawahara K, Hiroshima K, Oda Y, Nabeshima K. A combination of MTAP and BAP1 immunohistochemistry in pleural effusion cytology for the diagnosis of mesothelioma. Cancer Cytopathol. 2018 Jan;126(1):54-63.
12. Berg KB, Churg AM, Cheung S, Dacic S. Usefulness of methylthioadenosine phosphorylase and BRCA-associated protein 1 immunohistochemistry in the diagnosis of malignant mesothelioma in effusion cytology specimens. *Cancer Cytopathol*. 2020;128:126–132.
13. Hatem L, McIntire PJ, He B, Gogineni S, Ho M, Mathew S, Margolskee EM, Siddiqui MT, Rao RA. The role of BRCA1-associated protein 1 in the diagnosis of malignant mesothelioma in effusion and fine-needle aspiration cytology. Diagn Cytopathol. 2019 Mar;47(3):160-165.
14. Ozdemir HG, Kokten SC, Barisik NO. Can BAP1 expression loss in mesothelial cells be an indicator of malignancy? *J Pathol Transl Med*. 2020;54:497–503.
15. Girolami I, Lucenteforte E, Eccher A, Marletta S, Brunelli M, Graziano P, Pisapia P, Malapelle U, Troncone G, Scarpa A, Huang T, Pantanowitz L. Evidence-based diagnostic performance of novel biomarkers for the diagnosis of malignant mesothelioma in effusion cytology. Cancer Cytopathol. 2022 Feb;130(2):96-109.
16. Hashimoto M, Sato A, Kuroda A, Nakamura A, Nakamichi T, Kondo N, Yuki M, Nabeshima K, Tsujimura T, Hasegawa S. Clinical feature of diagnostic challenging cases for pleural biopsy in patient with malignant pleural mesothelioma. Gen Thorac Cardiovasc Surg. 2020 Aug;68(8):820-827.
17. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, de Santibañes E, Pekolj J, Slankamenac K, Bassi C, Graf R, Vonlanthen R, Padbury R, Cameron JL, Makuuchi M. The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg. 2009 Aug;250(2):187-96.
18. Jones TE, Geisler DL, Baskota SU, Ohori NP, Cuda J, Khader SN. Reliably making the primary diagnosis of mesothelioma utilizing serous fluid cytology specimens: an institutional experience. J Am Soc Cytopathol. 2024 May-Jun;13(3):174-182.
19. Şeker NS, Tekin E, Ak G, Metintaş M, Metintaş S, Dündar E. Investigation of MTAP and BAP1 staining loss and P16/CDKN2A deletion in pleural cytology specimens and its role in the diagnosis of mesothelioma. *Diagn. Cytopathol*. 2024;52:211–216..
20. Mansour MSI, Huseinzade A, Seidal T, Hejny K, Maty A, Taheri-Eilagh F, Mager U, Dejmek A, Dobra K, Brunnström H. Comparison of immunohistochemical mesothelial biomarkers in paired biopsies and effusion cytology cell blocks from pleural mesothelioma. Cytopathology. 2023 Sep;34(5):456-465.
21. Kinoshita Y, Hamasaki M, Yoshimura M, Matsumoto S, Iwasaki A, Nabeshima K. Hemizygous loss of NF2 detected by fluorescence in situ hybridization is useful for the diagnosis of malignant pleural mesothelioma. *Mod Pathol*. 2020;33:235–244.