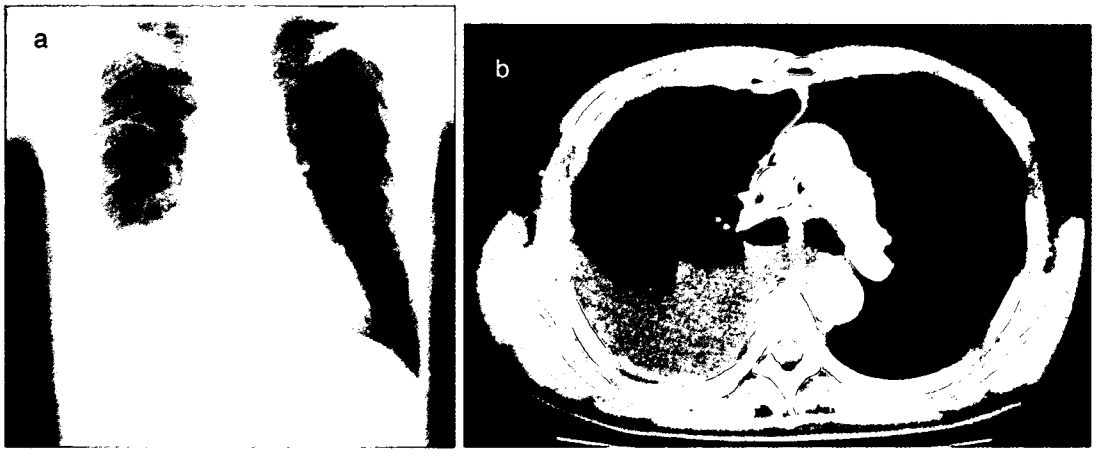

図2 症例1の初診時の胸部画像
初診時胸部X線正面像(a)で，右胸水貯留のみを認める．また，胸部CT像(b)では右胸水貯留と縦隔側から前胸壁にかけての胸膜肥厚像を認める．

さく，腫瘍の全層が採取されていない場合には反応性中皮細胞すなわち，線維性胸膜炎との鑑別が難しい場合もある[16]．胸腔鏡下生検の場合には肉眼的な観察が可能であるので，胸部画像を駆使しても診断困難な比較的早期病変の微妙な胸膜の色調の違いから腫瘍性病変を見出すことができるが，内視鏡的にまったく異常を認めない場合もあるので，その際にはなるべく全層性生検が行われるようにするべきである．一方，開胸生検では十分に観察し組織も十分量を採取できるため診断率がほぼ100%であるとも報告されている．しかし，生検の際に腫瘍が術部あるいはカテーテル挿入部位から周囲に進展する率が22%あるため，検査後の放射線照射などの対応が必要である．

胸膜中皮腫の進展様式

胸膜中皮腫の初発部位は壁側胸膜と考えられている．壁側胸膜に発生した中皮腫は中皮表層あるいは中皮下を進展する．壁側胸膜に腫瘍が限局する場合をInternational Mesothelioma Interest Group(IMIG)分類[17](表1)ではT1aとする．腫瘍はやがて臓側胸膜に進展する(T1b)．そして，腫瘍は葉間胸膜を含む臓側胸膜から胸膜直下の肺に浸潤する(T2)．この段階までの胸膜中皮腫症例は胸水貯留をきっかけとして診断される症例が多い．そして，遠隔転移やリンパ節転移がない場合には，IMIGのStage分類(表2)でStage II以下であり，70歳以下で，PSが0または1の症例では治療として，胸膜肺全摘出術の適応がある．腫瘍はさらに深達性に進展し，胸壁脂肪織，横隔膜脂肪層，筋層のみならず，胸郭内筋膜や縦隔脂肪層あるいは心膜(T3)に浸潤する．このような進展をした場合に，手術により切除可能である範囲の進展ならT3で，切除不可能となればT4と判定する．一方，胸膜中皮腫のリンパ節転移は手術適応を狭める因子であり，同側気管支周囲や肺門リンパ節転移(N1)であってもStage IIIとなり，手術適応が難しくなる．なぜなら，N因子が陽性である場合に肺胸膜全摘出術を行っても再発率が高いからである．胸腔内リンパ節に転移のある症例は約40%であるといわれるが，剖検ではリンパ節転移は約70%であることも報告されている．一方，肝臓，肺，骨，副腎に血行性に遠隔転移する頻度も低くなく，脳転移も報告されている．

胸膜中皮腫の臨床像と経過

症例1は60歳代男性で，内装工事を38年間行っており，石綿ばく露歴を有する．労作時呼吸困難で発症し，2か月後に岡山労災病院を受診した．初診時の胸部X線写真では右胸水貯留(図2-a)，胸部CTにて縦隔側と前胸壁の胸膜肥厚を示した(図2-b)．石綿ばく露歴があるため，胸膜中皮腫を疑い局所麻酔下胸腔鏡を施行した．肉眼所見では顆粒状の隆起病変を認め(図3)，胸膜生検にて，上皮型胸膜中皮腫の臨床病期Stage I (cT1N0M0)と診断したため，右胸膜肺全摘出術

図3　症例1の胸腔鏡肉眼所見
局所麻酔下胸腔鏡所見では顆粒状の隆起病変を認め，胸膜生検で上皮型中皮腫であると診断された．

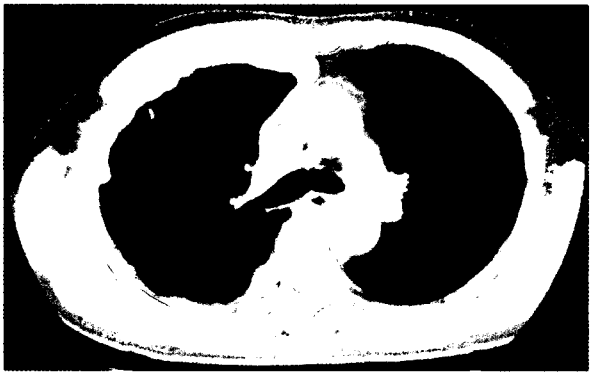

図4　症例1の初診後1か月目の胸部CT画像
初診後1か月目の胸部CT画像所見では，胸水は消失しているが，縦隔側から前胸壁の胸膜肥厚は顕著となり，腫瘍性肥厚像であると診断できる．

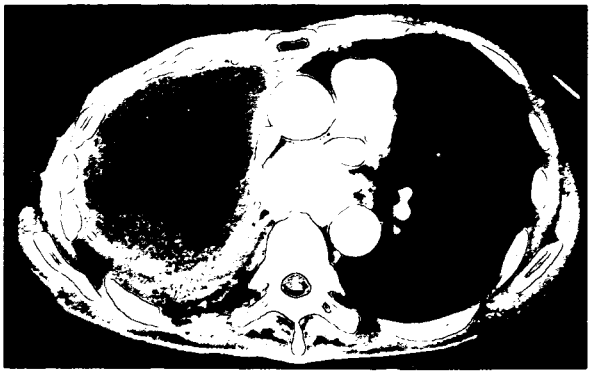

図5　術後再発時の胸部CT画像
胸膜肺全摘出後，中皮腫再発時の胸部CTでは，傍食道縦隔に腫瘤形成を認めた．

図6　症例2の初診時の胸部画像
胸部X線正面像では左胸水貯留像を認める．

をすすめたが，拒否して退院した．しかし，わずか1か月後の胸部CTでは胸膜肥厚は顕著となり，著明な病状の進展が示唆された(図4)．そこで，手術適応は現時点が限界であると説明したところ手術を受け入れ，即刻同手術を施行した．術後診断では一部で肺に浸潤していたが，Stage II(pT2N0M0)であり，放射線あるいは化学療法を加えずに経過を観察し，臨床経過は良好であった．術後24か月目に嚥下障害を訴え，胸部CTにて，傍食道部縦隔に再発を確認した(図5)．そのため，CDDP＋ALIMTA併用療法を行ったが，stable disease(SD)であり，肺炎を合併して術後34か月目に死亡した．胸膜肺全摘出術後の問題点は縦隔を含む原発部位への局所再発の頻度が高いことである．

症例2は60歳代男性で，造船所の艤装作業を37年間行っており，石綿ばく露歴を有する．自覚症状はなく，石綿健康診断の際に胸部X線写真で，左胸水貯留(図6)を指摘されたため，紹介された．胸部CTでは胸膜の腫瘍性肥厚像を認め(図7-a, b)，胸腔鏡下胸膜生検で，上皮型胸膜中皮腫と診断された〔Stage III(cT3N0M0)〕．そのため，CDDP＋ALIMTA併用療法を6コース施行し，効果(PR)が認められた．しかし，診断後14か月目に，心膜への直接浸潤による心不全により死亡した(図8-a, b)．心膜浸潤による心嚢水の貯留に対する処置は効果が得られにくく，予後不良の兆候である．

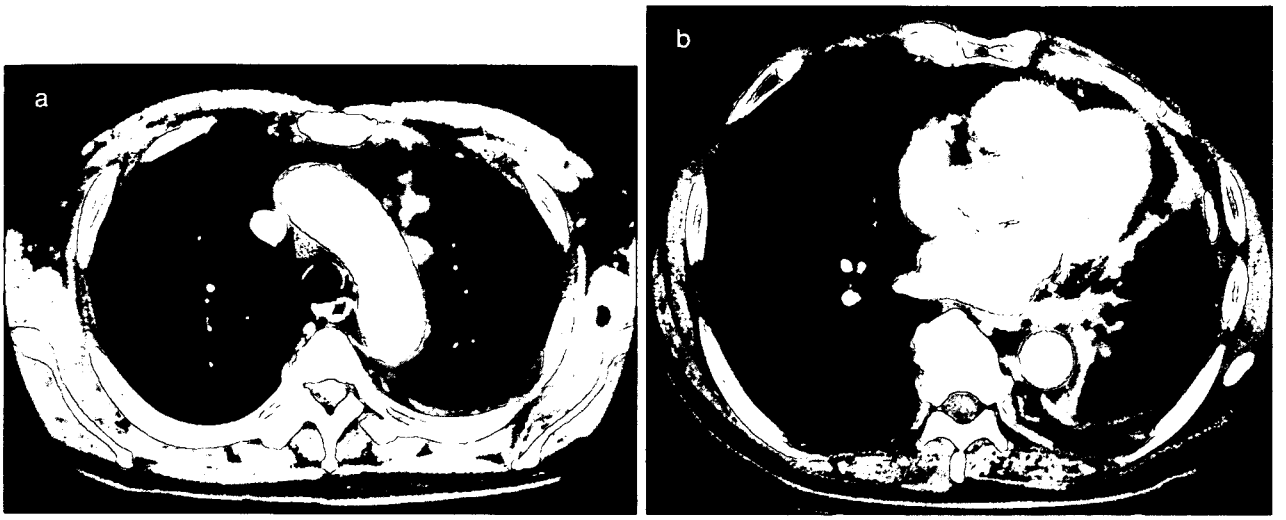

図 7　症例 2 の初診時の胸部CT画像
左上部縦隔側胸膜にはわずかな小結節状の腫瘤病変(a)が認められるだけであるが，下部胸膜の病変は心膜を含む縦隔への浸潤を示唆する病変(b)である．

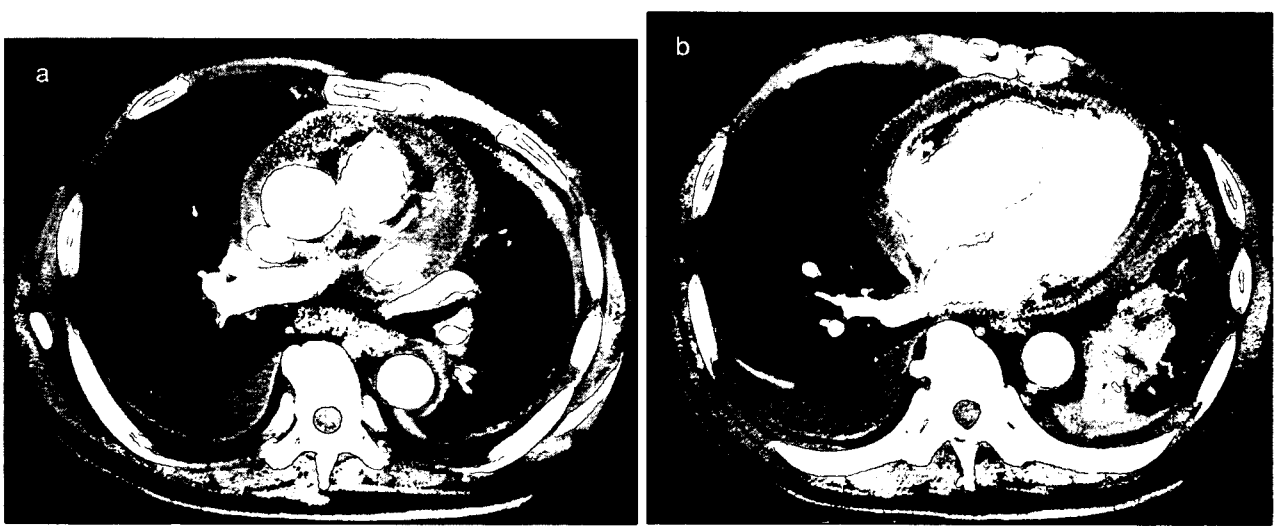

図 8　症例 2 の心膜浸潤時の胸部CT画像
経過観察中に胸部CT上，腫瘍の心膜浸潤により，心嚢液貯留が認められるとともに，右胸水と左下肺の無気肺像を認める(a, b)．

症例 3 は50歳代男性で，電気工事を22年間行っており，石綿ばく露歴を有する．胸痛を主訴として発症した．他院で胸腔鏡下胸膜生検を行ったが，非特異的胸膜肥厚との診断であり，びまん性胸膜肥厚として経過観察されていた．しかし，胸痛が持続するため岡山労災病院を紹介された．胸部画像上は左胸膜のびまん性腫瘍性胸膜肥厚像を呈しており，胸水を認めなかった．典型的な胸膜中皮腫の像(図 9-a, b)であったため，再度胸腔鏡下胸膜生検を行い，二相型胸膜中皮腫と診断した．胸膜病変の進展は緩徐であったが，腹膜腔への進展が速く(図10-a, b)，イレウスにより死亡した．剖検では，腫瘍は左胸膜から腹膜に浸潤し，腹腔内はほとんど腫瘍に置換されていた．二相型胸膜中皮腫であった．腹膜浸潤の腫瘍組織像は肉腫型で，CDDP＋ALIMTA併用療法が無効であり，診断から 2 か月で死亡に至った．

以上のように，胸膜中皮腫の大半は臨床経過が速く，腫瘍の進展による呼吸面積の低下，心膜浸潤や腹腔内進展を起こして死亡に至る．

2003年の全国における胸膜中皮腫死亡者の追跡調査で，臨床病理学的に胸膜中皮腫であると確定診断した158例の予後は中央値8.2か月であっ

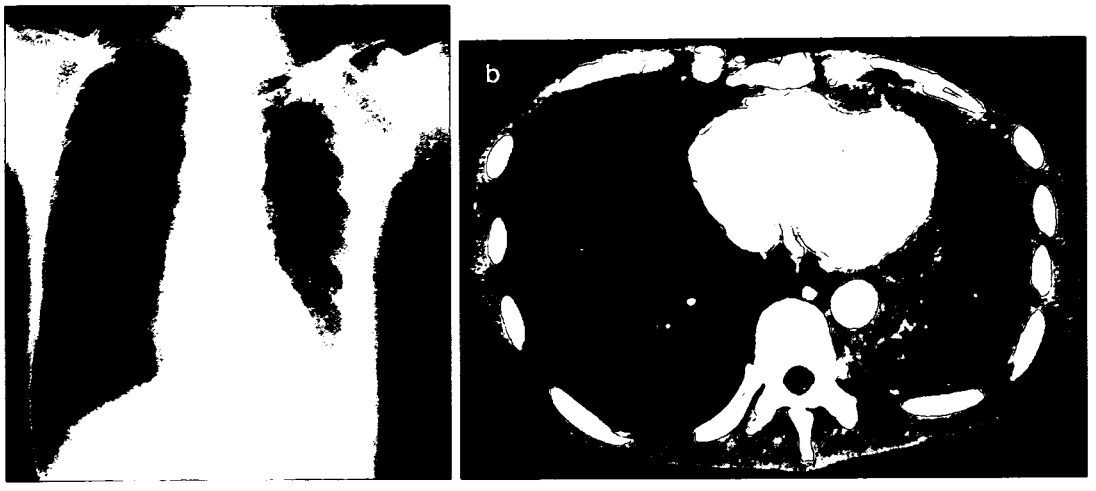

図9　症例3の初診時の胸部画像
初診時胸部X線所見では左下部胸壁の胸膜肥厚像を認める(a)．胸部CT上ではびまん性の不整な胸膜肥厚像(b)を認める．

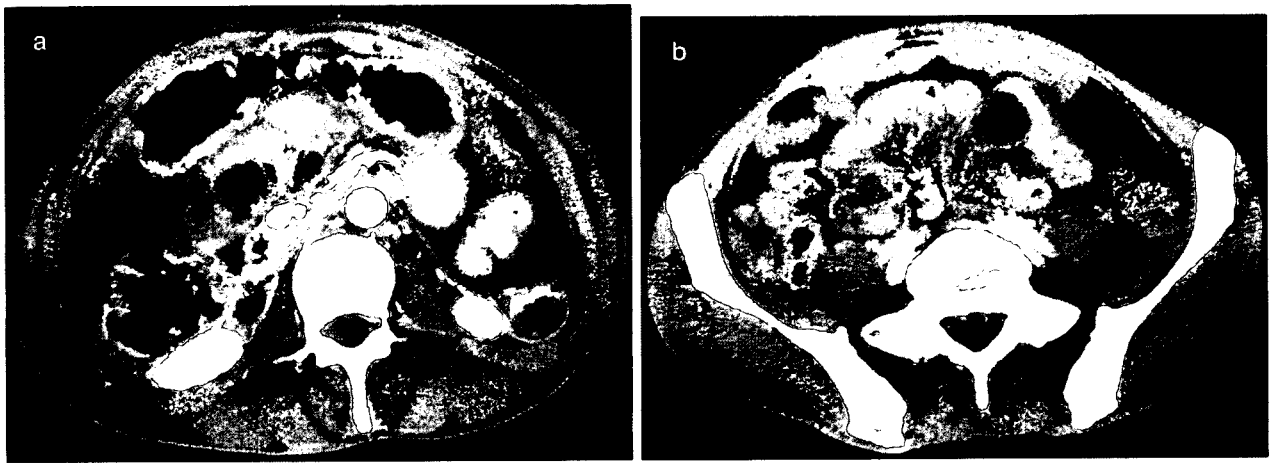

図10　腹膜播種時の腹部CT画像
病変が進展し，腹膜播種をきたした際の腹部CT像では腹水貯留とびまん性の腹膜肥厚像を認める(a, b)．

た．治療法別では，手術症例では11.4か月，化学療法を行った症例では8.8か月，対症療法のみの症例では4.8か月であり，予後不良な疾患であることを確認できた[18]．

文　献

1) Bang KM, Pinheiro GA, Wood JM, et al. Malignant mesothelioma mortality in the United States, 1999-2001. Int J Occup Environ Health 2006 ; 12 : 9.
2) 三浦溥太郎．第3章，第3節，中皮腫—臨床．In：森永謙二・編．職業性石綿ばく露と石綿関連疾患—基礎知識と労災補償—．東京：三信図書；2005. p. 184.
3) Bénard F, Sterman D, Smith RJ, et al. Metabolic imaging of malignant pleural mesothelioma with fluorodeoxyglucose positron emission tomography. Chest 1998 ; 114 : 713.
4) Flores RM, Akhurst T, Gonen M, et al. Positron emission tomography defines metastatic disease but not locoregional disease in patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2003 ; 126 : 11.
5) Reid A, de Klerk N, Ambrosini G, et al. The additional risk of malignant mesothelioma in former workers and residents of Wittenoom with benign pleural disease or asebstosis. Occup Environ Med 2005 ; 62 : 665.
6) Paganuzzi M, Onetto M, Marroni P, et al. Diagnostic value of CYFRA21-1 tumor marker as CEA in pleural effusion due to mesothelioma. Chest 2001 ;

119 : 1138.
7) Hiraki A, Aoe K, Ueoka H. Asbestos exposure and serum osteopontin. N Engl J Med 2006 ; 354 : 304.
8) Tigrani DY, Weydert JA. Immunohistochemical expression of osteopontin in epthelioid mesotheliomas and reactive mesothelial proliferations. Am J Clin Pathol 2007 ; 127 : 580.
9) Scherpereel A, Grigoriu B, Conti M, et al. Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. Am J Respir Crit Care Med 2006 ; 173 : 1155.
10) Hiraki A, Aoe K, Murakami T, et al. Clinical significance of the expression of tumor-associated antigen, RCAS1, and its soluble protein in pleural fluid in malignant mesothelioma. Oncol Rep 2005 ; 14 : 357.
11) Carletti AM, Roncella S, Canessa PA, et al. Expression of human mammaglobin gene in pleural effusions of patients with malignant mesothelioma. Thorax 2006 ; 61 : 271.
12) Saad RS, Lindner JL, Lin X, et al. The diagnostic utility of D2-40 for malignant mesothelioma versus pulmonary carcinoma with pleural involvement. Diagn Cytopathol 2006 ; 34 : 801.
13) Saad RS, Cho P, Liu YL, et al. The value of epithelial membrane antigen expression in separating benign mesothelial proliferation from malignant mesothelioma : a comparative study. Diagn Cytopathol 2005 ; 32 : 156.
14) Welker L, Müller M, Holz O, et al. Cytological diagnosis of malignant mesothelioma -improvement by additional analysis of hyaluronic acid in pleural effusions. Virchows Arch 2007 ; 450 : 455.
15) Agarwal PP, Seely JM, Matzinger FR, et al. Pleural mesothelioma : sensitivity and incidence of needle track seeding after image-guided biopsy versus surgical biopsy. Radiology 2006 ; 241 : 589.
16) Sakuraba M, Masuda K, Hebisawa A, et al. Diagnostic value of thoracoscopic pleural biopsy for pleurisy under local anaesthesia. ANZ J Surg 2006 ; 76 : 722.
17) Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. Chest 1995 ; 108 : 1122.
18) 玄馬顕一，岸本卓巳．中皮腫と職業性石綿ばく露に関する研究．In：平成17年度厚生労働科学特別研究報告書．2006. p. 13.

* * *

Pathology International 2007; **57**: 190–199 doi:10.1111/j.1440-1827.2007.02080.x

Original Article

Immunohistochemical marker panels for distinguishing between epithelioid mesothelioma and lung adenocarcinoma

Kei Kushitani, Yukio Takeshima, Vishwa Jeet Amatya, Osamu Furonaka, Akio Sakatani and Kouki Inai

Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

The distinction between epithelioid mesothelioma and lung adenocarcinoma remains an important diagnostic challenge for surgical pathologists. The aim of the present study was to select a limited and appropriate panel of antibodies that can differentiate between epithelioid mesothelioma and lung adenocarcinoma. Specimens of 90 epithelioid mesotheliomas and 51 lung adenocarcinomas obtained from Japanese cases were examined using calretinin, WT1, AE1/AE3, CAM5.2, cytokeratin (CK) 5/6, vimentin, epithelial membrane antigen (EMA), thrombomodulin, CEA, CA19-9, and CA125. Ninety-six percent of epithelioid mesotheliomas were positive for calretinin; 99% for WT1; 100% for AE1/AE; 97% for CAM5.2; 70% for CK 5/6; 91% for vimentin; 96% for EMA; 71% for thrombomodulin; 77% for mesothelin; 7% for CEA; 17% for CA19-9; and 85% for CA125. In contrast, 33% of lung adenocarcinomas were positive for calretinin; 16% for WT1; 100% for AE1/AE3, CAM5.2, and EMA; 41% for CK 5/6; 47% for vimentin; 20% for thrombomodulin; 69% for mesothelin; 98% for CEA; 73% for CA19-9; and 80% for CA125. For distinguishing between epithelioid mesothelioma and lung adenocarcinoma, the combination of CEA, calretinin and each WT1 or thrombomodulin was suggested to be the best panel of immunohistochemical markers.

Key words: adenocarcinoma, calretinin, CEA, immunohistochemistry, mesothelioma, thrombomodulin, WT1

The differential diagnosis between epithelioid mesothelioma and lung adenocarcinoma is a well-known diagnostic challenge in surgical pathology, and it is of critical importance for proper clinical management and in view of the increasing number of claims involving job-related asbestos exposure. Both the tumors may involve the pleural surfaces and, in some instances, their overlapping histological features preclude a firm diagnosis based on conventional light microscopic observations. Several ancillary diagnostic techniques, including histochemistry, electron microscopy, and immunohistochemistry (IHC), have been proposed to assist in the diagnosis of epithelioid mesothelioma. The diagnostic utility of conventional histochemical stains is limited. Lung adenocarcinomas are not consistently positive for intracytoplasmic mucicarmine and PAS after diastase digestion. Furthermore, this reaction has been observed in a few epithelioid mesotheliomas.[1] The alcian blue-positive, hyaluronidase-sensitive reaction has also been reported in lung adenocarcinomas.[1] Electron microscopy has proven to be useful and is often considered as the gold standard in the diagnosis of epithelioid mesothelioma.[2,3] However, electron microscopic study generally requires great expense and a lot of time compared with the other diagnostic techniques, and the morphological ultrastructural features of mesothelial differentiation may not be apparent in the less-differentiated tumors.

Since the last decade, various immunohistochemical markers that can facilitate the diagnosis of epithelioid mesothelioma have become available.[4,5] A particular issue is the lack of reliable positive markers for mesothelial cells in formalin-fixed, paraffin-embedded sections, although several reports have claimed variable results.[4,5] To date, many of the routinely used probes such as CEA, BerEP4, B72.3, and CD15 stain the carcinoma cells in adenocarcinomas but not those in mesotheliomas. Over the past few years, a number of markers that react with epithelioid mesotheliomas but not with adenocarcinomas have become commercially available; but the number of studies that have evaluated the practical use of these antibodies is limited, and the results are controversial.[5]

In the present study, 12 of the most promising commercially available antibodies were examined on routine histological specimens of epithelioid mesotheliomas and lung adenocarcinomas obtained from Japanese cases. These

Correspondence: Kouki Inai, MD, PhD, Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: koinai@hiroshima-u.ac.jp

Received 11 July 2006. Accepted for publication 27 November 2006.

Table 1 Antibodies used in this study

Antibody to	Clone	Source	Dilution	Retrieval†
Calretinin	Poly	Zymed, San Francisco, CA, USA	1:100	MW, 5 min
WT1	6F-H12	DakoCytomation, Glostrup, Denmark	1:400	AC, 120°C, 20 min
Cytokeratin-multi	AE1/AE3	Novocastra, Newcastle-upon-Tyne, UK	1:100	MW, 5 min
Cytokeratin (CAM5.2)	2A4	Becton-Dickinson, Mountainview, CA, USA	Pre-diluted	MW, 5 min
CK 5/6	D5/16B4	DakoCytomation, Glostrup, Denmark	1:50	MW, 5 min
Vimentin	V9	DakoCytomation, Glostrup, Denmark	1:100	MW, 5 min
EMA	E29	DakoCytomation, Glostrup, Denmark	1:100	MW, 5 min
Thrombomodulin	1009	DakoCytomation, Glostrup, Denmark	1:1600	No retrieval
Mesothelin	5B2	Novocastra, Newcastle-upon-Tyne, UK	1:20	MW, 5 min
CEA	Poly	Immuno-Biomedical Laboratories, Takasaki, Japan	1:40	MW, 5 min
CA19-9	OV185:1	TFB, Tokyo, Japan	1:1	MW, 5 min
CA125	116NS19-9	TFB, Tokyo, Japan	Pre-diluted	MW, 5 min

†In citrate buffer (pH 6.0).
AC, autoclave; CK, cytokeratin: EMA, epithelial membrane antigen; MW, microwave; WT1, Wilms' tumor gene product.

antibodies were tested to clarify the contribution of IHC in the differential diagnosis of these two tumor types and to confirm a specific type of battery of antibodies that can be used in any pathological department or laboratory.

MATERIALS AND METHODS

Patients and histological samples

We used paraffin-embedded specimens from 90 patients with a definite histological diagnosis of mesothelioma who had undergone thoracoscopic pleural biopsy, percutaneous needle biopsy, surgical decortication, or autopsy conducted between 1995 and 2005. These specimens were retrieved from the archives of the Department of Pathology at Hiroshima University and from 36 other institutes. These 90 cases were divided into 71 cases of epithelioid mesotheliomas and 19 cases of biphasic mesotheliomas. In the present study, immunohistochemical evaluation for the sarcomatoid component of the biphasic mesotheliomas was excluded. Paraffin-embedded histological samples of the surgical specimens from 51 patients with a histological diagnosis of primary lung adenocarcinoma were obtained at segmentectomy, lobectomy, or pneumonectomy conducted between 2003 and 2005. These samples were retrieved from the archives of the Department of Pathology at Hiroshima University.

Each of the tumor specimens was reviewed by three pathologists (K. I., Y. T., and K. K.), and all mesothelioma cases were diagnosed using the currently accepted histological criteria combined with the immunohistochemical features.

Immunohistochemical procedures

Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using the avidin–biotin–peroxidase method (ABC) with Histofine SAB-PO kit (Nichirei, Tokyo, Japan). The primary antibodies used in the present study are as follows: calretinin, WT1, pan-cytokeratin (AE1/AE3), CAM5.2, cytokeratin 5/6 (CK 5/6), vimentin, epithelial membrane antigen (EMA), thrombomodulin, mesothelin, CEA, CA19-9 and CA125. Dilution and incubation times of each marker are shown in Table 1.

Immunoreactivity was scored as negative (no immunostaining) or positive. The grade of the immunostained cells was recorded as follows: 1+, 1–25%; 2+, 26–50%; 3+, 51–100%. The scoring was performed based on the extent of positive cells, regardless of intensity. Several combinations of specific and sensitive immunohistochemical findings were analyzed in order to identify the most specific and sensitive-combination that can be used for the differential diagnosis of these two tumor types.

Table 2 Immunohistochemical findings in mesothelioma

Markers	Positive cases		Grading of reactivity			
	n	%	0	1+	2+	3+
Calretinin	85/89	95.5	4	13	8	64
WT1	85/86	98.8	1	23	14	48
AE1/AE3	89/89	100	0	6	5	78
CAM5.2	85/88	96.6	3	3	6	76
CK 5/6	56/80	70	24	28	14	14
Vimentin	81/89	91.0	8	26	18	37
EMA	85/89	95.5	4	17	17	51
Thrombomodulin	60/85	70.6	25	38	13	9
Mesothelin	65/84	77.4	19	5	15	45
CEA	6/88	6.8	82	4	1	1
CA19-9	7/41	17.1	34	5	2	0
CA125	35/41	85.4	6	6	10	19

CK, cytokeratin; EMA, epithelial membrane antigen.

Table 3 Immunohistochemical findings in adenocarcinoma

Markers	Positive cases		Grading of reactivity			
	n	%	0	1+	2+	3+
Calretinin	17/51	33.3	34	12	3	2
WT1	8/51	15.7	43	8	0	0
AE1/AE3	51/51	100	0	1	0	50
CAM5.2	51/51	100	0	0	0	51
CK 5/6	21/51	41.2	30	17	4	0
Vimentin	24/51	47.1	27	10	11	3
EMA	51/51	100	0	1	0	50
Thrombomodulin	10/51	19.6	41	6	3	1
Mesothelin	35/51	68.6	17	19	8	7
CEA	50/51	98.0	1	3	6	41
CA19-9	37/51	72.5	14	22	5	10
CA125	41/51	80.4	10	12	10	19

CK, cytokeratin; EMA, epithelial membrane antigen.

RESULTS

Immunohistochemical profiles of epithelioid mesotheliomas and lung adenocarcinomas

The immunohistochemical results are summarized in Tables 2 and 3, and a brief description of the reactivity of each antibody is presented here.

Calretinin

In epithelioid mesotheliomas, the staining reaction was generally strong and diffuse, and a positive finding was observed in both the cytoplasm and the nucleus (Fig. 1a). In contrast, the staining reaction was limited to <10% of the carcinoma cells in most lung adenocarcinomas.

WT1

In the majority of epithelioid mesotheliomas, the staining reaction was strong and diffuse, and it was confined to the nuclei (Fig. 1b). In all eight lung adenocarcinomas that had positive reactivity, the reaction was focal (1+) and weak.

AE1/AE3 and CAM5.2

All epithelioid mesotheliomas and most lung adenocarcinomas were positive for AE1/AE3 and CAM5.2. A cytoplasmic staining pattern was observed.

CK 5/6

The majority of epithelioid mesotheliomas had a staining reaction that was limited to <50% of the tumor cells. In most of the lung adenocarcinomas, the reaction was focal (1+).

Vimentin

In both epithelioid mesotheliomas and lung adenocarcinomas, vimentin was expressed throughout the cytoplasm.

EMA and thrombomodulin

In both epithelioid mesotheliomas and lung adenocarcinomas, a cytoplasmic staining pattern was observed with accentuation of the reaction along the cell membranes (Fig. 1c).

Mesothelin

In epithelioid mesotheliomas, the staining reaction was usually strong and diffuse, and it was characterized by thick membranous reactivity, particularly along the apical cell membrane (Fig. 2a). In lung adenocarcinomas, the reaction was focal and weak, and its pattern was less consistent; that is, in some cases the reaction was observed along the apical cell membrane, and in others it was cytoplasmic or mixed membranous and cytoplasmic (Fig. 2b).

CEA, CA19-9 and CA125

In lung adenocarcinomas, a cytoplasmic staining pattern was observed with accentuation of the reaction along the cell membrane (Fig. 3a). The grade was 1+ in a few epithelioid mesotheliomas that had a positive reaction.

Specificity and sensitivity of each immunohistochemical antibody for epithelioid mesothelioma

The comparison of the immunoreactivity between epithelioid mesotheliomas and lung adenocarcinomas is shown in Table 4. The results indicated that calretinin, CK 5/6, vimentin, and thrombomodulin are the positive markers for epithelioid mesothelioma, and CEA and CA19-9 are the negative markers.

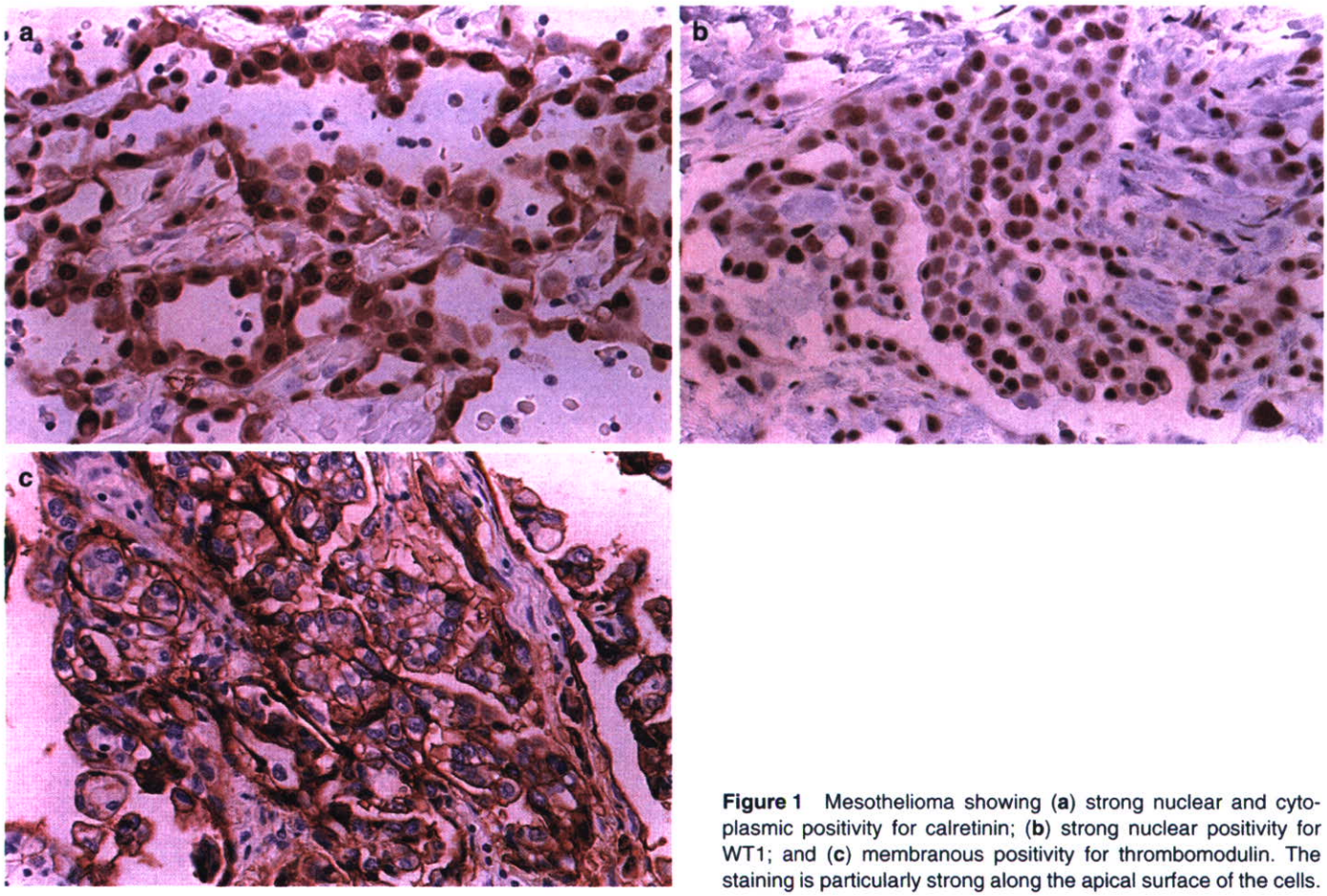

Figure 1 Mesothelioma showing (**a**) strong nuclear and cytoplasmic positivity for calretinin; (**b**) strong nuclear positivity for WT1; and (**c**) membranous positivity for thrombomodulin. The staining is particularly strong along the apical surface of the cells.

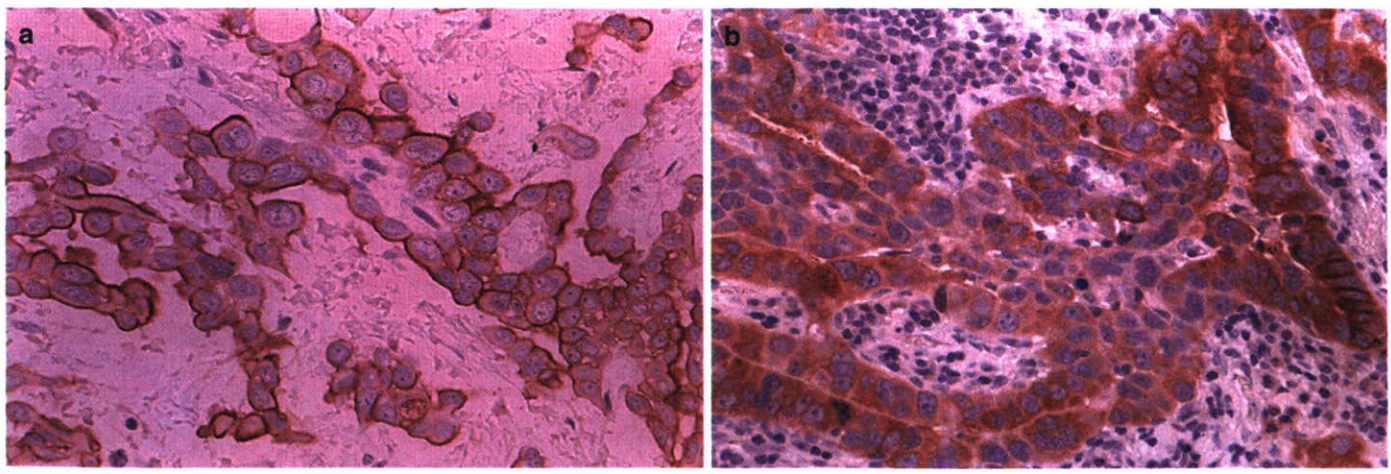

Figure 2 (**a**) Mesothelioma with strong positivity for mesothelin. The staining is particularly strong along the apical surface of the cells. (**b**) Adenocarcinoma with mesothelin positivity.

The sensitivity and specificity of one, two, or three antibodies for the diagnosis of epithelioid mesothelioma are indicated in Tables 5–7.

When each positive marker was observed, WT1 had the highest sensitivity and specificity in epithelioid mesotheliomas, and it was suggested to be the most useful positive marker of epithelioid mesothelioma. The sensitivity of calretinin was as high as WT1, but it was inferior to WT1 with respect to specificity. Thrombomodulin followed WT1 with respect to high specificity but it was inferior to WT1 and calretinin. Among the positive markers of lung adenocarcinoma, CEA had the highest sensitivity and specificity.

Table 4 Comparison of immunohistochemical findings between mesotheliomas and adenocarcinomas

	Positive cases				
	Mesotheliomas		Adenocarcinomas		*P*
Markers	*n*	(%)	*n*	(%)	(Fisher's exact test)
Calretinin	85/89	95.5	17/51	33.3	<0.001
WT1	85/86	98.8	8/51	15.7	<0.001
AE1/AE3	89/89	100	51/51	100	–
CAM5.2	85/88	96.6	51/51	100	0.251
CK 5/6	56/80	70	21/51	41.2	0.001
Vimentin	81/89	91.0	24/51	47.1	<0.001
EMA	85/89	95.5	51/51	100	0.159
Thrombomodulin	60/85	70.6	10/51	19.6	<0.001
Mesothelin	65/84	77.4	35/51	68.6	0.907
CEA	6/88	6.8	50/51	98.0	<0.001
CA19-9	7/41	17.1	37/51	72.5	<0.001
CA125	35/41	85.4	41/51	80.4	0.366

CK, cytokeratin; EMA, epithelial membrane antigen.

Table 5 Sensitivity and specificity of immunohistochemistry in mesotheliomas by one marker

One marker	Sensitivity (%)	Specificity (%)
Calretinin(+)	95.5	66.7
WT1(+)	98.8	84.3
CK 5/6(+)	70	58.8
Vimentin(+)	91.0	52.9
Thrombomodulin(+)	70.6	80.4
CEA(–)	93.2	98.0
CA19-9(–)	82.9	72.5

CK, cytokeratin.

Table 6 Sensitivity and specificity of immunohistochemistry in mesotheliomas by two markers

Two markers	Sensitivity (%)	Specificity (%)
Calretinin(+) or WT1(+)	100	62.7
Calretinin(+) or TM(+)	98.8	54.9
Calretinin(+) or CEA(–)	97.7	64.7
WT1(+) or TM(+)	97.6	70.6
WT1(+) or CEA(–)	100	82.4
TM(+) or CEA(–)	97.6	78.4
Calretinin(+) and WT1(+)	95.3	88.2
Calretinin(+) and TM(+)	70.5	92.2
Calretinin(+) and CEA(–)	91.8	100
WT1(+) and TM(+)	73.1	94.1
WT1(+) and CEA(–)	90.4	100
TM(+) and CEA(–)	69.1	100

TM, thrombomodulin.

Among the combinations of two antibodies, the combination of WT1 and CEA (either WT1 positivity or CEA negativity) had the highest sensitivity, and the combination of calretinin and CEA (both calretinin positivity and CEA negativity) had the highest specificity (Table 6).

Among the combinations of three antibodies, the combination of WT1, calretinin, and thrombomodulin (WT1-positivity and (calretinin positivity or thrombomodulin positivity)) had the highest sensitivity, but it was inferior to the combination of WT1 and CEA. Both the combination of CEA, calretinin and WT1 (CEA negativity and (calretinin positivity or WT1 positivity)) and the combination of CEA, calretinin and thrombomodulin (CEA negativity and (calretinin positivity or thrombomodulin positivity)) had the highest specificity (Table 7).

Table 7 Sensitivity and specificity of immunohistochemistry in mesotheliomas by three markers

Three markers	Sensitivity (%)	Specificity (%)
WT1(+) and (Cal(+) or CEA(–))	96.4	88.2
WT1(+) and (Cal(+) or TM(+))	97.6	86.3
WT1(+) and (TM(+) or CEA(–))	96.3	94.1
Cal(+) and (WT1(+) or CEA(–))	96.4	88.2
Cal(+) and (WT1(+) or TM(+))	95.2	84.3
Cal(+) and (TM(+) or CEA(–))	94.0	92.2
CEA(–) and (Cal(+) or WT1(+))	92.9	100
CEA(–) and (Cal(+) or TM(+))	92.9	100
CEA(–) and (WT1(+) or TM(+))	91.5	100

Cal, calretinin; TM, thrombomodulin.

In the present study, the proportion of epithelioid mesotheliomas that had partial reactivity to calretinin, WT1, and cytokeratin 5/6 (graded as 1+ or 2+) was higher than that recently reported by Ordóñez.[6] Precisely, these markers generally had diffuse (graded as 3+) and dense positive findings in well-differentiated epithelioid mesotheliomas, which have a distinct papillary or tubulopapillary growth pattern (Fig. 4a). In contrast, in the poorly differentiated cases, which have a solid

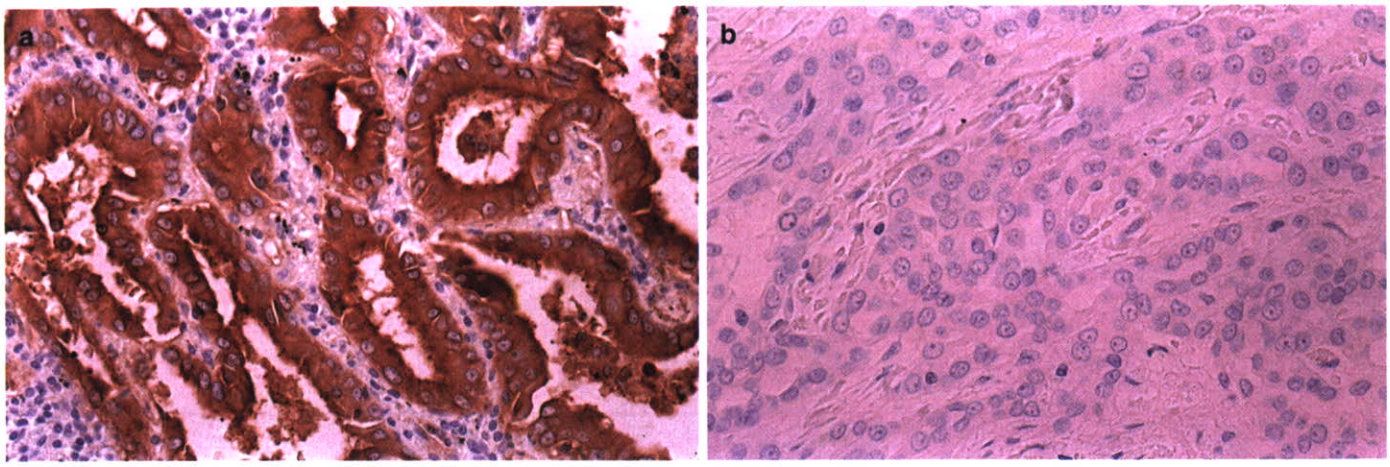

Figure 3 (**a**) Adenocarcinoma showing strong membranous and cytoplasmic positivity for CEA. (**b**) Mesothelioma cells are negative for CEA.

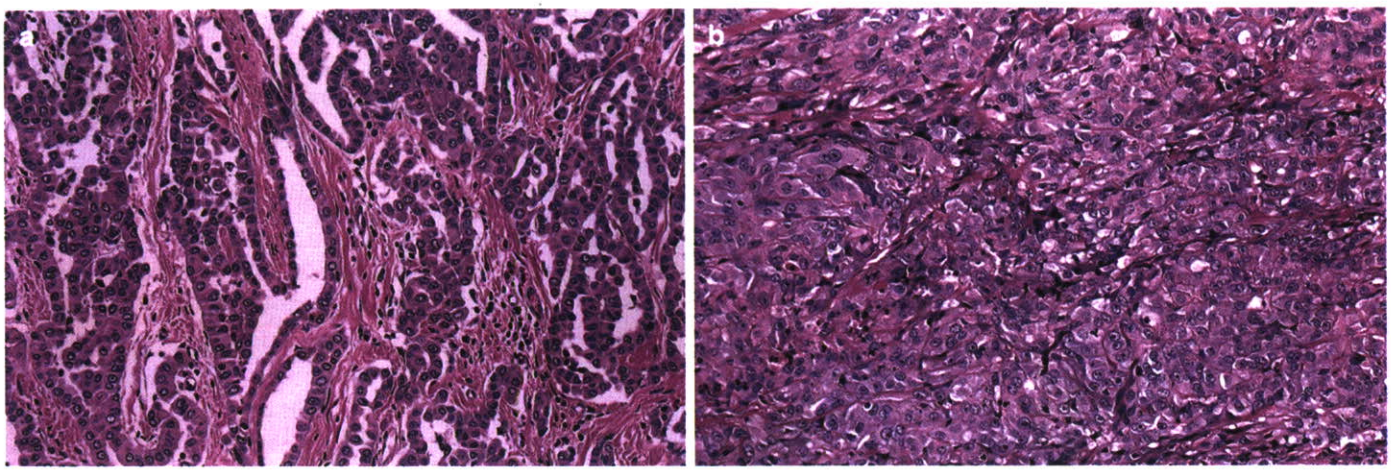

Figure 4 (**a**) Well-differentiated epithelioid mesothelioma showing distinct tubulopapillary growth pattern. (**b**) Poorly differentiated epithelioid mesothelioma showing solid and diffuse growth pattern and lack of papillary or tubulopapillary structure.

and diffuse growth pattern and lack papillary or tubulopapillary structure (Fig. 4b), these antibodies had weak and localized (graded as 1+ or 2+) reactions. In addition, calretinin and WT1 produced a nuclear-positive pattern in well-differentiated cases, whereas they produced a cytoplasmic pattern in the poorly differentiated cases. Based on the fact noted here, the discrepancy between the present results and that of the Ordóñez report may be due to the variation in the cases selected, that is, the proportion of the poorly differentiated cases included in the present study may be higher than that in the Ordóñez study. In addition, there might be some technical problems, for example, the time lapse after the sections were cut into thin sections, the vagaries of IHC or interlaboratory variability.

The comparison of the immunoreactivity between epithelioid mesotheliomas and epithelioid components of biphasic mesotheliomas is given in Table 8. CK 5/6 and mesothelin had wider reactivity in epithelioid mesothelioma. In contrast, vimentin had wider reactivity in the epithelioid component of biphasic mesothelioma. These findings suggest that biphasic mesothelioma indicated the loss or decrease of mesothelial phenotypes along the progression.

In the present study we could not obtain complete data on asbestos exposure of each patient. With regard to the data we could obtain, there were no significant differences in expression patterns with the presence and absence of asbestos exposure.

DISCUSSION

Among the so-called 'positive' markers of mesothelioma, calretinin is one of the most frequently used markers for the diagnosis of epithelioid mesothelioma. The results of the present study confirm those of other investigations.[6,7] However, the percentage of calretinin-positive lung adenocarcinoma cases in the present study was much higher than that previously reported.

Table 8 Comparison of immunoreactivity between epithelioid mesothelioma and epithelioid component of biphasic mesothelioma

	Epithelioid mesothelioma						Epithelioid component of biphasic mesothelioma						
	Positive cases		Grading of reactivity				Positive cases		Grading of reactivity				
Markers	*n*	%	0	1+	2+	3+	*n*	%	0	1+	2+	3+	*P*†
Calretinin	69/70	98.6	1	9	7	53	16/19	84.2	3	4	1	11	0.058
WT1	66/67	98.5	1	15	11	40	19/19	100	0	8	3	8	0.137
AE1/AE3	70/70	100	0	5	4	61	19/19	100	0	1	1	17	0.779
CAM5.2	66/69	95.7	3	3	3	60	19/19	95.7	0	0	3	16	0.878
CK 5/6	46/62	74.2	16	21	11	14	10/18	55.6	8	7	3	0	0.030
Vimentin	62/70	88.6	8	22	14	26	19/19	100	0	4	4	11	0.049
EMA	68/70	97.1	2	15	12	41	17/19	89.5	2	2	5	10	0.703
Thrombomodulin	47/66	71.2	19	30	10	7	13/19	68.4	6	8	3	2	0.902
Mesothelin	56/66	84.8	10	3	13	40	9/18	50	9	2	2	5	0.002
CEA	3/69	4.3	66	3	0	0	3/19	15.8	16	1	1	1	0.070
CA19-9	6/32	18.8	26	4	2	0	1/9	11.1	8	1	0	0	0.564
CA125	29/32	90.6	3	5	8	16	6/9	66.7	3	1	2	3	0.202

†Mann–Whitney *U*-test.
CK, cytokeratin; EMA, epithelial membrane antigen.

Recent studies have suggested that *WT1* suppressor gene plays an important role in both the development of the mesothelium and in the pathogenesis of mesothelioma.[8–13] The present results are similar to those obtained in other investigations in which 6F-H2 anti-WT1 mAb used was the same as that used in the present study.[6] Although the percentage of WT1-positive lung adenocarcinomas in the present study was higher than that previously reported, positive findings were observed only in a small area or a few cells (graded as 1+) in all the WT1-positive lung adenocarcinomas. In addition, WT1 had the highest sensitivity and specificity among all positive markers. Therefore, it is evident that WT1 is the most useful positive marker for the pathological diagnosis of epithelioid mesothelioma. However, when compared with calretinin, WT1 reactivity tends to be within a limited area (graded as 1+ or 2+). In addition, the density of WT1 reactivity tends to be weaker than calretinin reactivity. Therefore, it is possible that calretinin, rather than WT1, is more useful in distinguishing between these two malignancies, particularly in a small biopsy specimen.

In 1992 Collins *et al.* were the first to suggest that thrombomodulin (CD141) could be a useful positive immunohistochemical marker for the diagnosis of epithelioid mesothelioma.[14] In their study, thrombomodulin reactivity was reported in all 31 epithelioid mesotheliomas, whereas only four of the 48 lung adenocarcinomas were positive and only one of the four cases exhibited strong positivity. Since then, many other reports have been published and although the majority of the reports have confirmed the usefulness of thrombomodulin in distinguishing epithelioid mesotheliomas from lung adenocarcinomas,[14–18] others have not.[6,19–21] The results of the present study including the grading of reactivity are in almost complete agreement with the observation reported by Ordóñez.[6]

In 1985, using gel electrophoresis, Blobel *et al.* were the first to demonstrate significant differences in the cytokeratin expression pattern between epithelioid mesotheliomas and lung adenocarcinomas.[22] These investigators demonstrated that although both these malignancies expressed simple epithelial-type CK peptides (CK 7, 8, 18, and 19), other CK, including CK 5 and CK 6, were present in epithelioid mesotheliomas but not in lung adenocarcinomas. In 1989, Moll *et al.* confirmed this observation by immunofluorescence methods using the AE14 anti-CK 5 antibody.[23] Additional comparative studies on the expression of CK peptide 5 and 6 in epithelioid mesotheliomas and lung adenocarcinomas have become possible only recently with the introduction of the commercially available D5/16B4 anti-CK 5/6 mAb. In the present study, 70% of epithelioid mesotheliomas had reactivity to CK 5/6. This finding is in agreement with the observation reported by Chu and Weiss;[24] but this value is lower than those reported in other investigations.[6,7,16,25,26] In contrast, 41.2% of lung adenocarcinomas also expressed this marker. In the present study, the percentage of CK 5/6-positive lung adenocarcinomas was much higher than those previously reported. However, the reaction was focal (1+) and weak in most cases.

Although a large number of published reports have advocated the utility of CEA in the diagnosis of epithelioid mesotheliomas, some controversy still exists regarding the expression of this protein in epithelioid mesotheliomas.[6,16,17,19,20,27–50] In some earlier studies, the percentage of CEA positivity in epithelioid mesothelioma was reported to be as high as one-third to nearly one half of the cases.[30,36,42] At present, it is believed that these high values were due to the use of anti-CEA antibodies that cross-reacted with non-CEA antigens. However, in recent investigations, CEA expression has been consistently reported in epithelioid mesotheliomas, but in much lower percentages, ranging from 1% to 10% of the cases.[16,20,27,29,34,44] In the present study only 6.8% of epithelioid mesotheliomas had reactivity for CEA. This finding confirms those of other recent

investigations.[16,20,27,29,34,44] The findings related to the grading of reactivity are also very similar to those reported by Ordóñez.[6] Among the negative markers of epithelioid mesothelioma, CEA had the highest sensitivity and specificity. Due to its high sensitivity and specificity, CEA continues to be one of the best negative markers of mesothelioma.

The first investigation on the potential of CA19-9 as a marker for distinguishing between epithelioid mesothelioma and lung adenocarcinoma was conducted by Ordóñez in 1989.[41] In his study, CA19-9 positivity was reported in nine (39%) of the 23 lung adenocarcinomas, but not in any of the 19 epithelioid mesotheliomas. Since then, several other studies have been published.[27,51–53] In the most recent study, which is a comparative investigation of a variety of markers, CA19-9 reactivity was reported in 16 (53%) of the 30 adenocarcinomas at various sites, but not in any of the 28 epithelioid mesotheliomas. Ordóñez concluded that CA19-9 was useful and should be part of the four-marker panel recommended for distinguishing between epithelioid mesotheliomas and adenocarcinomas. The present results indicated that this marker is one of the negative markers of epithelioid mesothelioma; but its sensitivity and specificity in epithelioid mesothelioma is not sufficiently high to distinguish between epithelioid mesothelioma and lung adenocarcinoma.

In 1992, using the K1 anti-mesothelin antibody on frozen tissue specimens, Chang *et al.* reported the expression of this marker in all 15 epithelioid mesotheliomas, but not in any of the 23 lung adenocarcinomas.[54] These investigators concluded that mesothelin could be a useful immunohistochemical marker for discriminating between these malignancies. However, recent studies have shown that mesothelin is strongly expressed in other carcinomas, particularly serous carcinomas of the ovary, pancreatic adenocarcinomas, and in some squamous cell carcinomas.[55–59] In the present study there was no significant difference with respect to the immunoreactivity between epithelioid mesotheliomas and lung adenocarcinomas. These results indicate that this marker is not useful in discriminating these tumors.

Since the first investigation on the potential of vimentin immunostaining in the diagnosis of epithelioid mesothelioma by Churg in 1985,[60] a large number of reports have been published with conflicting conclusions, but some controversy remains regarding its potential in the diagnosis of epithelioid mesothelioma.[6,19,37,39,41,43,49,50,61] The results of the present study indicate that this marker is useful as one of the positive markers of epithelioid mesothelioma. However, its specificity in epithelioid mesothelioma is not sufficiently high to distinguish between epithelioid mesothelioma and lung adenocarcinoma.

To date, there are few reports that have examined the sensitivity and specificity of various combinations of antibodies for their usefulness in diagnosis of epithelioid mesotheliomas. In previous investigations Riera *et al.* recommended the combination of CEA, BG8, and BerEP4,[62] Abutaily *et al.* recommended E-cadherin and TTF-1,[7] and Yaziji *et al.* recommended calretinin, BG8, and MOC-31 as the first-line antibodies.[63] In the present study, among the combinations of two or three antibodies, the combination of WT1 and CEA had the highest sensitivity, and the combination of CEA, calretinin and either WT1 or thrombomodulin had the highest specificity. The combination of WT1 and CEA had 100% sensitivity, but specificity of this combination was insufficient to distinguish lung adenocarcinoma from mesothelioma. Consequently, the present results suggest that the first-line antibodies for the differential diagnosis of epithelioid mesothelioma and lung adenocarcinoma should be CEA, calretinin and either WT1 or thrombomodulin.

In the present study we did not evaluate podoplanin or D2-40 mAb, which appear to be highly specific and sensitive mesothelial markers.[64–66] The two reports describing podoplanin expression in epithelioid mesothelioma and adenocarcinoma suggest that it is highly specific for epithelioid mesothelioma.[65,66] However, D2-40 also recognized ovarian serous carcinomas in one of the two previous studies,[64] and therefore, does not appear to be highly specific for epithelioid mesothelioma. So far, it should be considered that their utility in routine diagnostic work has not yet been completely determined. Further research on these antibodies would clarify their utility in discriminating between these tumors.

CONCLUSION

It is suggested that the combination of CEA, calretinin and either WT1 or thrombomodulin is the most useful antibody panel for distinguishing between epithelioid mesothelioma and lung adenocarcinoma. Further studies, including whole genome expression profiles of epithelioid mesothelioma and lung adenocarcinoma, will clarify several useful positive and negative markers for the differential diagnosis of these tumors in the near future.[67]

ACKNOWLEDGMENTS

The authors thank the pathologists at 36 other institutions for allowance of immunohistochemical staining and the analysis of the results, and Ms Midori Nagai (Technical Center, Hiroshima University) for her excellent technical assistance.

REFERENCES

1 Hammar SP, Bockus DE, Remington FL *et al.* Mucin-positive epithelial mesotheliomas: A histochemical, immunohistochemi-

cal, and ultrastructural comparison with pulmonary mucin producing pulmonary adenocarcinoma. *Ultrastruct Pathol* 1996; **20**: 293–325.
2 Henderson DW, Shilkin KB, Whitaker D *et al.* The pathology of malignant mesothelioma, including immunohistology and ultrastructure. In: Henderson DW, Shilkin KB, Langlois SL *et al.*, eds. *Malignant Mesothelioma.* New York, NY: Hemisphere, 1992; 69–166.
3 Ordóñez NG, Mackay B. The roles of immunohistochemistry and electron microscopy in distinguishing epithelial mesothelioma of the pleura from adenocarcinoma. *Adv Anat Pathol* 1996; **3**: 273–93.
4 Henderson DW, Comin CE, Hammar SP *et al.* Malignant mesothelioma of the pleura: Current surgical pathology. In: Corrin B, ed. *Pathology of Lung Tumors.* New York, NY: Churchill Livingstone, 1997; 241–80.
5 Ordóñez NG. The immunohistochemical diagnosis of epithelial mesothelioma. *Hum Pathol* 1999; **30**: 313–23.
6 Ordóñez NG. The immunohistochemical diagnosis of mesothelioma: A comparative study of epithelioid mesothelioma and lung adenocarcinoma. *Am J Surg Pathol* 2003; **27**: 1031–51.
7 Abutaily AS, Addis BJ, Roche WR. Immunohistochemistry in the distinction between malignant mesothelioma and pulmonary adenocarcinoma: A critical evaluation of new antibodies. *J Clin Pathol* 2002; **55**: 662–8.
8 Amin KM, Litzky LA, Smythe WR *et al.* Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. *Am J Pathol* 1995; **146**: 344–56.
9 Gerwin BI. Asbestos and the mesothelial cell: A molecular trail to mitogenic stimuli and suppressor gene suspects. *Am J Respir Cell Mol Biol* 1994; **11**: 507–8.
10 Kleymenova EV, Yuan X, LaBate ME *et al.* Identification of a tumor-specific methylation site in the Wilms tumor suppressor gene. *Oncogene* 1998; **16**: 713–20.
11 Kumar-Singh S, Segers K, Rodeck U *et al.* WT1 mutation in malignant mesothelioma and WT1 immunoreactivity in relation to p53 and growth factor receptor expression, cell-type transition, and prognosis. *J Pathol* 1997; **181**: 67–74.
12 Park S, Schalling M, Bernard A *et al.* The Wilms tumour gene WT1 is expressed in murine mesoderm-derived tissues and mutated in a human mesothelioma. *Nat Genet* 1993; **4**: 415–20.
13 Walker C, Rutten F, Yuan X *et al.* Wilms' tumor suppressor gene expression in rat and human mesothelioma. *Cancer Res* 1994; **54**: 3101–6.
14 Collins CL, Ordóñez NG, Schaefer R *et al.* Thrombomodulin expression in malignant pleural mesothelioma and pulmonary adenocarcinoma. *Am J Pathol* 1992; **141**: 827–33.
15 Attanoos RL, Goddard H, Gibbs AR. Mesothelioma-binding antibodies: Thrombomodulin, OV 632 and HBME-1 and their use in the diagnosis of malignant mesothelioma. *Histopathology* 1996; **29**: 209–15.
16 Carella R, Deleonardi G, D'Errico A *et al.* Immunohistochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma: A study with logistic regression analysis. *Am J Surg Pathol* 2001; **25**: 43–50.
17 Ordóñez NG. Value of antibodies 44-3A6, SM3, HBME-1, and thrombomodulin in differentiating epithelial pleural mesothelioma from lung adenocarcinoma: A comparative study with other commonly used antibodies. *Am J Surg Pathol* 1997; **21**: 1399–408.
18 Ordóñez NG. Value of thrombomodulin immunostaining in the diagnosis of mesothelioma. *Histopathology* 1997; **31**: 25–30.
19 Brown RW, Clark GM, Tandon AK *et al.* Multiple-marker immunohistochemical phenotypes distinguishing malignant pleural mesothelioma from pulmonary adenocarcinoma. *Hum Pathol* 1993; **24**: 347–54.
20 Doglioni C, Dei Tos AP, Laurino L *et al.* Calretinin: A novel immunocytochemical marker for mesothelioma. *Am J Surg Pathol* 1996; **20**: 1037–46.
21 Foster MR, Johnson JE, Olson SJ *et al.* Immunohistochemical analysis of nuclear versus cytoplasmic staining of WT1 in malignant mesotheliomas and primary pulmonary adenocarcinomas. *Arch Pathol Lab Med* 2001; **125**: 1316–20.
22 Blobel GA, Moll R, Franke WW *et al.* The intermediate filament cytoskeleton of malignant mesotheliomas and its diagnostic significance. *Am J Pathol* 1985; **121**: 235–47.
23 Moll R, Dhouailly D, Sun T-T. Expression of keratin 5 as a distinctive feature of epithelial and biphasic mesotheliomas: An immunohistochemical study using monoclonal antibody AE14. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1989; **58**: 129–45.
24 Chu PG, Weiss LM. Expression of cytokeratin 5/6 in epithelial neoplasms: An immunohistochemical study of 509 cases. *Mod Pathol* 2002; **15**: 6–10.
25 Clover J, Oates J, Edwards C. Anti-cytokeratin 5/6: A positive marker for epithelioid mesothelioma. *Histopathology* 1997; **31**: 140–43.
26 Ordóñez NG. Value of cytokeratin 5/6 immunostaining in distinguishing epithelial mesothelioma of the pleura from lung adenocarcinoma. *Am J Surg Pathol* 1998; **22**: 1215–21.
27 Bateman AC, al-Talib RK, Newman T *et al.* Immunohistochemical phenotype of malignant mesothelioma: Predictive value of CA125 and HBME-1 expression. *Histopathology* 1997; **30**: 49–56.
28 Battifora H, Kopinski MI. Distinction of mesothelioma from adenocarcinoma: An immunohistochemical approach. *Cancer* 1985; **55**: 1679–85.
29 Comin CE, Novelli L, Boddi V *et al.* Calretinin, thrombomodulin, CEA, and CD15: A useful combination of immunohistochemical markers for differentiating pleural epithelial mesothelioma from peripheral pulmonary adenocarcinoma. *Hum Pathol* 2001; **32**: 529–36.
30 Corson JM, Pinkus GS. Mesothelioma: Profile of keratin proteins and carcinoembryonic antigen: An immunoperoxidase study of 20 cases and comparison with pulmonary adenocarcinomas. *Am J Pathol* 1982; **108**: 80–87.
31 Dejmek A, Hjerpe A. Carcinoembryonic antigen-like reactivity in malignant mesothelioma: A comparison between different commercially available antibodies. *Cancer* 1994; **73**: 464–9.
32 Delahaye M, Hoogsteden HC, van der Kwast TH. Immunocytochemistry of malignant mesothelioma: OV632 as a marker of malignant mesothelioma. *J Pathol* 1991; **165**: 137–43.
33 Dewar A, Valente M, Ring NP *et al.* Pleural mesothelioma of epithelial type and pulmonary adenocarcinoma: An ultrastructural and cytochemical comparison. *J Pathol* 1987; **152**: 309–16.
34 Garcia-Prats MD, Ballestin C, Sotelo T *et al.* A comparative evaluation of immunohistochemical markers for the differential diagnosis of malignant pleural tumours. *Histopathology* 1998; **32**: 462–72.
35 Gibbs AR, Harach R, Wagner JC *et al.* Comparison of tumour markers in malignant mesothelioma and pulmonary adenocarcinoma. *Thorax* 1985; **40**: 91–5.
36 Holden J, Churg A. Immunohistochemical staining for keratin and carcinoembryonic antigen in the diagnosis of malignant mesothelioma. *Am J Surg Pathol* 1984; **8**: 277–9.
37 Jasani B, Edwards RE, Thomas ND *et al.* The use of vimentin antibodies in the diagnosis of malignant mesothelioma. *Virchows Arch A Pathol Anat Histopathol* 1985; **406**: 441–8.
38 Kwee WS, Veldhuizen RW, Golding RP *et al.* Histologic distinction between malignant mesothelioma, benign pleural lesion and carcinoma metastasis: Evaluation of the application of morphometry combined with histochemistry and immun-

ostaining. *Virchows Arch A Pathol Anat Histopathol* 1982; **397**: 287–99.
39 Moch H, Oberholzer H, Dalquen N *et al.* Diagnostic tools for differentiating between pleural mesothelioma and lung adenocarcinoma in paraffin embedded tissue: I. Immunohistochemical findings. *Virchows Arch A Pathol Anat Histopathol* 1993; **423**: 19–27.
40 O'Hara CJ, Corson JM, Pinkus GS *et al.* ME1. A monoclonal antibody that distinguishes epithelial-type malignant mesothelioma from pulmonary adenocarcinoma and extrapulmonary malignancies. *Am J Pathol* 1990; **136**: 421–8.
41 Ordóñez NG. The immunohistochemical diagnosis of mesothelioma: Differentiation of mesothelioma and lung adenocarcinoma. *Am J Surg Pathol* 1989; **13**: 263–91.
42 Otis CN, Carter D, Cole S *et al.* Immunohistochemical evaluation of pleural mesothelioma and pulmonary adenocarcinoma: A bi-institutional study of 47 cases. *Am J Surg Pathol* 1987; **11**: 445–56.
43 Pfaltz M, Odermatt B, Christen B *et al.* Immunohistochemistry in the diagnosis of malignant mesothelioma. *Virchows Arch A Pathol Anat Histopathol* 1987; **411**: 387–93.
44 Roberts F, Harper CM, Downie I *et al.* Immunohistochemical analysis still has a limited role in the diagnosis of malignant mesothelioma: A study of thirteen antibodies. *Am J Clin Pathol* 2001; **116**: 253–62.
45 Sheibani K, Battifora H, Burke JS. Antigenic phenotype of malignant mesotheliomas and pulmonary adenocarcinomas: An immunohistologic analysis demonstrating the value of Leu M1 antigen. *Am J Pathol* 1986; **123**: 212–19.
46 Szpak CA, Johnston WW, Roggli V *et al.* The diagnostic distinction between malignant mesothelioma of the pleura and adenocarcinoma of the lung as defined by a monoclonal antibody (B72.3). *Am J Pathol* 1986; **122**: 252–60.
47 Wang NS, Huang SN, Gold P. Absence of carcinoembryonic antigen-like material in mesothelioma: An immunohistochemical differentiation from other lung cancers. *Cancer* 1979; **44**: 937–43.
48 Whitaker D, Sterrett GF, Shilkin KB. Detection of tissue CEA-like substance as an aid in the differential diagnosis of malignant mesothelioma. *Pathology* 1982; **14**: 255–8.
49 Wick MR, Loy T, Mills SE *et al.* Malignant epithelioid pleural mesothelioma versus peripheral pulmonary adenocarcinoma: A histochemical, ultrastructural, and immunohistologic study of 103 cases. *Hum Pathol* 1990; **21**: 759–66.
50 Wirth PR, Legier J, Wright GL Jr. Immunohistochemical evaluation of seven monoclonal antibodies for differentiation of pleural mesothelioma from lung adenocarcinoma. *Cancer* 1991; **67**: 655–62.
51 Chenard-Neu MP, Bellocq JP, Maier A *et al.* Mésothéliomes malins de la plévre: Analyse de leurs aspects immunohistochemiques. *Ann Pathol* 1990; **10**: 20–27.
52 Chenard-Neu MP, Kabou A, Mechine A *et al.* L'immunohistochimie dans le diagnostic différentiel entre mésothéliome et adénocarcinome: Évaluation de 5 nouveaux anticorps et réévaluation de 6 anticorps traditionnels. *Ann Pathol* 1998; **18**: 460–65.
53 Oshio G, Yamaki K, Imamura T *et al.* Distribution of the carbohydrate antigens, DU-PAN-2 and CA19-9, in tumors of the lung. *Tumori* 1995; **81**: 67–73.
54 Chang K, Pai LH, Pass H *et al.* Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg Pathol* 1992; **16**: 259–68.
55 Argani P, Iacobuzio-Donahue C, Ryu B *et al.* Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: Identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res* 2001; **7**: 3862–8.
56 Chang K, Pai LH, Batra JK *et al.* Characterization of the antigen (CAK1) recognized by monoclonal antibody K1 present on ovarian cancers and normal mesothelium. *Cancer Res* 1992; **52**: 181–6.
57 Chang K, Pastan I, Willingham MC. Frequent expression of the tumor antigen CAK1 in squamous-cell carcinomas. *Int J Cancer* 1992; **51**: 548–54.
58 Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. *Int J Cancer* 1992; **50**: 373–81.
59 Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci USA* 1996; **93**: 136–40.
60 Churg A. Immunohistochemical staining for vimentin and keratin in malignant mesothelioma. *Am J Surg Pathol* 1985; **9**: 360–65.
61 Mullink H, Henzan-Logmans SC, Alons-van Kordelaar JJM *et al.* Simultaneous immunoenzyme staining of vimentin and cytokeratins with monoclonal antibodies as an aid in the differential diagnosis of malignant mesothelioma from pulmonary adenocarcinoma. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1986; **52**: 55–65.
62 Riera JR, Astengo-Osuna C, Longmate JA *et al.* The immunohistochemical diagnostic panel for epithelial mesothelioma: A reevaluation after heat-induced epitope retrieval. *Am J Surg Pathol* 1997; **21**: 1409–19.
63 Yaziji H, Battifora H, Barry TS *et al.* Evaluation of 12 antibodies for distinguishing epithelioid mesothelioma from adenocarcinoma: Identification of a three-antibody immunohistochemical panel with maximal sensitivity and specificity. *Mod Pathol* 2006; **19**: 514–23.
64 Chu AY, Litzky LA, Pasha TL *et al.* Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. *Mod Pathol* 2005; **18**: 105–10.
65 Kimura N, Kimura I. Podoplanin as a marker for mesothelioma. *Pathol Int* 2005; **55**: 83–6.
66 Ordóñez NG. D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. *Hum Pathol* 2005; **36**: 372–80.
67 Gordon GJ, Rockwell GN, Jensen RV *et al.* Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. *Am J Pathol* 2005; **166**: 1827–40.

Pathology International 2008; **58**: 75–83 doi:10.1111/j.1440-1827.2007.02193.x

Original Article

Differential diagnosis of sarcomatoid mesothelioma from true sarcoma and sarcomatoid carcinoma using immunohistochemistry

Kei Kushitani, Yukio Takeshima, Vishwa Jeet Amatya, Osamu Furonaka, Akio Sakatani and Kouki Inai

Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

Differentiation of sarcomatoid mesothelioma from other sarcomatoid tumors involving the pleura and other structures by light microscopy remains an important diagnostic challenge for surgical pathologists. The purpose of the present study was to investigate the utility of diagnostic immunohistochemistry for differentiating sarcomatoid mesothelioma from its histological mimics: true sarcoma and pulmonary sarcomatoid carcinoma. A total of 39 specimens of mesotheliomas with sarcomatoid components, 43 specimens of true sarcomas, and nine specimens of pulmonary sarcomatoid carcinomas were obtained from Japanese patients and examined using a 10-antibody panel (calretinin, WT1, AE1/AE3, CAM5.2, epithelial membrane antigen, desmin, α-smooth muscle actin, S-100 protein, CD34, and CD68). CAM5.2 had the highest sensitivity and specificity for differentiating sarcomatoid mesothelioma from true sarcoma. The combination of CAM5.2, WT1, and AE1/AE3 is recommended for routine pathological diagnosis. Accurate clinical information is necessary for differentiating sarcomatoid mesothelioma from sarcomatoid carcinoma.

Key words: CAM5.2, immunohistochemistry, sarcomatoid carcinoma, sarcomatoid mesothelioma, true sarcoma

Differential diagnosis of sarcomatoid mesothelioma from sarcomatoid tumors involving the pleura, peritoneum, pericardium, and tunica vaginalis is of critical importance for optimal clinical management; but this can be very challenging using light microscopy alone. Additional clinical information based on clinical history and diagnostic imaging is necessary, together with ancillary diagnostic procedures on the tissue, including immunohistochemistry and electron microscopy. A number of immunohistochemical markers have been proposed in recent years. However, most of these markers have been used for differentiating epithelioid mesothelioma from adenocarcinoma but not for characterizing sarcomatoid mesothelioma.[1–8] Moreover, the effectiveness of immunohistochemistry for differentiating sarcomatoid mesothelioma from other sarcomatoid tumors is unclear.

Therefore the purpose of the present study was to clarify the use of immunohistochemistry in the differential diagnosis of sarcomatoid mesothelioma from other sarcomatoid tumors, including true sarcoma involving the pleura and sarcomatoid carcinoma.

MATERIALS AND METHODS

Patients and histological samples

We used paraffin-embedded specimens from 39 patients with a definite histological diagnosis of mesothelioma who had undergone biopsy, surgical resection, or autopsy between 1995 and 2005. Nine specimens were obtained by biopsy; nine specimens by surgical resection; and 21 specimens by autopsy. All 39 mesotheliomas originated from the pleura. These specimens were provided by 27 external institutes. The 39 mesotheliomas were classified into two groups: the first consisted of 23 sarcomatoid mesotheliomas and the second consisted of 16 cases of biphasic mesothelioma with a definite sarcomatoid component. In the second group only the sarcomatoid component was examined.

For comparison, 43 true sarcomas showing spindle cell or pleomorphic morphology (Table 1) and nine pulmonary sarcomatoid carcinomas were examined according to the World Health Organization (WHO) criteria.[9,10] All true sarcomas originated from the non-pleural soft, visceral, or osseous tissue. All the sarcomatoid carcinomas were confirmed to have intrapulmonary tumor as the primary site. True sarcomas were obtained by surgical resection conducted between 1996 and

Correspondence: Kouki Inai, MD, PhD, Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: koinai@hiroshima-u.ac.jp

Received 12 December 2006. Accepted for publication 1 October 2007.

2005. Sarcomatoid carcinomas were obtained by lobectomy or autopsy conducted between 2002 and 2006. These samples were obtained from the archives of the Department of Pathology, Hiroshima University. In the present study we evaluated only the spindle cell component of sarcomatoid carcinomas. Informed consent was obtained from all subjects.

Each tumor specimen was reviewed by three pathologists (K.I., Y.T., and K.K.). All the mesothelioma cases were diagnosed based on the currently accepted histological criteria[10,11] associated with the immunohistochemical features; furthermore, the histological classification of true sarcoma and sarcomatoid carcinoma was confirmed according to the WHO classification.[9,10]

Immunohistochemical procedures

Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using the avidin–biotin–peroxidase method (ABC) with the Histofine SAB-PO kit (Nichirei, Tokyo, Japan), following an antigen retrieval procedure using a microwave or autoclave. The primary antibodies used in the present study are as follows: calretinin, WT1, pan-cytokeratin (AE1/AE3), CAM5.2, epithelial membrane antigen (EMA), desmin, α-smooth muscle actin (α-SMA), S-100 protein (S-100p), CD34, and CD68. Dilution and incubation periods of these markers are shown in Table 2. The grade of positivity was determined by the proportion of immunostained cells and was recorded as follows: 1+, 1–25%; 2+, 26–50%; and 3+, 51–100%.

Table 1 Sarcomatoid tumor phenotypes

Histology	*n*
Leiomyosarcoma	15
Malignant fibrous histiocytoma	6
Malignant peripheral nerve sheath tumor	6
Desmoid tumor	4
Dedifferentiated liposarcoma	3
Rhabdomyosarcoma	3
Fibrosarcoma	2
Myxofibrosarcoma	2
Intimal sarcoma	1
Malignant gastrointestinal stromal tumor	1
Pulmonary sarcomatoid carcinoma	9

RESULTS

Immunohistochemical profiles of sarcomatoid mesothelioma, true sarcoma, and sarcomatoid carcinoma

The results of immunohistochemical staining are summarized in Tables 3,4. A brief description of the reactivity of each antibody is as follows.

Calretinin

In sarcomatoid mesothelioma, positive results were observed mainly in the cytoplasm, with a great difference from epithelioid mesothelioma (Fig. 1a). Thirty percent of true sarcomas (four leiomyosarcomas, four desmoids, two malignant fibrous histiocytomas (MFH), one dedifferentiated liposarcoma, one fibrosarcoma, and one malignant peripheral nerve sheath tumor (MPNST)) and 66.7% of sarcomatoid carcinomas had calretinin positivity, mainly in the cytoplasm; but >51% reactivity was found only in two tumors (one desmoid and one sarcomatoid carcinoma).

WT1

Sarcomatoid mesothelioma generally had positive results in the cytoplasm (Fig. 1b), and the percentages of WT1-positive cells were greater than those of calretinin-positive cells. Forty-seven percent of true sarcomas (nine leiomyosarcomas, four MPNST, two MFH, two myxofibrosarcomas, one desmoid, one fibrosarcoma, and one malignant gastrointestinal stromal tumor) and 44.4% of sarcomatoid carcinomas had varying degrees of WT1 positivity in the cytoplasm.

Table 2 Antibodies used

Antibody to	Clone	Source	Dilution	Retrieval†
Calretinin	Poly	Zymed, San Francisco, CA, USA	1:100	MW, 5 min
WT1	6F-H12	DakoCytomation, Glostrup, Denmark	1:400	AC, 20 min
Cytokeratin-multi	AE1/AE3	Novocastra, Newcastle-upon-Tyne, UK	1:100	MW, 5 min
Cytokeratin (CAM5.2)	2A4	Becton-Dickinson, Mountain View, CA, USA	Pre-diluted	MW, 5 min
EMA	E29	DakoCytomation, Glostrup, Denmark	1:100	MW, 5 min
Desmin	D33	DakoCytomation, Glostrup, Denmark	1:100	MW, 5 min
α-SMA	1A4	DakoCytomation, Glostrup, Denmark	1:100	MW, 5 min
S-100p	Poly	DakoCytomation, Glostrup, Denmark	1:1000	MW, 5 min
CD34	QBEnd/10	Novocastra, Newcastle-upon-Tyne, UK	1:50	MW, 5 min
CD68	KP-1	DakoCytomation, Glostrup, Denmark	1:100	MW, 5 min

†In citrate buffer (pH 6.0).

AC, autoclave; α-SMA, c-smooth muscle actin; EMA, epithelial membrane antigen; MW, microwave.

Table 3 Positive mesothelioma markers

	Sarcomatoid mesothelioma						True sarcoma						Sarcomatoid carcinoma					
	Positive cases		Grading of reactivity				Positive cases		Grading of reactivity				Positive cases		Grading of reactivity			
Markers	*n*	%	0	1+	2+	3+	*n*	%	0	1+	2+	3+	*n*	%	0	1+	2+	3+
Calretinin	34/39	87.2	5	23	7	4	13/43	30.2	30	9	3	1	6/9	66.7	3	4	1	1
WT1	35/39	89.7	4	16	11	8	20/43	46.5	23	7	5	8	4/9	44.4	5	1	1	2
AE1/AE3	33/39	84.6	6	12	9	12	2/43	4.7	41	2	0	0	8/9	88.9	1	2	0	6
CAM5.2	36/39	92.3	3	6	3	27	3/43	7.0	40	2	1	0	7/9	77.8	2	1	0	6
EMA	19/39	48.7	20	14	5	0	5/43	11.6	38	5	0	0	8/9	88.9	1	5	0	3

Table 4 Mesenchymal markers on sarcomatoid mesotheliomas

	Positive cases		Grading of reactivity			
Markers	*n*	%	0	1+	2+	3+
Desmin	3/39	7.7	36	2	1	0
α-SMA	22/37	59.5	15	8	6	8
S-100p	13/36	36.1	23	12	1	0
CD34	2/32	6.3	30	1	0	1
CD68	22/36	61.1	14	13	5	4

AE1/AE3

Eighty-five percent of sarcomatoid mesotheliomas had cytoplasmic positive staining for AE1/AE3 (Fig. 1c), although the proportion of positive cells varied from 1+ to 3+. Two sarcomas (one leiomyosarcoma and one MFH) were positive for AE1/AE3, but the reaction was focal and weak (1+). Sarcomatoid carcinomas generally had diffuse and strong positive staining (3+) for AE1/AE3 in the cytoplasm.

CAM5.2

More than 90% of sarcomatoid mesotheliomas had diffuse and strong cytoplasmic positive staining (3+) for CAM5.2 (Fig. 1d). The reaction was focal (1+ or 2+) in three CAM5.2-positive true sarcomas (two leiomyosarcomas and one MPNST). Sarcomatoid carcinomas generally had diffuse and strong positive staining (3+) for CAM5.2 in the cytoplasm.

EMA

Forty-nine percent of sarcomatoid mesotheliomas and 12% of true sarcomas (four leiomyosarcomas and one MPNST) had focal (1+ or 2+) and weak cytoplasmic positive staining for EMA. A diffuse (3+) and strong positive staining for EMA in the cytoplasm was observed in 89% sarcomatoid carcinomas and three EMA-positive sarcomatoid carcinomas (Fig. 2).

Desmin and α-SMA

The results for desmin and α-SMA are shown in Table 4. In sarcomatoid mesothelioma, positive staining for desmin was observed only in three cases (8%), but the reaction was focal (1+ or 2+) and weak. In addition, 60% of sarcomatoid mesotheliomas were positive for α-SMA, and the positive reactions for α-SMA were wider and stronger than those for desmin. In contrast, among true sarcomas, myogenic sarcoma was naturally positive for α-SMA and desmin in the cytoplasm. Sarcomatoid carcinoma was completely negative for desmin; but 57% of sarcomatoid carcinomas (5/9) had positive staining for α-SMA, while two had diffuse (3+) and strong cytoplasmic positive staining (data not shown).

S-100p, CD34, and CD68

In sarcomatoid mesothelioma, cytoplasmic positive staining for S-100p was observed in 13 of 36 cases (31%) but the reaction was focal (1+) and weak in most of the positive cases (12/13). Positive results for CD34 were observed only in two sarcomatoid mesotheliomas (6%); but one of these two had diffuse (3+) and strong cytoplasmic positive staining. CD34 positive staining is often observed in solitary fibrous tumors;[12] but the one that had diffuse positive staining for CD34 also had the same pattern for AE1/AE3, CAM5.2, and WT1. Therefore, it can be inferred with a fair amount of certainty that this case was of sarcomatoid mesothelioma and not of solitary fibrous tumor. Sixty-one percent (22 of 36 cases) of sarcomatoid mesotheliomas had cytoplasmic positive staining for CD68, and diffuse (3+) and strong positive staining was observed in four. In contrast, among true sarcomas, neurogenic sarcoma was naturally positive for S-100p in the cytoplasm. In addition, 48% and 82% of sarcomas had positive staining for CD34 and CD68 irrespective of histological classification. In sarcomatoid carcinoma, 67% and 100% had positive staining for S-100p and CD68, respectively, but CD34 was completely negative.

Immunohistochemistry for sarcomatoid mesothelioma and true sarcoma

Comparison of the immunohistochemical findings of sarcomatoid mesothelioma and true sarcoma is shown along with statistical analysis in Fig. 3(a). The results indicate that WT1, calretinin, AE1/AE3, and CAM5.2 were statistically significantly different between sarcomatoid mesothelioma and true sarcoma.

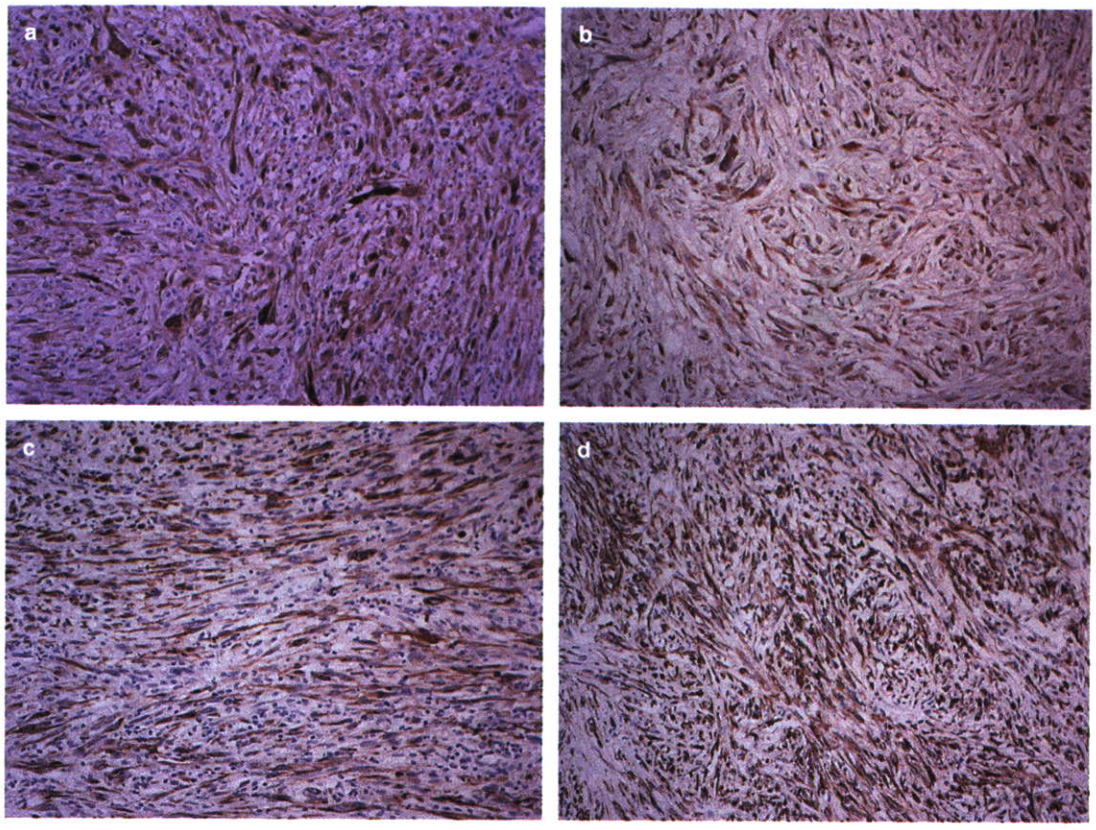

Figure 1 Immunohistochemistry of sarcomatoid mesothelioma: (**a**) nuclear and cytoplasmic positivity for calretinin; (**b**) cytoplasmic positivity for WT1; (**c**) cytoplasmic positivity for AE1/AE3; (**d**) strong cytoplasmic positivity for CAM5.2.

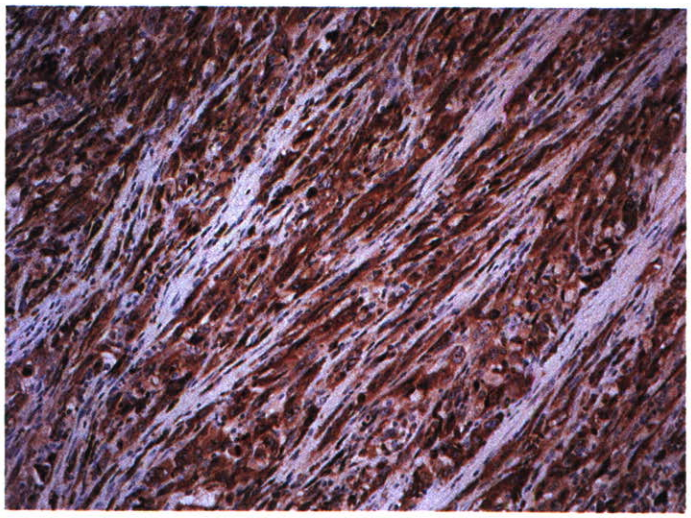

Figure 2 Immunohistochemistry of sarcomatoid carcinoma: cytoplasmic positivity for epithelial membrane antigen.

The sensitivity and specificity of one, two, or three of these positive markers for the differentiation of sarcomatoid mesothelioma and true sarcoma are shown in Tables 5–7.

With regard to each positive marker, CAM5.2 had the highest sensitivity while AE1/AE3 had the highest specificity in sarcomatoid mesotheliomas. The specificity of CAM5.2 was slightly inferior to that of AE1/AE3, but the reactivity of CAM5.2 was considerably wider and stronger than that of AE1/AE3 in sarcomatoid mesothelioma. Therefore, CAM5.2 is suggested to be the most useful positive marker for differentiating sarcomatoid mesothelioma from true sarcoma. The sensitivity of calretinin and WT1 was higher than that of AE1/AE3 but their specificities were considerably inferior to that of AE1/AE3 (Table 5).

Among the combinations of two antibodies, both the combination of WT1 and AE1/AE3 (either WT1 positivity or AE1/AE3 positivity) and that of WT1 and CAM5.2 (either WT1 positivity or CAM5.2 positivity) had the highest sensitivity, while the combination of calretinin and CAM5.2 (both calretinin and CAM5.2 positivity) and that of AE1/AE3 and CAM5.2 (both calretinin and CAM5.2 positivity) had the highest specificity (Table 6).

Among the combinations of three antibodies, the combination of CAM5.2, WT1, and AE1/AE3 (CAM5.2 positivity and (WT1 positivity or AE1/AE3 positivity)) had the highest sensitivity, but the sensitivity of this combination was inferior to that of WT1 and AE1/AE3 combination and WT1 and CAM5.2 combination. The combination of AE1/AE3, calretinin, and WT1 (AE1/AE3 positivity and (calretinin positivity or WT1 positivity)), the combination of AE1/AE3, calretinin, and CAM5.2 (AE1/AE3 positivity and (calretinin positivity or CAM5.2 positivity)), and the combination of AE1/AE3, WT1, and CAM5.2 (AE1/AE3 positivity and (WT1 positivity or CAM5.2 positivity)) had the highest specificity, and the specificity of these combinations was equal to that of calretinin and CAM5.2 combination and AE1/AE3 and CAM5.2 combination (Table 7).

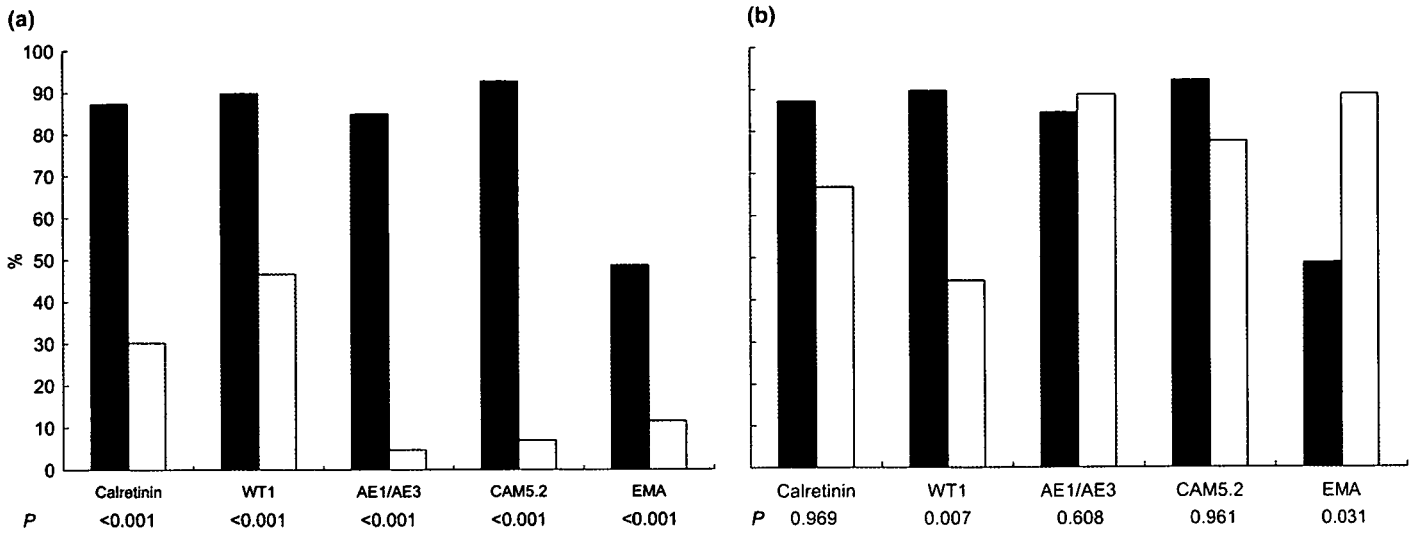

Figure 3 Positivity of each marker for (**a**) (■) sarcomatoid mesothelioma and (□) true sarcoma; and (**b**) (■) sarcomatoid mesothelioma and (□) sarcomatoid carcinoma (Fisher's exact test).

Table 5 Immunohistochemistry in sarcomatoid mesothelioma for one marker

One marker	Sensitivity (%)	Specificity (%)
Calretinin(+)	87.2	69.8
WT1(+)	89.7	53.5
AE1/AE3(+)	84.6	95.3
CAM5.2(+)	92.3	93.0

Table 6 Immunohistochemistry in sarcomatoid mesothelioma for two markers

Two markers	Sensitivity (%)	Specificity (%)
Calretinin(+) or WT1(+)	100	27.9
Calretinin(+) or AE1/AE3(+)	92.3	67.4
Calretinin(+) or CAM5.2(+)	94.9	67.4
WT1(+) or AE1/AE3(+)	100	48.8
WT1(+) or CAM5.2(+)	100	48.8
AE1/AE3(+) or CAM5.2(+)	92.3	90.7
Calretinin(+) and WT1(+)	76.9	95.3
Calretinin(+) and AE1/AE3(+)	79.5	97.7
Calretinin(+) and CAM5.2(+)	84.6	97.7
WT1(+) and AE1/AE3(+)	74.4	95.3
WT1(+) and CAM5.2(+)	82.1	97.7
AE1/AE3(+) and CAM5.2(+)	84.6	97.7

Immunohistochemistry findings of sarcomatoid mesothelioma and sarcomatoid carcinoma

Comparison of the immunohistochemical findings of sarcomatoid mesothelioma and sarcomatoid carcinoma is shown along with statistical analysis results in Fig. 3(b): It appears that WT1 is a positive marker while EMA is a negative marker for sarcomatoid mesothelioma; but the specificity of these markers for the differentiation of sarcomatoid mesothelioma and sarcomatoid carcinoma is relatively low. Therefore, these markers are not useful for differentiating these tumors.

Table 7 Immunohistochemistry in sarcomatoid mesothelioma for three markers

Three markers	Sensitivity (%)	Specificity (%)
Calretinin(+) and (WT1(+) or AE1/AE3(+))	87.2	93.0
Calretinin(+) and (WT1(+) or CAM5.2(+))	87.2	90.7
Calretinin(+) and (AE1/AE3(+) or CAM5.2(+))	84.6	95.3
WT1(+) and (Calretinin(+) or AE1/AE3(+))	82.1	95.3
WT1(+) and (Calretinin(+) or CAM5.2(+))	84.6	93.0
WT1(+) and (AE1/AE3(+) or CAM5.2(+))	82.1	97.7
AE1/AE3(+) and (Calretinin(+) or WT1(+))	84.6	97.7
AE1/AE3(+) and (Calretinin(+) or CAM5.2(+))	84.6	97.7
AE1/AE3(+) and (WT1(+) or CAM5.2(+))	84.6	97.7
CAM5.2(+) and (Calretinin(+) or WT1(+))	92.3	93.0
CAM5.2(+) and (Calretinin(+) or AE1/AE3(+))	89.7	95.3
CAM5.2(+) and (WT1(+) or AE1/AE3(+))	92.3	95.3

Diagnostic flowchart based on immunohistochemical scoring of sarcomatoid mesothelioma and true sarcoma

A diagnostic flowchart based on immunohistochemical scoring of sarcomatoid mesothelioma and true sarcoma is shown in Fig. 4. If tumor cells have diffuse (3+) positive results for CAM5.2, the tumor is probably mesothelioma. If tumor cells are CAM5.2 negative and 1+ or 2+ and AE1/AE3 negative, the tumor is probably true sarcoma. If any discrepancy exists between pathological diagnosis and clinical findings, WT1 can be used to differentiate mesothelioma from true sarcoma.

DISCUSSION

Malignant mesothelioma is subclassified into three types: epithelioid, sarcomatoid, and biphasic.[10] In the case of