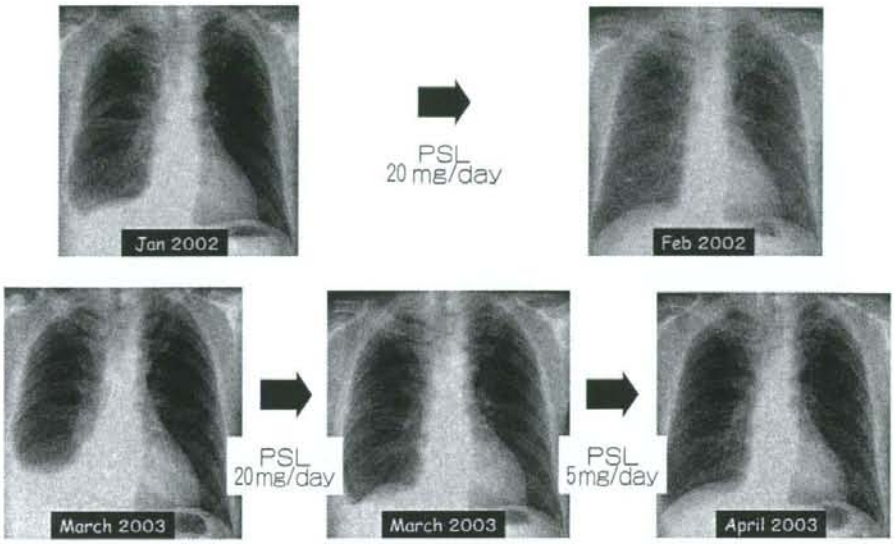

図 4　2002年から2003年までの胸部単純X線所見の変化
右胸水はプレドニゾロンの内服で軽快を繰り返したため，良性石綿胸水であると診断した。

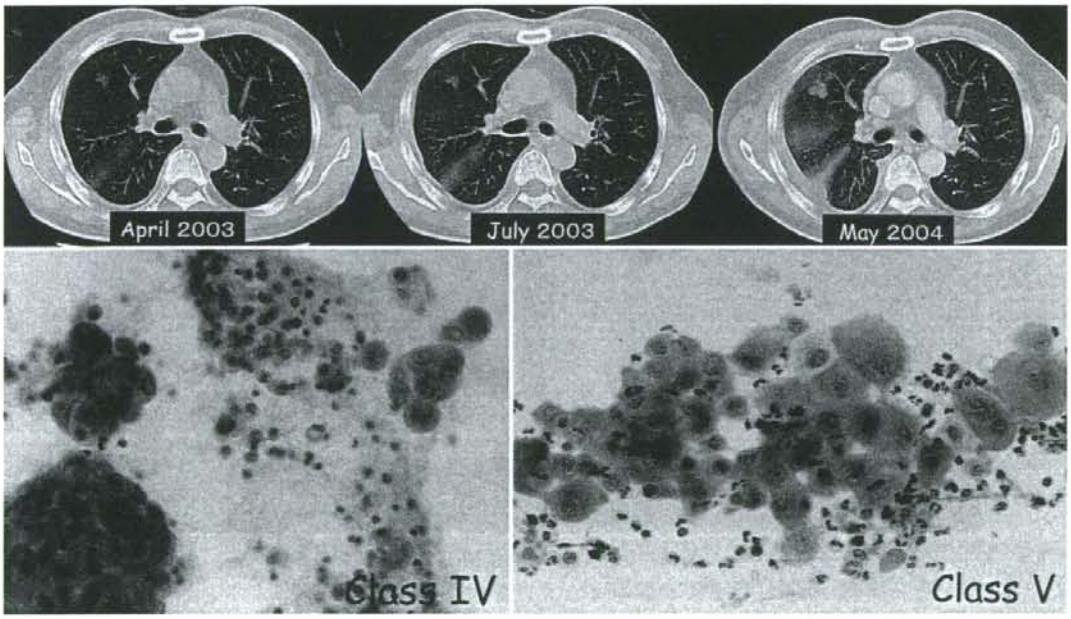

図 5　2003～2004年の胸部CT画像の変化と細胞診像
2003年7月から葉間胸膜の肥厚が目立ち始めるとともに胸水に中皮腫を示唆する大型の異型細胞が出現するようになった。

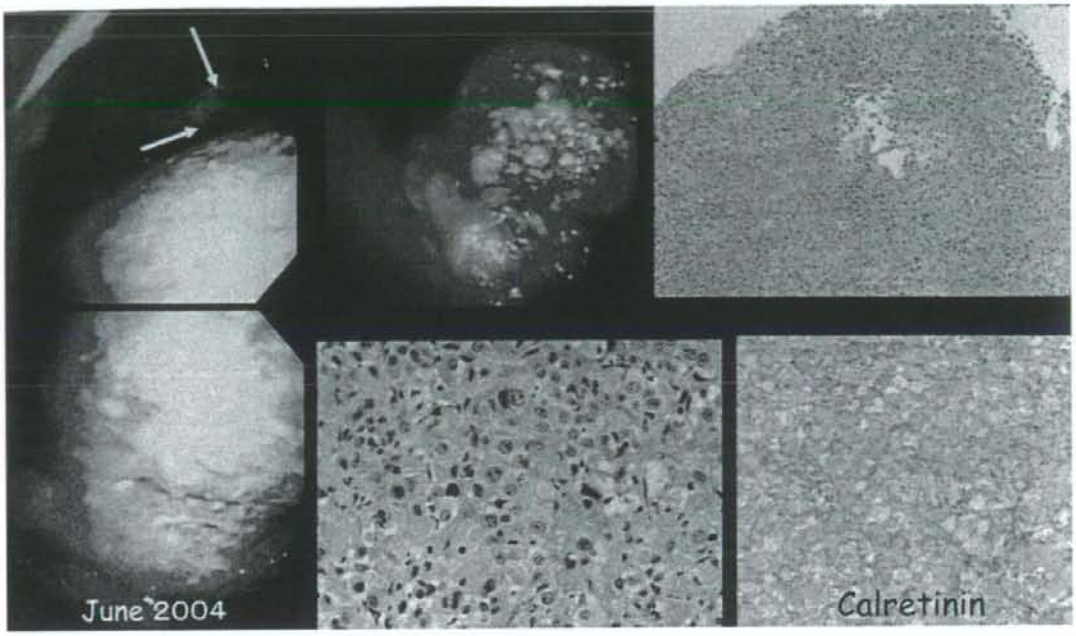

図 6　2004年の局所麻酔下胸腔鏡肉眼像と組織所見
壁側および臓側胸膜は腫瘍性肥厚を示すとともに胸膜プラーク（→）を認める。
病理組織所見ではカルレチニンが濃染する腫瘍細胞が認められ，上皮型中皮腫であると診断した。

たが，胸部画像上に異常な肥厚像はなく，プレドニゾロンの内服のみで胸水の消失が得られた（**図4**）。しかし，2003年4月からは右葉間胸膜の肥厚像（**図5**）が認められるようになったため，2004年6月に局所麻酔下胸腔鏡検査を行った。その結果は**図6**に示すような不整な隆起病変を認め，胸膜中皮腫に一致した所見であった。そして，生検組織より上皮型胸膜中皮腫の確定診断を得た。一方，胸部CT上では認めることができなかった胸膜プラークを肉眼的に認めた（→）。この時点でのstagingはIMIG分類によるとT3N0M0のstage IIIであったが，2004年7月に胸膜肺全摘出術を行った。術後の病理学的所見で中皮腫は確定され，stagingでは心膜側での脂肪織への浸潤を認めていた（**図7**）。そのため，6カ月後腫瘍性心膜炎で再発した。心膜ドレナージとともにシスプラチンの局所注入療法で軽快した。その後，左胸膜あるいは腹膜に再発を来したため，現在はビノレルビン＋ゲムシタビンの併用療法で病状はコントロール可能である。本症例の場合，どの時点で中皮腫の発症があったかをレトロスペクティブに決定することは難しいが，このような症例は少なくないものと思われる。

中皮腫症例では初診時から不規則なびまん性胸膜肥厚を伴う典型像を示す症例がある。しかし，本症例のように，胸水貯留のみで腫瘤を形成しない場合もあるので，石綿曝露歴のある症例では早期に胸腔鏡を行い，確定診断できない場合でも臨床経過が中皮腫を疑う場合には再度胸腔鏡検査を行い，診断を確定

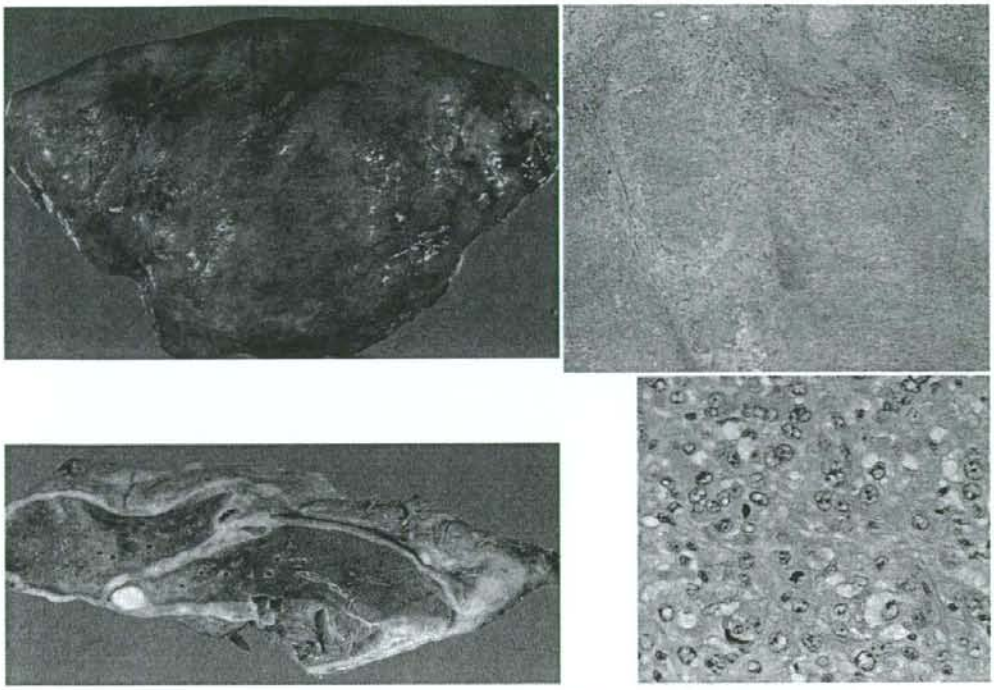

図 7　胸膜肺全摘出術により摘出した胸膜肺の肉眼所見と病理組織像
肉眼的に中皮腫は臓側胸膜から一部肺に浸潤していた。腫瘍細胞は胸腔鏡下生検で得られた上皮型中皮腫細胞のみで，肉腫成分は認められなかった。

する必要があると思われる。

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A Useful Antibody Panel for Differential Diagnosis Between Peritoneal Mesothelioma and Ovarian Serous Carcinoma in Japanese Cases

Yukio Takeshima, MD, Vishwa J. Amatya, MD, Kei Kushitani, MD, and Kouki Inai, MD

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Abstract

Malignant mesothelioma is increasing in incidence worldwide, including in Japan. However, the accurate pathologic diagnosis of pleural or peritoneal mesothelioma (PM) is sometimes difficult if adequate histologic and immunohistochemical analyses are not undertaken. The aim of this study was to identify a useful antibody panel for distinguishing PM from ovarian serous papillary adenocarcinoma (SC). We obtained 29 PMs (23 epithelioid mesotheliomas and 6 biphasic mesotheliomas) and 20 SCs from our surgical pathology files. Immunohistochemical analysis was undertaken using 13 commercially available antibodies. No significant sex differences in antigen expression among the 29 PMs were observed. The results identified calretinin and thrombomodulin as positive markers and Ber-EP4, MOC-31, CA19-9, and estrogen receptor as negative markers with relatively high sensitivity and specificity for the differential diagnosis of PM and SC. The combination of these positive and negative markers may contribute to accurate diagnosis and adequate therapy for PM and ovarian SC.

Malignant mesothelioma is a relatively rare tumor in Japan; however, its incidence has been increasing owing to the past importation and use of asbestos in Japan and other countries.[1-3]

The majority of mesotheliomas occur in the pleura, followed by the peritoneum, pericardium, and tunica vaginalis testis. The percentage of peritoneal mesotheliomas (PMs) is rather small, accounting for only 10% of all mesotheliomas.[4,5] The diagnosis of malignant mesothelioma is difficult owing to its rare frequency, histologic variety, and heterogeneity. Four histologic subtypes, including epithelioid, sarcomatoid, desmoplastic, and biphasic, are described in the 2004 World Health Organization classification.[6] Differential diagnosis depends on the tumor location and its histologic type. For example, pleural mesothelioma must be differentiated from pulmonary adenocarcinoma, pulmonary pleomorphic carcinoma, reactive mesothelial hyperplasia, fibrous pleuritis, and "true" sarcoma invading the pleura and chest wall.

On the other hand, PM is often disseminated in the peritoneal cavity with formation of multiple nodules and ascites, occasionally forming a localized abdominal mass, including in the ovaries.[7] Therefore, serous papillary adenocarcinoma (SC) of ovarian or peritoneal origin[8] is the most important and difficult malignant tumor from which it must be differentiated owing to these clinical and histologic similarities.[9] The differential diagnosis of these tumors from PM is important because chemotherapy and/or radiotherapy can significantly improve patient survival and decrease recurrence, especially for primary and secondary SC.[10-12]

There are a relatively large number of immunohistochemical studies of PM focused on differential diagnosis from pulmonary adenocarcinoma[13-17]; however, immunohistochemical

studies on the differential diagnosis of PM and ovarian carcinoma are limited[18-22] because of the rare frequency of PM.

In this study, therefore, immunohistochemical analyses were conducted using commercially available antibodies to elucidate the usefulness of immunohistochemical analysis for the differential diagnosis of PM and ovarian SC in Japanese cases.

Materials and Methods

We selected 29 cases of PM, including 23 of the epithelioid type and 6 of the biphasic type (from 23 men and 6 women), and 20 cases of ovarian SC, collected from 1987 to 2007, from the surgical archives of the Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan. The average patient age was 63 years (range, 22-84 years) for men with mesothelioma, 50 years (range, 22-78 years) for women with mesothelioma, and 59 years (range, 23-84 years) for women with ovarian SC. The diagnosis in each case was based on the recommended criteria listed in the 2004 World Health Organization classification.[6] All of the patients were Japanese.

Immunohistochemical staining of sections from formalin-fixed, paraffin-embedded tissue samples was performed using the streptavidin-biotin-peroxidase method (SAB) with the Histofine SAB-PO kit (Nichirei, Tokyo, Japan) with or without antigen retrieval. The list of primary antibodies, including the 13 antibodies, clone, source, dilution, and antigen retrieval, is shown in ▮Table 1▮.

The scoring of immunohistochemical staining was semiquantitative as follows: 0, no or trace staining; 1+, fewer than 25% of tumor cells positive; 2+, 26% to 50% of tumor cells positive; and 3+, 51% or more of tumor cells positive. The definition of a positive case in this study is a case having a score of 1+, 2+, or 3+. The scoring was performed on the epithelioid component of the epithelioid and biphasic mesotheliomas. The immunohistochemical evaluation for the sarcomatoid component of the biphasic mesotheliomas was excluded in this study. The abbreviation "PM" indicated in the present study includes epithelioid mesothelioma and the epithelioid component of biphasic mesothelioma.

Statistical analyses were performed by using the Fisher exact test and Mann-Whitney U test. Sensitivity and specificity were calculated for each marker by using a simple 2 × 2 table.

Results

Positivity of Antibodies for PM and Ovarian SC

The positive results of each antigen for PM and ovarian SC are given in ▮Table 2▮. Representative immunohistochemical staining panels for PM and SC are shown in ▮Image 1▮ and ▮Image 2▮. The staining pattern for each antibody for the 2 tumor types is described in brief in the following paragraphs.

Calretinin

All PM cases were positive, with most in the 3+ staining category. The staining pattern in the PM cases was fairly strong staining in the nucleus and weak in the cytoplasm. The SC cases showed the same staining pattern as the PM cases; however, most of the positive cases had a lower staining score (ie, 1+ or 2+).

D2-40

PM cases and SC cases showed an intense membranous staining pattern. All positive cases of PM had a high

▮Table 1▮
Antibodies Used in the Study

Antibody	Source	Clone	Pretreatment	Dilution
Calretinin	Zymed, San Francisco, CA	Polyclonal	Autoclave	1:50
D2-40	Nichirei BioScience, Tokyo, Japan	D2-40	Autoclave	Prediluted
WT1	DAKO, Glostrup, Denmark	6F-H2	Autoclave	1:400
Thrombomodulin	DAKO	1009	None	1:500
CK5/6	DAKO	D5/16B4	Autoclave	1:50
Mesothelin	Novocastra, Newcastle upon Tyne, England	5B2	Autoclave	1:20
Ber-EP4	DAKO	Ber-EP4	None	1:100
MOC-31	DAKO	MOC-31	None	1:50
CD15	DAKO	C3D-1	Autoclave	1:30
CA19-9	TFB, Tokyo, Japan	NS19-9	Autoclave	Prediluted
CEA	Nichirei BioScience	COL-1	Autoclave	Prediluted
h-Caldesmon	DAKO	H-CD	Microwave	1:50
ER	DAKO	1D5	Autoclave	1:75

CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor.

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Table 2
Immunohistochemical Findings for Mesothelioma and Ovarian Serous Carcinomas for Various Antibodies

	Peritoneal Mesothelioma					Serous Papillary Adenocarcinoma						
		Staining Grade*					Staining Grade*					
Marker	No./Total (%)	0	1+	2+	3+	No./Total (%)	0	1+	2+	3+	$P^{\dagger}$	$P^{\ddagger}$
Calretinin	29/29 (100)	0	2	1	26	8/20 (40)	12	5	3	0	<.0001	<.0001
D2-40	22/23 (96)	1	0	8	14	9/20 (45)	11	7	2	0	.002	<.0001
WT1	22/24 (92)	2	8	3	11	18/20 (90)	2	1	10	7	.623	.741
Thrombomodulin	21/22 (95)	1	11	7	3	1/20 (5)	19	1	0	0	<.0001	<.0001
CK5/6	19/21 (90)	2	6	6	7	16/20 (80)	4	9	4	3	.307	.040
Mesothelin	20/20 (100)	0	2	3	15	19/20 (95)	1	1	3	15	.5	.972
Ber-EP4	0/21 (0)	21	0	0	0	20/20 (100)	0	2	5	13	<.0001	<.0001
MOC-31	1/19 (5)	18	1	0	0	18/20 (90)	2	3	7	8	<.0001	<.0001
CD15	0/12 (0)	12	0	0	0	12/20 (60)	8	10	2	0	.012	<.001
CA19-9	3/23 (13)	20	3	0	0	16/20 (80)	4	9	4	3	<.0001	<.0001
CEA	0/29 (0)	29	0	0	0	7/20 (35)	13	5	0	2	<.001	<.001
h-Caldesmon	0/17 (0)	17	0	0	0	0/20 (0)	20	0	0	0	NA	NA
ER	0/15 (0)	15	0	0	0	16/20 (80)	4	1	5	10	<.0001	<.0001

CEA, carcinoembryonic antigen; CK, cytokeratin; ER, estrogen receptor; NA, not available.
* The scoring of immunohistochemical staining was semiquantitative, as follows: 0, no or trace staining; 1+, fewer than 25% of tumor cells positive; 2+, 26%-50% of tumor cells positive; and 3+, 51% or more of tumor cells positive.
† The difference in the positive rate between peritoneal mesothelioma and serous papillary adenocarcinoma for each antibody calculated by the Fisher exact test.
‡ The difference in the distribution of reactivity scores between peritoneal mesothelioma and serous papillary adenocarcinoma for each antibody calculated by the Mann-Whitney *U* test.

staining score (ie, 2+ or 3+); however, most positive cases of SC had a low score.

WT1

For WT1 staining, 90% or more of the PM and SC cases were positive. The staining location in each tumor was the nucleus. Little or trace cytoplasmic staining was observed in some positive cases.

Thrombomodulin

More than 90% of the PM cases showed a primarily membranous staining pattern. However, half of the positive cases had a 1+ staining score. Only 1 SC case showed focal membranous staining.

Cytokeratin 5/6

For cytokeratin (CK)5/6, 80% or more of the PM and SC cases were positive. The staining location was the cytoplasm.

Mesothelin

All PM cases and 19 (95%) of 20 SC cases were positive. The distribution of scores for PM and SC was similar. Most of the positive cases showed a membranous staining pattern.

Ber-EP4 and MOC-31

No PM case was positive for Ber-EP4, whereas all SC cases were positive. Most of the positive SC cases were graded 3+. Only 1 PM case was weakly positive for MOC-31, whereas 18 (90%) of 20 SC cases were positive for this antibody. The location of expression of both antigens was the cell membrane for both tumor types.

CA19-9, CD15, and Carcinoembryonic Antigen

Only 3 (13%) of 23 PM cases were focally or weakly positive (1+) for CA19-9, whereas 16 (80%) of 20 SC cases showed a cytoplasmic staining pattern for this antibody. No PM cases were positive for CD15 or carcinoembryonic antigen (CEA). For SC cases, the positive rates for these antibodies were 60% (12/20) and 35% (7/20), respectively. The staining location was the cytoplasm in both tumor types. Most of the CD15+ SC cases had a 1+ score.

Estrogen Receptor and h-Caldesmon

No PM cases were positive for estrogen receptor (ER), whereas 16 (80%) of 20 SC cases showed a strong nuclear staining pattern for ER. No cases were positive for h-caldesmon for either tumor type.

Statistical Results

The *P* values for the difference in positive rates (cases with a score of 1+, 2+, or 3+) by the Fisher exact test between PM and ovarian SC are shown in Table 2. Calretinin, D2-40, and thrombomodulin showed a significantly higher expression rate in PM; however, Ber-EP4, MOC-31, CA19-9, CD15, CEA, and ER showed significantly lower expression than in SC.

The *P* values according to the Mann-Whitney *U* test for differences in the distribution of staining grades between PM and SC for each antibody are also shown in Table 2. Nearly

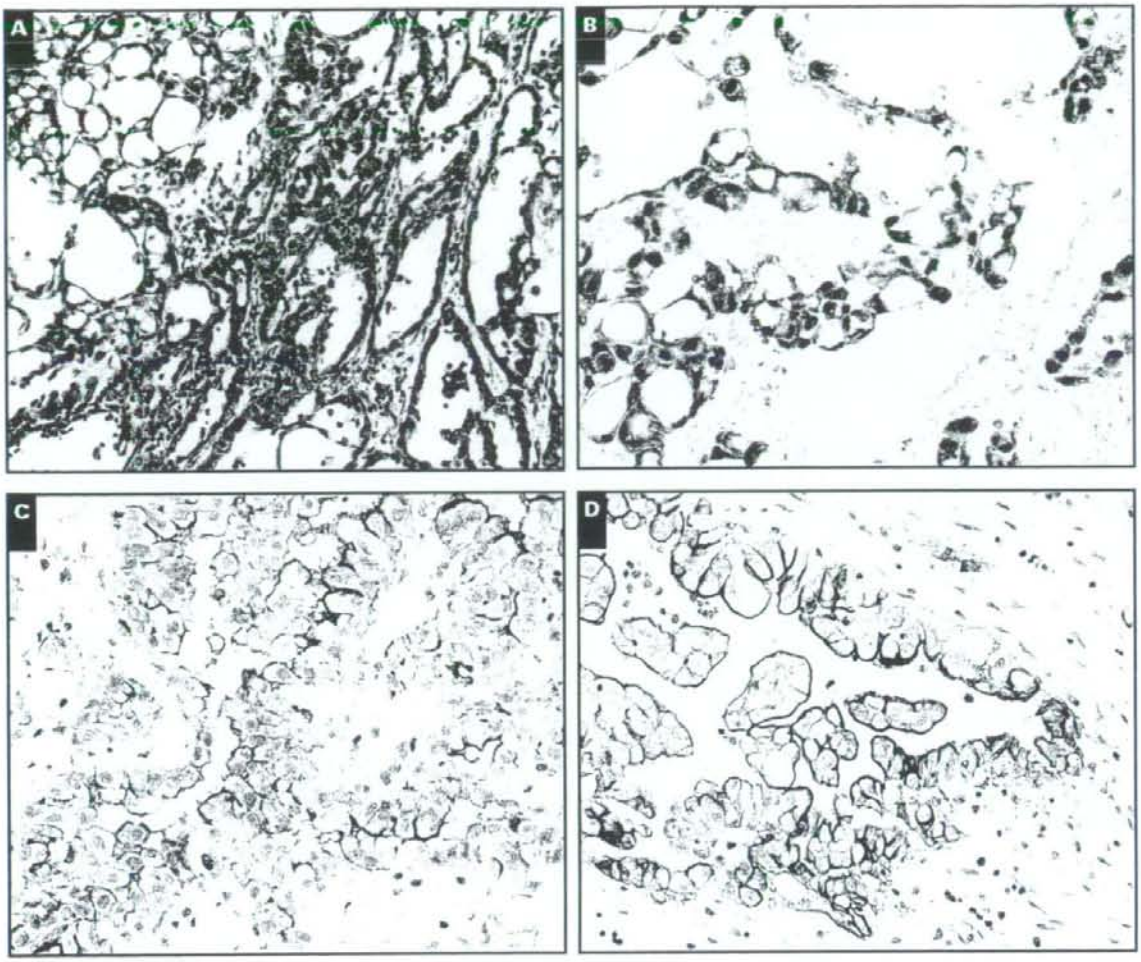

Image 1 Immunohistochemical staining panel of a peritoneal epithelioid mesothelioma in a 48-year-old woman. **A**, Epithelioid peritoneal mesothelioma showing a tubular growth pattern with microcystic change (H&E, ×200). **B**, Calretinin is expressed primarily in the nuclei and weakly in the cytoplasm (×200). **C** and **D**, D2-40 (**C**, ×200) and thrombomodulin (**D**, ×200) are expressed with a strong membranous staining pattern. **E**, WT1 is expressed strongly at the nuclei (×200). No estrogen receptor, Ber-EP4, or CA19-9 expression is shown in this tumor.

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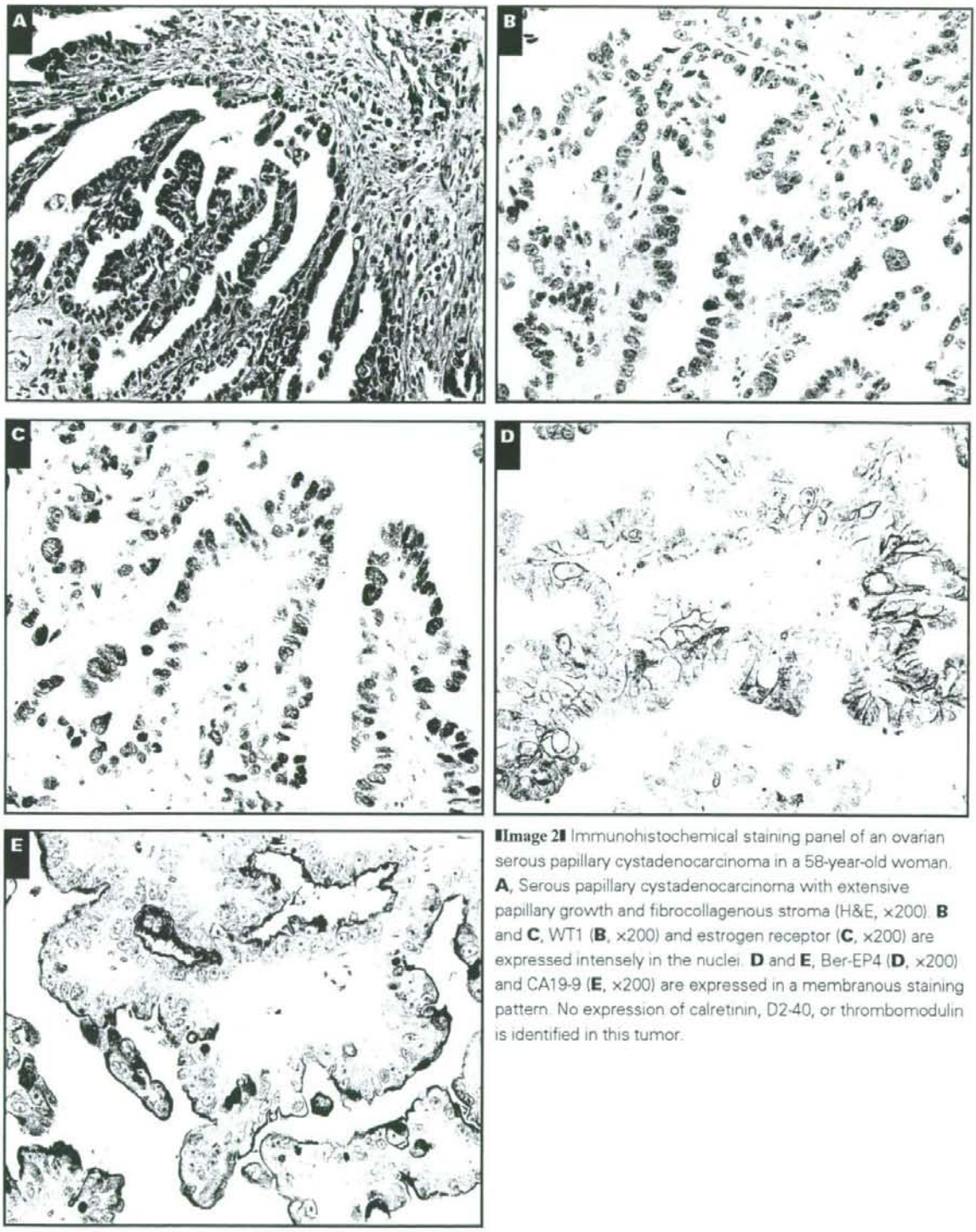

Image 2 Immunohistochemical staining panel of an ovarian serous papillary cystadenocarcinoma in a 58-year-old woman. **A**, Serous papillary cystadenocarcinoma with extensive papillary growth and fibrocollagenous stroma (H&E, ×200). **B** and **C**, WT1 (**B**, ×200) and estrogen receptor (**C**, ×200) are expressed intensely in the nuclei. **D** and **E**, Ber-EP4 (**D**, ×200) and CA19-9 (**E**, ×200) are expressed in a membranous staining pattern. No expression of calretinin, D2-40, or thrombomodulin is identified in this tumor.

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the same pattern of significant differences was observed among the antibodies as for the simple positive rate described in the preceding paragraph.

Sex Differences in Expression of Various Markers for PM

In this study, there was a relatively higher number of cases among men than among women (23 men and 6 women). Therefore, to exclude the possibility of sex bias in the comparisons between PM and SC, differences according to sex were analyzed. As indicated in ▮Table 3▮, no significant sex differences were observed for any antibody, although the number of cases was limited.

Sensitivity and Specificity of Each Antibody for Differential Diagnosis Between PM and Ovarian SC

The sensitivity and specificity of each antibody in terms of differential diagnosis between PM and ovarian SC cases are indicated in ▮Table 4▮. For SC, calretinin and thrombomodulin as positive markers and Ber-EP4, MOC-31, CA19-9, and ER as negative markers showed relatively high sensitivity and specificity (>60%). Although the negativity of CD15 showed the same sensitivity and specificity as calretinin, this antigen was excluded as a negative marker because many of the CD15+ SC cases had a low staining score.

▮Table 3▮
Sex Differences in Expression of Various Antibodies in Peritoneal Mesothelioma Cases*

	Male	Female	$P^{\dagger}$
Calretinin	23/23 (100)	6/6 (100)	NA
D2-40	17/18 (94)	5/5 (100)	.783
WT1	18/19 (95)	4/5 (90)	.38
Thrombomodulin	16/17 (94)	5/5 (100)	.772
Cytokeratin 5/6	14/16 (88)	5/5 (100)	.571
Mesothelin	15/15 (100)	5/5 (100)	NA
Ber-EP4	0/16 (0)	0/5 (0)	NA
MOC-31	1/14 (7)	0/5 (0)	.737
CD15	0/8 (0)	0/4 (0)	NA
CA19-9	2/18 (11)	1/5 (20)	.893
Carcinoembryonic antigen	0/23 (0)	0/6 (0)	NA
h-Caldesmon	0/13 (0)	0/4 (0)	NA
Estrogen receptor	0/11 (0)	0/4 (0)	NA

NA, not available
* Data are given as number/total (percentage).
† For sex differences in expression calculated by the Fisher exact test.

▮Table 4▮
Sensitivity and Specificity of Each Antibody for Differential Diagnosis Between Mesothelioma and Ovarian Serous Carcinoma

Marker	Sensitivity (%)	Specificity (%)
Calretinin+	100	60
D2-40+	95.7	55
WT1+	91.7	10
Thrombomodulin+	94.4	95
Cytokeratin 5/6+	90.5	20
Mesothelin+	100	5
Ber-EP4–	100	100
MOC-31–	94.7	90
CD15–	100	60
CA19-9–	87	80
Carcinoembryonic antigen–	100	35
h-Caldesmon–	100	0
Estrogen receptor–	100	80

Discussion

The accurate diagnosis of PM and exclusion of other peritoneal malignant tumors is sometimes difficult when pathologists are limited to observing histologic features such as H&E and classical mucin staining, even when associated with clinical information. This distinction is especially difficult because disseminated SC of the ovary and SC originating from the female peritoneum are among the most important malignant neoplasms to be differentiated.[9]

Baker et al[9] described the morphologic differences between PM and SC based on their experience with 75 PMs in females. Specifically, PM might show more papillary growth with prominent, often hyalinized stromal cores and have more conspicuous eosinophilic cytoplasm than SC. On the other hand, SC might have more frequent slit-like spaces and psammoma bodies, more hierarchical branching, cellular stratification and detached cell clusters, a greater degree of nuclear atypia, and more mitotic figures than PM. However, the differential diagnosis between PM and SC is difficult through purely histologic observations, especially when higher grades of these tumors are encountered.

Recently, many so-called mesothelial markers, including calretinin, D2-40, WT1, thrombomodulin, mesothelin, and others, have been certified.[23,24] However, to date, no mesothelioma-specific markers that are expressed only in mesothelioma cells have been identified. This fact indicates that a combination of positive and negative mesothelial markers is important to accurately diagnose mesothelioma. Several comparative immunohistochemical studies for differential diagnosis between PM and SC have been reported.[18-22,24,25] Attanoos et al[19] reported that calretinin and Ber-EP4 are useful discriminant markers for distinguishing PM in women from serous papillary ovarian and peritoneal carcinoma. They also indicated that thrombomodulin, cytokeratin 5/6, and CD44H as mesothelial markers and CEA and CD15 as carcinoma markers have too low a sensitivity for practical use.[19] Ordonez[24] reported that a combination of the best positive markers (D2-40 and calretinin) and negative markers (Ber-EP4 and MOC-31) is useful for discriminating between 2 tumors. Ordonez[20] and Barnetson et al[25] also reported that ER immunohistochemical analysis is very useful for the differential diagnosis owing to its high sensitivity and specificity,

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ie, high expression in serous carcinoma and no expression in epithelioid mesothelioma. In the present study, the combination of positive and negative markers, such as calretinin and thrombomodulin as positive markers and Ber-EP4, MOC-31, CA19-9, and ER as negative markers, was helpful.

Calretinin is a 29-kDa calcium-binding protein that is a member of the large family of EF-hand proteins and is expressed especially strongly in epithelioid mesotheliomas of the pleura or peritoneum.[26] Calretinin positivity in PM has been reported as 100%,[26] 88%,[19] 100%,[24] 100%,[22] and 100% (present study), and positivity in SC has been reported as 6%,[26] 0%,[19] 31%,[24] 12.5%,[22] and 40% (present study). The calretinin positivity of SC in the present study is the highest among this and the previous studies,[19,22,24,26] considering its sensitivity, specificity, and positive intensity. These discrepancies of positivity, especially for SC, may be due to differences in antigen-retrieval and immunoscoring methods. In comparing the degree of positivity between the 2 tumor types in the present study, the PM cases with positive scores were mainly 3+; however, the SC cases had a relatively lower score (ie, 1+ or 2+). By considering the positive rate and positive staining grade, calretinin could be a "positive marker" for differential diagnosis between PM and SC, even though the specificity was relatively low (ie, 60%).

Commercially available D2-40 has recently been used as an antibody that reacts with the 40-kDa antigen of the M2A oncofetal membrane antigen originally detected in germ cell neoplasia.[27] This antibody is also expressed in the lymphatic endothelium, so is applied to the study of lymphatic invasion in many tumors.[28,29] Chu et al[30] first described the usefulness of D2-40 in the diagnosis of mesothelioma, showing 100% positivity in epithelioid mesothelioma, 96% in reactive pleura, 7% in pulmonary adenocarcinoma, and 65% in ovarian serous carcinoma; they concluded that it is a useful positive marker for differential diagnosis between epithelioid mesothelioma and pulmonary adenocarcinoma. Its relatively high expression in ovarian serous adenocarcinoma may be related to the close histogenetic relationship between the mesothelium and serous lining cells of müllerian origin.[30] Ordonez[23,24] reported that 93% of PMs and 13% of SCs were positive for the D2-40 antibody and that the positive intensity in PM was higher than that in SC and concluded that D2-40 is a good discriminator between the 2 tumors. Comin et al[22] also reported that 100% of PMs and 40% of SCs were positive for D2-40 and concluded that D2-40 is a good marker, although with a slightly lower sensitivity and specificity than shown by h-caldesmon and calretinin.

The present study on D2-40 in PM and SC had almost the same results as these previous studies.[23,24] However, most PMs showed higher expression compared with SCs in terms of the positive staining pattern, and D2-40 was expressed with a focal or weak pattern in SC in the 1+ scoring category. The difference in expression pattern and the degree of expression between PM and SC may be useful for differential diagnosis. However, it was not possible to determine the usefulness of this antibody for diagnosis of PM and SC because of its relatively low specificity. Further studies are necessary to draw a definite conclusion.

WT1 has been reported to be expressed in rat and human mesotheliomas[31,32] and to be a very useful marker for differentiating between epithelioid mesothelioma and pulmonary adenocarcinoma.[15,17] Ordonez[15] summarized the positivity of epithelioid mesothelioma as 71% to 95% and pulmonary adenocarcinoma as 0% to 22% in a review article. He also summarized data for serous adenocarcinoma as having a positivity of 83% to 100%. In the present study, the positivity for WT1 was 22 (92%) of 24 cases for PM and 18 (90%) of 20 cases for SC. WT1 also showed an intense nuclear staining pattern in PM and SC. Therefore, this antibody is not useful for distinguishing between PM and SC. Acs et al[33] reported that WT1 expression was seen in 24 of 28 SCs, 4 of 18 clear cell adenocarcinomas, and in none of 11 endometrioid adenocarcinomas or 11 mucinous carcinomas. These results suggest that WT1 is differentially expressed depending on cellular differentiation among the tumors of the female genital tract, including the peritoneum.[33] Accordingly, WT1 could not be used for differential diagnosis between PM and SC, in contrast with use for differential diagnosis between pulmonary adenocarcinoma and pleural mesothelioma.[17]

Thrombomodulin, also known as CD141, is a relatively old positive marker for mesothelioma.[34] The positivity of thrombomodulin was reported as 33% to 73% in PM and 2% to 35% in SC in a review article by Ordonez.[24] Ordonez reported that 73% of PMs and 4% of SCs are positive for thrombomodulin and concluded that it is a less sensitive and more specific marker than calretinin or D2-40. In the present study, the positivity of thrombomodulin was 21 (96%) of 22 cases for PM and 1 (5%) of 20 cases for SC. These results also indicated that thrombomodulin is a relatively highly specific and sensitive marker for distinguishing between PM and SC compared with findings of other studies.[19,24] The disadvantage of this antibody is that most of the positive cases had a lower staining grade (ie, 1+, 11 cases; 2+, 7 cases). Although thrombomodulin is a classical positive marker with a relatively weak positive staining pattern, its usefulness as a positive marker is suggested by its high specificity.

CK5/6, a high-molecular-weight keratin, has been reported as a positive mesothelioma marker in differentiating from pulmonary adenocarcinoma,[13,15] although the data are not consistent in our experience.[17] The CK5/6+ percentage reported for PM is 53% to 100% and for SC is 24% to 31%.[18,19,21] Also, the present results showed no significant difference in staining pattern between PM and SC.

Ber-EP4 and MOC-31 have been recognized as epithelial cell adhesion molecules[35] and reported to be useful negative

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markers for differentiating between PM and SC.[24,25] The present study also indicated no or only focal Ber-EP4 or MOC-31 staining for PM and, on the other hand, very high intensity and dense staining for SC. Therefore, these antibodies are also recommended as negative markers.

Recently, ER has been reported as a significant negative marker for PM,[20,25] and it was reported that no case of PM but more than 85% of SC cases (87% by Ordonez[20] and 93% by Barnetson et al[25]) were positive for ER. The results for ER expression in the present study are similar to these previous reports.

The present study includes a relatively large percentage of cases among men; however, sex differences could be excluded based on the analysis of positive rates for each antibody (Table 3). Barnetson et al[25] also reported no significant differences in the expression pattern of various positive and negative markers, including hormone receptors, between peritoneal mesothelioma cases in males and females. Attanoos et al[19] reported mesothelial and epithelial markers in PM cases only in females. Ordonez[24] included 25 female cases among 40 PM cases; however, there was no discussion of sex related to the evaluation of the expression of various antigens, including ER and progesterone receptor.

CA19-9 is a classical adenocarcinoma marker.[36] Ordonez[21] reported that 31 (67%) of 45 SCs and 0 (0%) of 40 PMs were positive for this marker and concluded that the limiting factor for its practical use was its low sensitivity in SC. However, in the present study, only a weak staining pattern (1+) was observed in 3 (13%) of 23 PMs, compared with the relatively higher percentage of staining in 80% of SCs (16/20). These results indicate that CA19-9 could be used as a negative marker.

CD15, also known as Leu-M1, is also reported as a possible negative marker for PM or a positive marker for adenocarcinoma.[24] In the present study, no mesothelioma cases were positive; however, 12 (60%) of 20 SC cases were positive for this antibody, although most of the positive cases were in the 1+ staining category. This tendency was also observed in CEA immunostaining. The relatively lower specificity and lower staining degree of these adenocarcinoma markers may not be adequate for a differential diagnosis.

Recently, Comin et al[37] reported the usefulness of h-caldesmon for differential diagnosis of pleural epithelioid mesothelioma from pulmonary adenocarcinoma, as well as PM from SC involving the peritoneum.[22] Although this antibody was included in the present study, no mesotheliomas or SC showed positive staining. A preliminary study of 60 pleural epithelioid mesotheliomas in our department also showed no reactivity (data not shown). This discrepancy may be due to different immunohistochemical procedures or different case selection. Therefore, the usefulness of h-caldesmon could not be determined at this time.

Mesothelin is a known 40-kDa glycosyl-phosphatidylinositol–linked glycoprotein and a differentiation antigen[38] and is highly expressed in normal mesothelial cells and epithelioid mesotheliomas.[39] In this study, all PM cases and 19 (95%) of 20 SC cases were positive for mesothelin, suggesting no usefulness of this antibody for differential diagnosis. Ordonez[39] reported the same result.

Conclusions

Each of the antibodies evaluated in the present study has some weak points for differential diagnosis. A combination of positive and negative markers, such as calretinin and thrombomodulin as positive markers and Ber-EP4, MOC-31, CA19-9, and ER as negative markers, which showed relatively high sensitivity and specificity for differential diagnosis between PM and SC, may contribute to accurate diagnosis and adequate therapy. Recently, Davidson et al[40] reported differences in the gene expression profile by using microarray expression and the GeneChip technique between ovarian/peritoneal serous carcinoma and pleural and/or peritoneal mesothelioma. These genome-wide expression analyses will provide new potential markers for the differential diagnosis of PM and ovarian SC.

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Pathology of mesothelioma

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Abstract The incidence of mesothelioma has been gradually increasing in Japan, and the underlying factor for this is considered to be the increase in the amount of asbestos imported into Japan between 1960 and 1975. Mesothelioma can be roughly divided into localized and diffuse types, but the former is extremely rare. In making a diagnosis of mesothelioma, it is important to confirm the location of tumor and the specific gross findings before histological examination. Mesothelioma can be categorized histologically as epithelioid type, sarcomatoid type, biphasic type, desmoplastic type, among others. It can take many forms; consequently, there are many diseases to be differentiated when the diagnosis of mesothelioma is based on histological analyses. Immunohistochemical stains are useful for making a diagnosis, but the correct combination of antibodies as positive or negative markers should be selected and a comprehensive assessment of the staining results is necessary. The accuracy of the pathological diagnosis is very important to the patients because they can be receive official compensation or relief when the diagnosis of mesothelioma is confirmed. Under present conditions, both clinicians and pathologists must make a concerted effort to improve the accuracy of the diagnosis of mesothelioma.

Keywords Antibody · Diagnostic accuracy · Differential diagnosis · Immunohistochemistry · Mesothelioma

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Incidence of mesothelioma

Mesothelioma is a malignant tumor that originates in the pleura, peritoneum, pericardium and tunica vaginalis, all locations where a lining of normal mesothelial cell is present. According to the official data of Ministry of Health, Labor and Welfare in Japan, the number of death due to mesothelioma was 500 in 1995, increasing gradually to 953 in 2004. This increase appears to parallel the increase of the amount of asbestos imported into Japan between 1960 and 1975, given that the latent period from the beginning of exposure to asbestos and an initial diagnosis of mesothelioma is approximately 40 years.

More detailed examination of the 878 patients who officially died from mesothelioma in 2003 reveals that the male:female ratio was almost 3:1 and that the peak of age of patients was 60+ in males and 70+ in females. The location of the mesothelioma was in the pleura in 84% of the cases, in the peritoneum in 12% and in the pericardium in 1%; there were not cases of mesothelioma in the tunica vaginalis. A larger proportion of females had mesothelioma in the peritoneum and pericardium than in the pleura [1].

General findings on mesothelioma

Mesothelioma can be roughly classified into localized and diffuse types, with the incidence of the latter being much higher than that of the former [2]. The localized form of mesothelioma was more frequently diagnosed in the past; however, most of the localized forms are currently diagnosed as a solitary fibrous tumor [3], which is a separate entity from mesothelioma based on immunohistochemical

phenotyping (positive for CD34, a marker of primitive endothelial cell) and has no relation to asbestos exposure [4].

At the early stage of pleural mesothelioma, small nodules are found in the parietal pleura (not in the visceral pleura) that eventually extend along the pleural surface. Eventually, parietal and visceral pleurae show adhesion, and the tumor encloses the entire lung parenchyma. Very few cases of peritoneal mesothelioma have been reported at the early stage and, consequently, little is known in terms of pathology and disease progression during the early stage [5]. Most of peritoneal mesothelioma is found as a diffusely extensive tumor involving intestinal serosa or a large tumor located at the omentum or mesentery.

Following the initial diagnosis of mesothelioma, it is important to confirm the location of the tumor and its gross findings before histological examination. In the case of a pleural mesothelioma, a tumor in the lung parenchyma suggests lung cancer with a pleural extension. In females with peritoneal extension, the ovary should be carefully examined as the primary site of the tumor because the differential diagnosis between ovarian cancer and peritoneal mesothelioma is difficult and can only be made on the basis of histological analyses.

Histology of mesothelioma

Histological classification of mesothelioma is shown in Table 1 [6]. There are three major types—epithelioid type, sarcomatoid type and biphasic type—and the proportion of each is approximately 60, 20 and 20%, respectively (Figs. 1, 2, 3) [7]. The desmoplastic type is rare (probably 1–2%) (Fig. 4), and special variants only appear sporadically (several percentages). However, the proportion of each histological type varies among the reports because of the large variety of histological analyses used [8–11].

Table 1 Histological classification of mesothelioma [6]

1. Epithelioid mesothelioma
2. Sarcomatoid mesothelioma
(1) Desmoplastic mesothelioma
3. Biphasic mesothelioma
4. Variants
(1) Lymphohistiocytoid mesothelioma
(2) Deciduoid mesothelioma
(3) Anaplastic mesothelioma
(4) Well differentiated papillary mesothelioma
(5) Others

Differential diagnosis

It is important to remember that there are many diseases to be differentiated when making a pathological diagnosis and that the tissue to be differentiated varies in terms of histological type (Table 2). Epithelioid types must be differentiated from lung adenocarcinoma for pleural mesothelioma [12], ovarian serous papillary adenocarcinoma or peritoneal serous carcinoma for peritoneal mesothelioma [13]. In terms of sarcomatoid types, sarcoma originating in the chest wall, lung, pleura, abdominal wall, peritoneum and intestine must be excluded. Sarcomatoid carcinoma (spindle cell sarcoma or pleomorphic carcinoma) of the lung is very difficult to differentiate from sarcomatoid pleural mesothelioma [14]. In terms of the biphasic type, carcinosarcoma or pulmonary blastoma of the lung, biphasic synovial sarcoma [15] of the pleural mesothelioma and carcinosarcoma of the female genital organs must be differentiated from their peritoneal counterpart. The desmoplastic type has a similar histology to fibrous pleuritis [16], and the differential diagnosis is very difficult, particularly if only a small biopsy specimen is available. Reactive mesothelial hyperplasia associated with pleuritis or other lung or pleural disease must be differentiated from early-stage epithelioid mesothelioma [17].

Usefulness of immunohistochemistry in an accurate diagnosis

The histochemical staining of hyarulonic acid and electron microscopic studies have been widely used in the past for making a differential diagnosis between mesothelioma and other tumors. However, immunohistochemical stains are currently the method of choice because of the simplicity and ease of these techniques. Many antibodies have been detected for use in immunohistochemical staining techniques aimed at diagnosing mesothelioma, but as yet there is no antibody that is completely specific for mesothelioma and on which a pathological diagnosis of mesothelioma can be singly based. Therefore, the combination of a number of antibodies as positive or negative markers is important, and an assessment of the results in a comprehensive manner is necessary (Table 3).

In the case of epithelioid mesothelioma, calretinin, WT1, thrombomodulin, mesothelin and D2-40 can be applied as a mesothelial cell marker [18–22]. CEA, TTF-1, Napsin A and surfactant apoprotein are used as markers for lung adenocarcinoma. In the case of ovarian serous papillary adenocarcinoma, we recommend CEA, Ber-EP4, MOC-31 and ER (estrogen receptor) as positive markers [23].

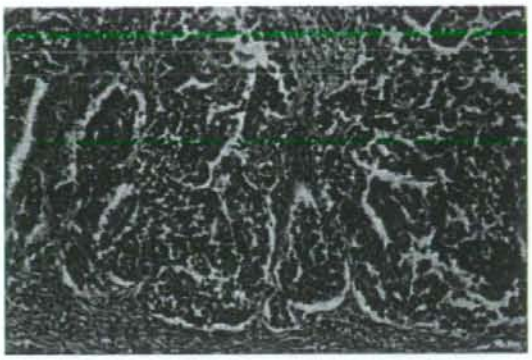

Fig. 1 Microphotograph of epithelioid mesothelioma (H&E stain). Papillo-tubular structure is prominent

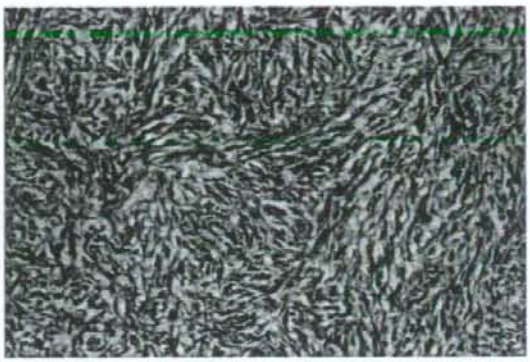

Fig. 2 Microphotograph of sarcomatoid mesothelioma (H&E stain). Proliferation of spindle cells mimies true sarcoma

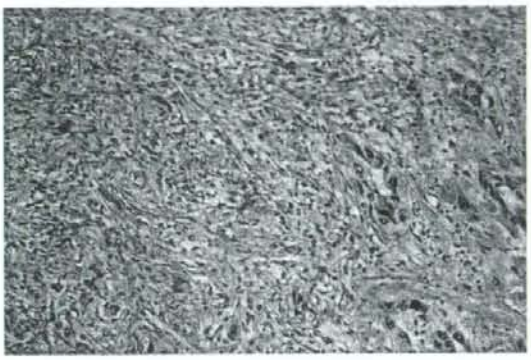

Fig. 3 Microphotograph of biphasic mesothelioma (H&E stain). The features of epithelioid mesothelioma and that of sarcomatoid mesothelioma are mixed within one tumor

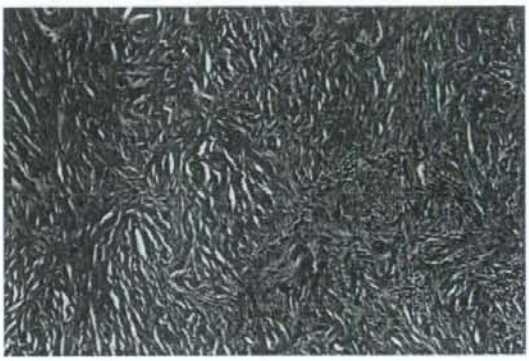

Fig. 4 Microphotograph of desmoplastic mesothelioma (H&E stain). The feature of granulation or fibrous pleuritis are dominant

The antibodies chosen for sarcomatoid mesothelioma are very different from those used for epithelioid mesothelioma. In the case of sarcomatoid mesothelioma, cytokeratin (AE1/AE3 or CAM5.2 as antibodies) exhibits a high specificity and is the most useful [24]. On the other hand, because the diagnosis for true sarcoma is based on the specific differentiation of tumor cells, mesothelioma is eliminated by making its differentiation clear. For example, the following antibodies are known to be useful: MyoD1, desmin and myoglobin for rhabdomyosarcoma; desmin, α-SMA and h-caldesmon for leiomyosarcoma; S-100p for malignant nerve sheath tumor; KP-1 for malignant fibrous histiocytoma [25].

The most difficult tumor to be differentiated from sarcomatoid mesothelioma of pleura is sarcomatoid carcinoma (spindle cell carcinoma, pleomorphic carcinoma) of the lung. When immunohistochemical stainings are used, both respond positively to cytokeratin [24]. In this case, therefore, the gross finding or the clinical diagnosis by imaging is very important, as already mentioned.

Immunohistochemical stains may be useful in differentiating between fibrous pleuritis and desmoplastic mesothelioma. Desmin is positive for spindle cells of the fibrous pleuritis, while desmin is negative for tumor cells of the sarcomatoid mesothelioma [26]. The combination of EMA, desmin and p53 is useful for differentiating between reactive mesothelial hyperplasia and early-stage epithelioid mesothelioma. Reactive mesothelial cells are positive for desmin and negative for EMA and p53 [27].

Compensation or relief of patients

In the compensation system for occupational exposures to asbestos and in the new law for non-occupational exposure to asbestos, if the diagnosis of mesothelioma is certain, it

Table 2 Differential diagnosis of mesothelioma

1. Epithelioid mesothelioma	
←→ Pleura	Adenocarcinoma of lung
	Metastatic adenocarcinoma involving pleura
	Reactive mesothelial hyperplasia
←→ Peritoneum	Serous papillary adenocarcinoma of ovary
	Serous carcinoma of peritoneum
2. Sarcomatoid mesothelioma	
←→ Pleura	Sarcoma, chest wall, pleura or lung
	Sarcomatoid carcinoma of lung
←→ Peritoneum	Sarcoma, abdomical wall, peritoneum or intestine
3. Desmoplastic mesothelioma	
←→ Pleura	Fibrous pleuritis
4. Biphasic mesothelioma	
←→ Pleura	Carcinosarcoma or pulmonary blastoma of lung
	Biphasic synovial sarcoma of pleura
←→ Peritoneum	Carcinosarcoma of ovary, uterus

Table 3 Antibodies used in the immunohistochemical staining for differential diagnosis

Type of mesothelioma	Positive markers	Negative markers
Epithelioid type	Calretinin	CEA
	WT1	TTF-1
	Thrombomodulin	Napsin A
	Mesothelin	Surfactant apoprotein
	D2-40	Ber EP4
		MOC31
		ER
Sarcomatoid type	AE1/AE3	Myo D1, Myoglobin
	CAM5.2	Desmin, h-calredesmon
		S-100p, KP-1

can almost always be presumed to be related to asbestos exposure and the patients can receive compensation or relief. Therefore, the accuracy of the pathological diagnosis as mesothelioma is very important. However, a rough estimate is that approximately 10–15% of mesothelioma patients receive an inadequate diagnosis. In the committee for the judgement of patients' relief, 30% of applicants are judged as not having mesothelioma or the decision is deferred until additional evidence is provided. It is therefore essential that Japanese clinicians and pathologists make an effort to improve the accuracy of the diagnosis as mesothelioma. In particular, the pathologists must improve the accuracy of the pathological diagnosis using adequate immunohistochemical stains.

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