Am J Cancer Res 2011;1(1):14-24
www.ajcr.us /ISSN:2156-6976/ajcr0000001

Original Article

The immunohistochemical characterization of sarcomatoid malignant mesothelioma of the pleura

Lucian R. Chirieac[1,3] Geraldine S. Pinkus[1,3] Jack L. Pinkus[1,3], John Godleski[1,3] David J. Sugarbaker[2,3], Joseph M. Corson[1,3]

Departments of [1]Pathology, and [2]Surgery, Brigham and Women's Hospital, and [3]Harvard Medical School, Boston, MA, USA.

Received August 26, 2010; accepted September 20, 2010; Epub September 30, 2010; published January 1, 2011

Abstract: The immunohistochemical characteristics of epithelioid malignant mesothelioma are well described. However, immunohistochemical analyses of sarcomatoid mesothelioma, the less common type, are limited and its distinction from other tumors of the chest wall, lung and pleura is often problematic. We evaluated 24 patients with pleural sarcomatoid mesothelioma who had surgery (12 extrapleural pneumonectomies, 9 pleurectomies and 3 large biopsies) between 1989 and 2005. Clinicopathologic features and demographic data were recorded. We describe immunohistochemical results for 10 antibodies: AE1/AE3, CAM5.2 and MNF-116 keratins, calretinin, WT-1 protein, bcl-2, CD34, desmin, D2-40 and podoplanin. The patients were 23 men and one woman with a median age at diagnosis of 64.7 years (range 47 to 76). Tumor cells were positive for the keratin proteins AE1/AE3 in 18/24 cases, CAM 5.2 in 23/24 cases and MNF-116 in 21/21 cases. Calretinin was positive in 6/24 cases, WT-1 (nuclear) in 8/24 cases, bcl-2 in 0/24 cases, CD34 in 0/24 cases, desmin in 0/24 cases, D2-40 in 24/24 cases and podoplanin in 24/24 cases. This panel of antibodies may be helpful in establishing a pathologic diagnosis of sarcomatoid mesothelioma. In our study, D2-40 and podoplanin are highly sensitive immunohistochemical markers for sarcomatoid mesothelioma. Additional studies are required to define their role in the differential diagnosis of other spindle cell tumors.

Keywords: Malignant mesothelioma, sarcomatoid, pleura, immunohistochemistry

Introduction

Diffuse malignant mesothelioma (DMM) is an aggressive neoplastic proliferation derived from cells lining the serosal membranes [1,2]. Malignant mesothelioma has been classified pathologically into epithelioid, sarcomatoid, mixed epithelioid and sarcomatoid (biphasic) types (some authors prefer epithelial and sarcomatous terminology) [3]. Pathologic classification is based on the histologic patterns and has prognostic value [1,2]

The epithelioid type of mesothelioma is more common and has a well-characterized immunophenotype that is used widely in the distinction from adenocarcinoma of lung and other tumors. Sarcomatoid mesotheliomas are uncommon tumors and published series are infrequent [4,5]. Epithelioid mesothelioma has a well-characterized immunophenotype, [6-15] but relatively few immunohistochemical studies of sarcomatoid mesothelioma have been reported and the role of immunohistochemistry in the histopathologic characterization of this type of mesothelioma is still limited [4,5,16-21] Therefore we examined clinical and pathological features, including histopathologic and immunophenotypic findings, of 24 patients with sarcomatoid malignant mesothelioma of the pleura who had available surgical pathology specimens [22]. In order to define a panel that could prove useful in establishing a pathologic diagnosis of sarcomatoid mesothelioma, we used commercial antibodies for AE1/AE3, CAM5.2 and MNF-116 keratins, calretinin and WT-1 protein, and bcl-2, CD34 and desmin. We also investigated the expression of D2-40 and podoplanin, two

Table 1. Summary of characteristics of patients with sarcomatoid malignant mesothelioma (N = 24 Cases)

Characteristics	Number (%)*
Age (yrs)	
Mean ± SD	64.7 ± 6
Median	63.6
Range	47-76
Gender – n (%)	
Male	23 (96)
Female	1 (4)
Histology – n (%)	
Sarcomatoid NOS	18 (75)
Desmoplastic	6 (25)
Anatomic location – n (%)	
Left pleura	6 (25)
Right pleura	18 (75)
Type of Surgery – n (%)	
Large biopsy	3 (13)
Pleurectomy	9 (38)
Extrapleural pneumonectomy	12 (50)
Asbestos body count (per g of lung)†	
Mean ± SD	2344.7 ± 1523.8
Median	2306
Range	356-5576

*Due to rounding, not all percentages total 100.
†Asbestos body count was available in 10 of 12 extrapleural pneumonectomies.

markers that have been reported to be highly specific and sensitive for epithelioid mesothelioma [12,23].

Materials and methods

Patients and specimens

We studied 24 patients with pleural sarcomatoid mesothelioma who had surgery at Brigham and Women's Hospital between 1989 and 2005. The demographic information, tumor site, type of surgical resection and asbestos body count (**Table 1**) were obtained from the files of the Department of Pathology. The study was approved by the Institutional Review Board.

Surgical pathology specimens were 12 extrapleural pneumonectomies, 9 pleurectomies and 3 large biopsies (with a mean of 82 mm^2 evaluable tumor area). We excluded cases with limited pathologic material or cases referred to our institution for consultation. Hematoxylin and eosin-stained slides were reviewed, and the histologic diagnosis of sarcomatoid mesothelioma was confirmed by two pathologists (LRC and JMC) using established criteria [3,24]. A tumor was diagnosed as desmoplastic sarcomatoid mesothelioma if at least 50% of the tumor had areas of abundant hyalinization. Both pathologists scored additional pathologic variables independently; discrepancies were reviewed to achieve a consensus.

Asbestos body counts are performed as part of the pathological assessment of mesotheliomas in our department. Methodology used has been previously described [25].

Immunohistochemistry

Selected sections from each case were examined using an immunohistochemical panel of ten commercial antibodies. **Table 2** lists the primary antibodies and technical conditions for immunohistochemical studies. The incubation

Table 2. Primary antibodies and technical conditions for immunohistochemical studies

Antibody	Titer	Source	Pretreatment	Detection System
Keratin Proteins				
Clone MNF-116	1:100	Dako Carpinteria, CA	Trypsin	Envision + (Dako)
Clone CAM 5.2	1:20	Becton-Dickinson San Jose, CA	Steamer/ EDTA	Envision +
Clone AE1/AE3	1:400	Dako	Steamer/ EDTA	Envision +
Calretinin	1:150	Zymed S. San Francisco, CA	Pressure cooker/ citrate	Envision +
Rabbit polyclonal				
WT-1	1:100	Dako	Steamer/ EDTA	Envision +
Clone 6F-H2				
CD34	1:50	Coulter Miami, FL	Pressure cooker/ citrate	Envision +
Clone QBEnd/10				
Bcl-2	1:30	Dako	Steamer/ EDTA	Envision +
Clone 124				
Desmin	1:50	Dako	Steamer/ EDTA	Envision +
Clone DE-R-11				
Podoplanin	1:100	AngioBio Co. Del Mar, CA	Pressure cooker/ citrate	Envision +*
Hybridoma supernatant				
D2-40 lymphatic endothelial marker	1:100	Covance/Signet Labs Dedham, MA	Pressure cooker/ citrate	Envision +*
Clone D2-40				

*Amplified 3-step technique (rabbit anti-mouse immunoglobulins antibody followed by rabbit Envision+)

was 1 hour at room temperature for all antibodies. In order to evaluate the specificity of the antibody, known positive and negative tissues were used as controls. The evaluated antibodies were clustered into four groups: (1) Keratins AE1/AE3, CAM 5.2 and MNF 116 known to be positive in epithelioid malignant mesothelioma, as well as in sarcomatoid carcinomas but not in most sarcomas [6,19]; (2) Calretinin and WT-1 known to be useful markers for epithelioid malignant mesothelioma [15,26-28]; (3) Bcl-2 reported to be positive in synovial sarcomas [29-35] CD34, positive in most solitary fibrous tumors (SFT) [36], and desmin, positive in leiomyosarcomas [37], and negative in limited studies of sarcomatoid malignant mesothelioma [38,39]; (4) and D2-40 and podoplanin, reported initially in epithelioid malignant mesothelioma as having high specificity and sensitivity [23,40]. The monoclonal antibody D2-40 recognizes the membrane protein podoplanin, which is a marker for germ cell tumors, mesotheliomas, and other tumor types [41-44]. To eliminate background staining from endogenous biotin, the EnVision technique was used.[45] The stained sections were reviewed by three pathologists (L.R.C., G.S.P., and J.M.C.), and a score was given for percentage of cell staining. The extent of immunoreactivity in neoplastic cells was graded according to the percentage of reactive cells: 0 if staining was absent in neoplastic cells or present in less than 1% of tumor cells; and 1 through 4 for each 25% increment in positive tumor cells (**Table 3**). We reported each case with grade 1 or higher as positive and cases with grade 0 as negative (**Table 3**). The number of cases positive for each specific antibody was also analyzed and reported as a percentage of all cases immunostained.

Results

Patient demographics and pathologic characteristics

The patient demographics and pathologic characteristics of the sarcomatoid malignant mesothelioma are summarized in **Table 1** and illus-

Table 3. Grading system used for each case according to the extent of immunoreactivity in neoplastic cells

Percentage of immunoreactive cells	Grade*
Less than 1%.	0
1-25%.	1
26-50%.	2
51-75%.	3
76-100%.	4

* Each case with grade 1 or higher was reported as positive and cases with grade 0 as negative.

trated in **Figure 1**. There were 23 men and one woman. The median age was 63.6 years (range 47-76 years). Most of the primary tumors were in the right pleura [n=18 (75%)] and the remainder of the evaluated cases were located in left pleura [n=6 (25%)]. Twelve patients (50%) had extrapleural pneumonectomy, nine (37.5 %) had pleurectomy and 3 had biopsy (12.5 %). These characteristics reflected the patient population in the malignant mesothelioma group. The majority of tumors (75%) were sarcomatoid mesotheliomas, with predominant spindle tumor cells with a subtle wavy architecture, and a diffuse infiltrating growth pattern. This pattern has a

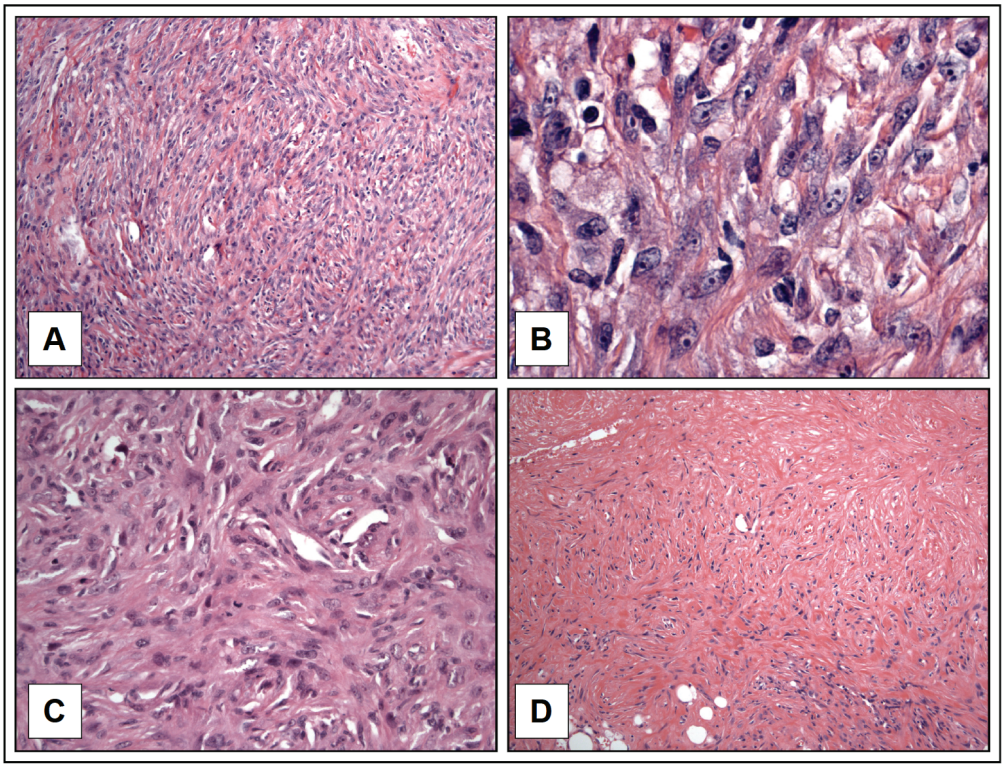

Figure 1. Histopathology of sarcomatoid DMM. **Panels A and B.** One of the more common patterns of sarcomatoid malignant mesothelioma. Note a diffuse, infiltrative growth pattern. Predominant spindle tumor cells with a subtle wavy pattern. This pattern has a striking resemblance to sarcoma or sarcomatoid carcinoma. (**A.** H&E 200x, **B.** H&E 600x). **Panel C.** Higher power magnification from a sarcomatoid mesothelioma showing spindle tumor cells with high nuclear pleomorphism and scattered mitoses. (H&E 400x). **Panel D.** This photomicrograph shows a typical desmoplastic mesothelioma. The tumor is hypocellular with cells arranged in a storiform pattern, in a heavily collagenized stroma, invading into the adipose tissue of the thoracic wall. Cells are small with small uniform nuclei. (H&E 400x).

Table 4. Immunohistochemical profile of sarcomatoid malignant mesothelioma of the pleura.

Case Nr.	AE1/AE3	CAM5.2	MNF-116*	Calretinin	WT-1	CD34	Bcl-2	Desmin	D2-40	Podoplanin
1	4	4	4	0	0	0	0	0	1	1
2	1	4	1	4	1	0	0	0	3	3
3	3	3	NP	1	0	0	0	0	2	2
4	2	2	3	0	0	0	0	0	3	4
5	1	2	NP	0	0	0	0	0	2	1
6	3	1	1	0	0	0	0	0	2	2
7	3	4	2	2	0	0	0	0	1	1
8	4	4	4	0	0	0	0	0	1	3
9	0	3	1	0	3	0	0	0	3	4
10	2	2	1	0	0	0	0	0	3	3
11	2	1	NP	0	0	0	0	0	1	1
12	0	2	1	0	0	0	0	0	4	4
13	1	3	3	0	0	0	0	0	1	2
14	0	1	3	0	1	0	0	0	1	2
15	0	3	3	0	0	0	0	0	1	2
16	3	3	1	2	2	0	0	0	1	1
17	1	1	2	0	1	0	0	0	2	2
18	0	3	1	0	0	0	0	0	1	1
19	1	3	3	0	0	0	0	0	1	2
20	2	3	4	1	0	0	0	0	1	1
21	3	4	2	1	0	0	0	0	2	2
22	1	3	1	0	1	0	0	0	2	2
23	0	0	1	0	1	0	0	0	4	4
24	2	3	2	0	1	0	0	0	3	4
Total Positive Cases	18	23	21	6	8	0	0	0	24	24

*NP: not performed.

striking resemblance to sarcoma or sarcomatoid carcinoma (**Figure 1A** and **1B**). Some cases displayed spindle tumor cells with high nuclear pleomorphism and scattered mitoses (**Figure 1C**). Six cases (25%) were desmoplastic mesothelioma. The desmoplastic tumors were hypocellular with cells arranged in a storiform pattern, in a heavily collagenized stroma, invading into the adipose tissue of the thoracic wall. Desmoplastic tumor cells had small, hyperchromatic nuclei (**Figure 1D**). The median overall survival of all patients (N=26) with sarcomatoid mesothelioma in this study was 5.1 months (range 1-28 months).

The asbestos body count results are also included in **Table 1**. Patients had a median of 2306 asbestos bodies per gram of wet lung tissue (range 356-5576). Mesothelioma had a broad range of asbestos body counts, (**Table 1**) reflecting high asbestos exposure. The median control level in our laboratory is 20.

Immunohistochemical characteristics of sarcomatoid malignant mesothelioma

The immunohistochemical characteristics that we observed in sarcomatoid malignant mesothelioma are shown in **Table 4** and **Figures 2** and **3**. Tumor cells were positive for: AE1/AE3 in 18 of 24 cases, for CAM 5.2 in 23 of 24 cases and for MNF-116 in 21 of 21 cases. In three cases MNF-116 was not performed due to exhaustion of tissue from the paraffin block. Although all cases analyzed were positive for MNF-116 keratin, thirteen (56.5%) were positive in less than 50% of tumor cells (**Figure 4**). However, none of the examined mesotheliomas were concomitantly negative for all of the epithelial markers (**Table 4**). Tumor cells were

positive for: WT-1 in 8 of 24 cases (33.3) and for calretinin in 6 of 24 cases (25%) (**Figure 5**). Six mesotheliomas (25%) were positive for WT-1 only, four (16.6%) for calretinin only, twelve (50%) were negative for both WT-1 and calretinin and only two mesotheliomas (8.3%) reacted simultaneously with both WT-1 and calretinin. Both tumors had a classic sarcomatoid mesothelioma pattern and a large biopsy was performed in each case. 24 out of 24 cases from our study had tumor cells negative for CD34, bcl-2, and desmin. All cases showed some degree of positivity when stained with both D2-40 and podoplanin (**Figure 4**). However in most of the cases (17 of 24, 70.8% and 16 of 24, 66.7%) positive tumor cells were present in less than 50% of the tumor for both D2-40 and podoplanin, respectively (**Table 4** and **Figure 4**). Therefore in only 7 of 24 (29.2%) and in 8 of 24 (33.3%) cases were tumor cells positive in more than 50% of the tumor for both D2-40 and podoplanin, respectively. Only one tumor (case nr. 8, **Table 4**) showed less than 50% positivity for D2-40 and more than 50% positivity for podoplanin. Some areas of the tumor were negative with appropriate positive staining of lymphatic endothelial cells.

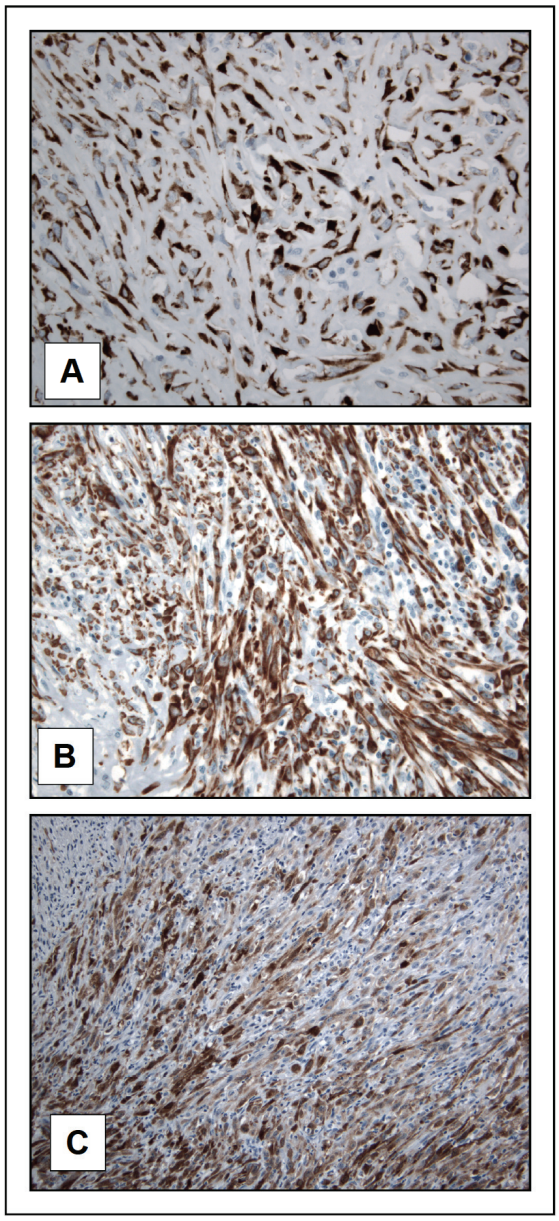

Figure 2. Immunophenotype of sarcomatoid malignant mesothelioma. **Panel A.** This photomicrograph illustrates positive immunostaining with AE1/AE3 in desmoplastic malignant mesothelioma (H&E 400x). **Panel B.** This photomicrograph illustrates positive immunostaining with CAM5.2 in sarcomatoid malignant mesothelioma (H&E 200x). **Panel C.** Positive immunostaining with calretinin. Almost all tumor cells are immunoreactive with calretinin. This case was scored 4 since more than 75% tumor cells had positive nuclear or cytoplasmic staining (H&E 400x).

Discussion

Distinction of sarcomatoid mesothelioma from sarcomatoid carcinoma, various sarcomas and other tumors of the chest wall, lung and pleura is often problematic. In the present study we examined clinical and pathological features, including histopathologic and immunophenotypic findings, in patients with sarcomatoid malignant mesothelioma of the pleura who had available large surgical pathology specimens and excluded cases with limited pathologic material. We confirmed presence of asbestos exposure by finding high asbestos body counts by quantitative asbestos analysis in the lungs of the majority of patients with extrapleural pneumonectomy. In addition, we investigated expression of two additional markers in sarcomatoid malignant mesothelioma, D2-40 and podoplanin, which are reported to be expressed in 90% of epithelioid mesotheliomas, but not in any other carcinomas [23] and in approximately 75% of sarcomatoid mesotheliomas [5].

Epithelioid malignant mesothelioma is the most common subtype and many studies have described a number of immunohistochemical markers that can make possible the distinction between epithelioid pleural mesothelioma and pulmonary peripheral adenocarcinomas [6,46]. Pleural epithelioid malignant mesothelioma has distinctive clinical-pathologic features and a

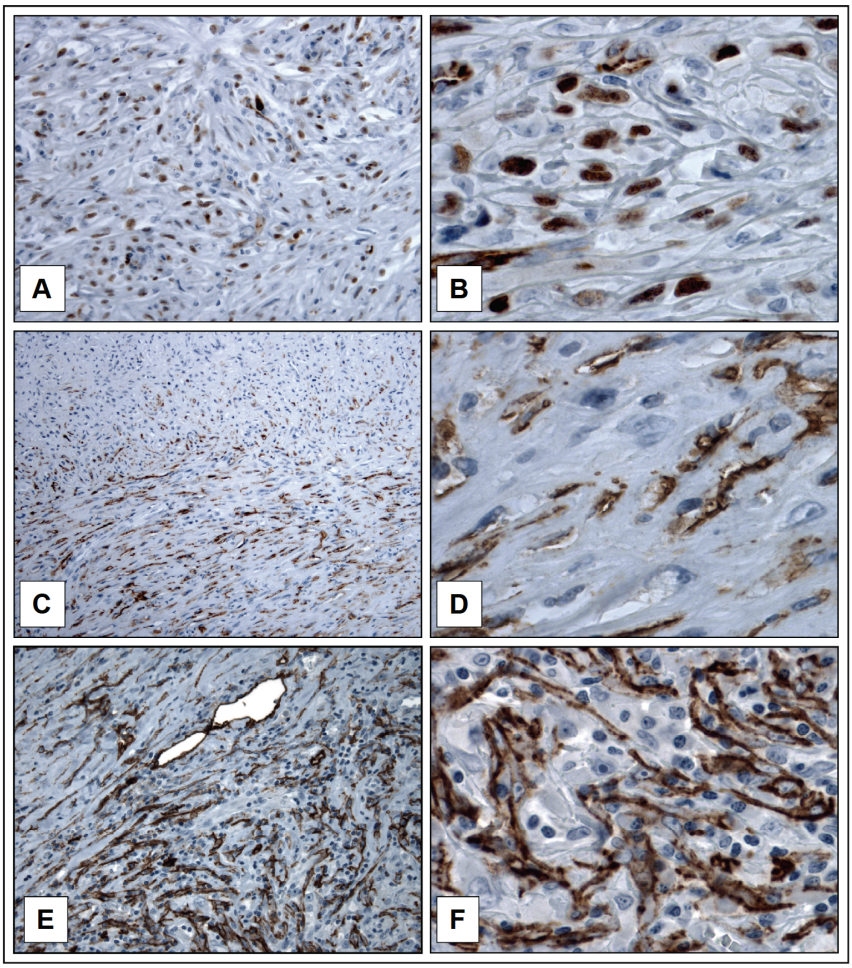

Figure 3. Immunophenotype of sarcomatoid malignant mesothelioma. **Panels A and B.** Sarcomatoid mesothelioma cells positive for WT-1. This case was scored as 4 since more than 75% tumor cells had positive nuclear staining (**A.** H&E 200x, **B.** H&E 600x). **Panels C and D.** This slide shows that the tumor cells in the lower part of the tumor stain with D2-40, whereas the tumor cells in the upper part do not. (**C.** H&E 200x, **D.** H&E 600x). **Panels E and F.** These photomicrographs show sarcomatoid malignant mesothelioma positive for podoplanin, with positive lymphatic channels (**E.** H&E 200x, **F.** H&E 600x). Note the positive internal control lymphatic endothelial cells.

characteristic immunophenotype. Recent reports have acknowledged two additional markers, D2-40 and podoplanin which are expressed in 90% of epithelioid mesotheliomas, but not in any other carcinomas [23,47,48]. D2-40 is a monoclonal antibody that recognizes the membrane protein podoplanin, which is a marker for germ cell tumors, mesotheliomas, other tumor types and in a variety of normal cells, including endothelial cells of lymphatic vessels, and mesothelial cells [10,23,43,44,47,48]. Podoplanin, a 36 kDa membrane glycoprotein of podocytes, is expressed in the endothelium of lymphatic vessels and distinctive types of angiosarcoma [47,49-51]. Because they are highly sensitive and specific for epithelioid mesotheliomas, D2-40 and podoplanin may be considered for inclusion in a series of antibodies to distinguishing epithelioid mesotheliomas from carcinomas metastatic to the serosal membranes.

In our current study, we show that tumor cells were positive for the keratin proteins AE1/AE3, CAM 5.2 and MNF-116 (**Figure 5**). The low AE1/AE3 sensitivity (75%) of sarcomatoid mesotheliomas in our study is somewhat surprising and would justify the addition of another keratin in the work up of sarcomatoid mesotheliomas that are AE1/AE3 negative. Calretinin was positive in 6/24 cases, WT-1 (nuclear) in 8/24 cases (**Figure 5**). We confirmed bcl-2, CD34 as negative markers of sarcomatoid malignant mesotheliomas as reported in previous studies (**Figure 5**). In addition, we show that D2-40 and podoplanin are highly sensitive immunohistochemical markers for sarcomatoid mesothelioma. In contrast to previous published analyses that reported the immunophenotype of sarcomatoid mesothelioma on tissue microarrays [5], our study evaluated the immunohistochemistry characteristics on larger sections that

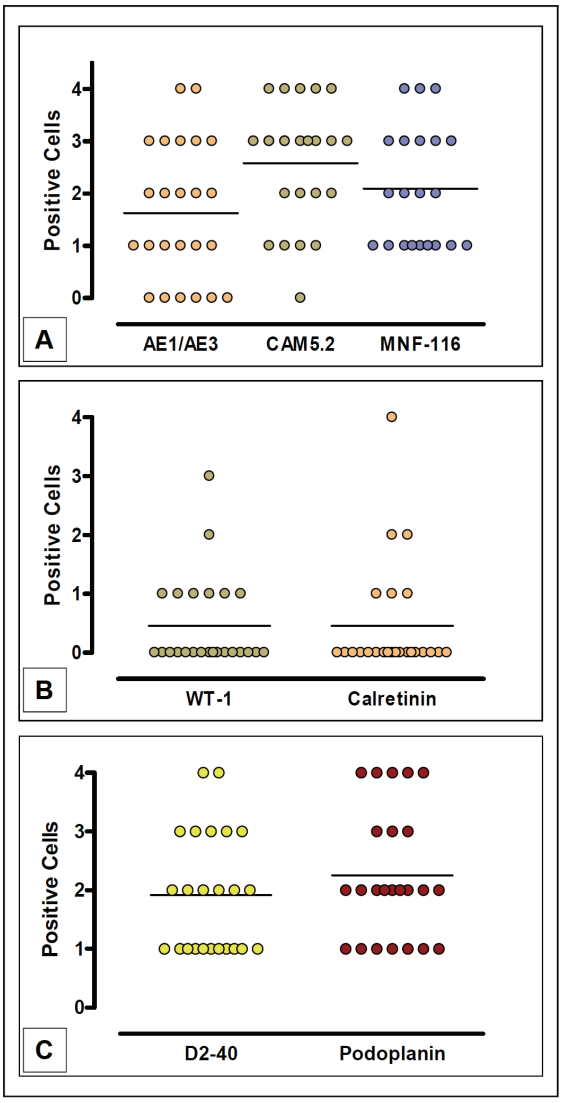

Figure 4. Scatter plot graph illustrating the immunophenotype of sarcomatoid malignant mesothelioma and the percentage of positive immunoreactive tumor cells in our study. **Panel A.** Distribution of cases according to the degree of immunoreactive positive cells with the keratin markers, CAM 5.2, AE1/AE3, and MNF-116. In addition, all of the examined mesotheliomas were positive for at least one of the keratin antibodies. **Panel B** shows the distribution of cases according to the degree of immunoreactive positive cells with WT-1 and calretinin. **Panel C** shows the distribution of cases according to the degree of immunoreactive positive cells with D2-40 and podoplanin. Horizontal lines represent mean values.

will minimize the rate of false negative results due to tumor heterogeneity (**Figure 3**). Although some investigators extrapolated the immunohistochemistry findings of the sarcomatoid component of mixed mesotheliomas to the "pure" sarcomatoid mesotheliomas [4], our study is unique since we were able to evaluate large amounts of tumor tissue from well-characterized sarcomatoid mesotheliomas. As previously reported, the median overall survival of all patients with sarcomatoid mesothelioma in this study was 5.1 months (range 1-28 months), similar to that observed in other studies [2].

Chronic fibrosing pleuritis is not a differential diagnosis when invasion is identified; however, in biopsy specimens, the distinction from chronic fibrosing pleuritis can be more problematic. Although superficial extension of reactive fibroblasts in parallel and linear layers may be

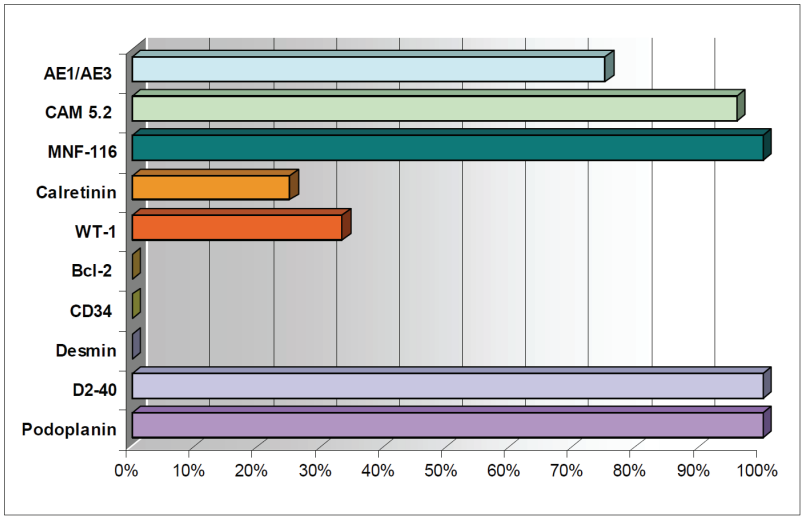

Figure 5. Bar graph illustrating the immunophenotype of sarcomatoid malignant mesothelioma and the percentage of positive cases in our study. D2-40 and podoplanin together with keratin proteins are highly sensitive immunohistochemical markers for sarcomatoid mesothelioma. A positive staining for CD34, bcl-2 and desmin should virtually exclude sarcomatoid mesothelioma. Since only a small proportion of sarcomatoid mesothelioma were positive for calretinin or WT-1 protein, a negative stain does not exclude MM.

seen in chronic fibrosing pleuritis and should not be confused with evidence of invasion, immunohistochemistry in general is not thought to be effective in differentiating chronic fibrosing pleuritis and mesothelioma.

Our study demonstrates that D2-40 and podoplanin together with keratin proteins are highly sensitive immunohistochemical markers for sarcomatoid mesothelioma. A positive staining for CD34, bcl-2 and/or desmin should virtually exclude sarcomatoid mesothelioma. Since only a small number of cases of sarcomatoid mesothelioma were positive for calretinin or WT-1 protein, a negative stain does not exclude sarcomatoid DMM. This panel of antibodies can be helpful in distinguishing sarcomatoid mesothelioma from other tumors.

Acknowledgments

This study was supported by the International Mesothelioma Program. This study was presented in part at the annual meeting of the United States and Canadian Academy of Pathology, February 14, 2006 [abstract published in Modern Pathology 2006, 19 (Suppl. 1): 305A]. We thank Ms. Brittany MacFarland and Ms. Kasey Forgione for secretarial assistance.

Please address correspondence to: Lucian R. Chirieac, MD, Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston MA 02115, USA. Tel: 617 732-8126, Fax: 617 264-5118, E-mail: lchirieac@partners.org

References

[1] Robinson BW, Lake RA. Advances in malignant mesothelioma. N Engl J Med 2005;353:1591-603.

[2] Sugarbaker DJ, Flores RM, Jaklitsch MT, Richards WG, Strauss GM, Corson JM, DeCamp MM Jr, Swanson SJ, Bueno R, Lukanich JM, Baldini EH, Mentzer SJ. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117:54-63.

[3] Churg A, Cagle PT, Roggli VL. Tumors of the serosal membranes. Washington, DC, American Registry of Pathology in collaboration with the Armed Forces Institute of Pathology, 2006 pp. 42-82.

[4] Kushitani K, Takeshima Y, Amatya VJ, Furonaka O, Sakatani A, Inai K. Differential diagnosis of sarcomatoid mesothelioma from true sarcoma and sarcomatoid carcinoma using immunohistochemistry. Pathol Int 2008;58:75-83.

[5] Padgett DM, Cathro HP, Wick MR, Mills SE. Podoplanin is a better immunohistochemical marker for sarcomatoid mesothelioma than calretinin. Am J Surg Pathol 2008;32:123-7.

[6] Ordonez NG: The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol 2003;27:1031-51.

[7] Comin CE, Saieva C, Messerini L: h-caldesmon, calretinin, estrogen receptor, and Ber-EP4: a useful combination of immunohistochemical markers for differentiating epithelioid peritoneal mesothelioma from serous papillary carcinoma of the ovary. Am J Surg Pathol 2007;31:1139-48.

[8] Marchevsky AM, Wick MR: Evidence-based guidelines for the utilization of immunostains in diagnostic pathology: pulmonary adenocarcinoma versus mesothelioma. Appl Immunohistochem Mol Morphol 2007;15:140-4.

[9] Kushitani K, Takeshima Y, Amatya VJ, Furonaka O, Sakatani A, Inai K. Immunohistochemical marker panels for distinguishing between epithelioid mesothelioma and lung adenocarcinoma. Pathol Int 2007;57:190-9.

[10] Mimura T, Ito A, Sakuma T, Ohbayashi C, Yoshimura M, Tsubota N, Okita Y, Okada M. Novel marker D2-40, combined with calretinin, CEA, and TTF-1: an optimal set of immunodiagnostic markers for pleural mesothelioma. Cancer 2007;109:933-8.

[11] Suster S, Moran CA: Applications and limitations of immunohistochemistry in the diagnosis of malignant mesothelioma. Adv Anat Pathol 2006;13:316-29.

[12] Ordonez NG: What are the current best immunohistochemical markers for the diagnosis of epithelioid mesothelioma? A review and update. Hum Pathol 2007;38:1-16.

[13] Yaziji H, Battifora H, Barry TS, Hwang HC, Bacchi CE, McIntosh MW, Kussick SJ, Gown AM. 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.

[14] King JE, Thatcher N, Pickering CA, Hasleton PS. Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data. Histopathology 2006;48:223-32.

[15] Ordonez NG: Immunohistochemical diagnosis of epithelioid mesothelioma: an update. Arch Pathol Lab Med 2005;129:1407-14.

[16] Knuuttila A, Jee KJ, Taskinen E, Wolff H, Knuutila S, Anttila S. Spindle cell tumours of the pleura: a clinical, histological and comparative genomic hybridization analysis of 14 cases. Virchows Arch 2006;448:135-41.

[17] Marchevsky AM: Application of immunohistochemistry to the diagnosis of malignant mesothelioma. Arch Pathol Lab Med 2008;132:397-401.
[18] Rdzanek M, Fresco R, Pass HI, Carbone M. Spindle cell tumors of the pleura: differential diagnosis. Semin Diagn Pathol 2006;23:44-55.
[19] Attanoos RL, Dojcinov SD, Webb R. Antimesothelial markers in sarcomatoid mesothelioma and other spindle cell neoplasms. Histopathology 2000;37:224-31.
[20] Montag AG, Pinkus GS, Corson JM: Keratin protein immunoreactivity of sarcomatoid and mixed types of diffuse malignant mesothelioma: an immunoperoxidase study of 30 cases. Hum Pathol 1988;19:336-42.
[21] Lucas DR, Pass HI, Madan SK, Adsay NV, Wali A, Tabaczka P, Lonardo F. Sarcomatoid mesothelioma and its histological mimics: a comparative immunohistochemical study. Histopathology 2003;42:270-9.
[22] Saad AG, Yeap BY, Thunnissen FB, Pinkus GS, Pinkus JL, Loda M, Sugarbaker DJ, Johnson BE, Chirieac LR. Sarcomatoid malignant mesothelioma: Immunohistochemical characteristics of 24 cases, Nature Publishing Group, 2006, pp 1422.
[23] Ordonez NG: D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. Hum Pathol 2005;36:372-80.
[24] Travis WD, World Health Organization., International Agency for Research on Cancer., et al: Pathology and genetics of tumours of the lung, pleura, thymus and heart. Lyon, IARC Press, 2004 .
[25] Christensen BC, Godleski JJ, Marsit CJ, Houseman EA, Lopez-Fagundo CY, Longacker JL, Bueno R, Sugarbaker DJ, Nelson HH, Kelsey KT. Asbestos exposure predicts cell cycle control gene promoter methylation in pleural mesothelioma. Carcinogenesis 2008;29:1555-9.
[26] Doglioni C, Dei Tos AP, Laurino L, Iuzzolino P, Chiarelli C, Celio MR, Viale G. Calretinin: a novel immunocytochemical marker for mesothelioma. Am J Surg Pathol 1996;20:1037-46.
[27] Granville LA, Younes M, Churg A, Roggli VL, Henderson DW, Cagle PT. Comparison of monoclonal versus polyclonal calretinin antibodies for immunohistochemical diagnosis of malignant mesothelioma. Appl Immunohistochem Mol Morphol 2005;13:75-9.
[28] Miettinen M, Limon J, Niezabitowski A, Lasota J. Calretinin and other mesothelioma markers in synovial sarcoma: analysis of antigenic similarities and differences with malignant mesothelioma. Am J Surg Pathol 2001;25:610-7.
[29] Suster S, Moran CA. Primary synovial sarcomas of the mediastinum: a clinicopathologic, immunohistochemical, and ultrastructural study of 15 cases. Am J Surg Pathol 2005;29:569-78.
[30] Hirakawa N, Naka T, Yamamoto I, Fukuda T, Tsuneyoshi M. Overexpression of bcl-2 protein in synovial sarcoma: a comparative study of other soft tissue spindle cell sarcomas and an additional analysis by fluorescence in situ hybridization. Hum Pathol 1996;27:1060-5.
[31] Lopes JM, Nesland JM, Reis-Filho JS, Holm R. Differential Ki67 and bcl-2 immunoexpression in solid-glandular and spindle cell components of biphasic synovial sarcoma: a double immunostaining assessment with cytokeratin and vimentin. Histopathology 2002;40:464-71.
[32] van de Rijn M, Barr FG, Xiong QB, Hedges M, Shipley J, Fisher C. Poorly differentiated synovial sarcoma: an analysis of clinical, pathologic, and molecular genetic features. Am J Surg Pathol 1999;23:106-12.
[33] Suster S, Fisher C, Moran CA: Expression of bcl-2 oncoprotein in benign and malignant spindle cell tumors of soft tissue, skin, serosal surfaces, and gastrointestinal tract. Am J Surg Pathol 1998;22:863-72.
[34] Carbone M, Rizzo P, Powers A, Bocchetta M, Fresco R, Krausz T. Molecular analyses, morphology and immunohistochemistry together differentiate pleural synovial sarcomas from mesotheliomas: clinical implications. Anticancer Res 2001;22:3443-8.
[35] Essary LR, Vargas SO, Fletcher CD: Primary pleuropulmonary synovial sarcoma: reappraisal of a recently described anatomic subset. Cancer 2002;94:459-69.
[36] Vallat-Decouvelaere AV, Dry SM, Fletcher CD: Atypical and malignant solitary fibrous tumors in extrathoracic locations: evidence of their comparability to intra-thoracic tumors. Am J Surg Pathol 1998;22:1501-11.
[37] Moran CA, Suster S, Abbondanzo SL, Koss MN. Primary leiomyosarcomas of the lung: a clinicopathologic and immunohistochemical study of 18 cases. Mod Pathol 1997;10:121-8.
[38] Chilosi M, Facchettti F, Dei Tos AP, Lestani M, Morassi ML, Martignoni G, Sorio C, Benedetti A, Morelli L, Doglioni C, Barberis M, Menestrina F, Viale G. bcl-2 expression in pleural and extrapleural solitary fibrous tumours. J Pathol 1997;181:362-7.
[39] Cappello F, Barnes L: Synovial sarcoma and malignant mesothelioma of the pleura: review, differential diagnosis and possible role of apoptosis. Pathology 2001;33:142-8.
[40] Ordonez NG: Podoplanin: a novel diagnostic immunohistochemical marker. Adv Anat Pathol 2007;13:83-8.
[41] Kaddu S, Leinweber B: Podoplanin expression in fibrous histiocytomas and cellular neurothekeomas. Am J Dermatopathol 2009;31:137-9.
[42] Kalof AN, Cooper K: D2-40 immunohistochemistry--so far! Adv Anat Pathol 2009;16:62-4.
[43] Chang MC, Vargas SO, Hornick JL, Hirsch MS, Crum CP, Nucci MR. Embryonic Stem Cell Transcription Factors and D2-40 (Podoplanin) as Diagnostic Immunohistochemical Markers in

Ovarian Germ Cell Tumors. Int J Gynecol Pathol 2009;28:347-55.
[44] Marsee DK, Pinkus GS, Hornick JL: Podoplanin (D2-40) is a highly effective marker of follicular dendritic cells. Appl Immunohistochem Mol Morphol 2009;17:102-7.
[45] Wiedorn KH, Goldmann T, Henne C, Kühl H, Vollmer E. EnVision+, a new dextran polymer-based signal enhancement technique for in situ hybridization (ISH). J Histochem Cytochem 2001;49:1067-71.
[46] Riera JR, Astengo-Osuna C, Longmate JA, Battifora H. The immunohistochemical diagnostic panel for epithelial mesothelioma: a reevaluation after heat-induced epitope retrieval. Am J Surg Pathol 1997;21:1409-19.
[47] Chu AY, Litzky LA, Pasha TL, Acs G, Zhang PJ. Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. Mod Pathol 2005;18:105-10.
[48] Kimura N, Kimura I. Podoplanin as a marker for mesothelioma. Pathol Int 2005;55:83-6.
[49] Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo K, Kerjaschki D. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol 1999;154:385-94.
[50] Breiteneder-Geleff S, Soleiman A, Horvat R, Amann G, Kowalski H, Kerjaschki D. [Podoplanin --a specific marker for lymphatic endothelium expressed in angiosarcoma]. Verh Dtsch Ges Pathol 1999;83:270-5.
[51] Zimmer G, Oeffner F, Von Messling V, Tschernig T, Gröness HJ, Klenk HD, Herrler G. Cloning and characterization of gp36, a human mucin-type glycoprotein preferentially expressed in vascular endothelium. Biochem J 1999;341 (Pt 2):277-84.