Value of Claudin-4 Immunostaining in the Diagnosis of Mesothelioma

Nelson G. Ordóñez, MD

Key Words: Claudin-4; Immunohistochemistry; Mesothelioma; Adenocarcinoma

DOI: 10.1309/AJCP0B3YJBXWXJII

Upon completion of this activity you will be able to:
- identify the best broad-spectrum immunohistochemical carcinoma marker to assist in distinguishing between epithelioid mesotheliomas and metastatic carcinomas to the serosal membranes.
- incorporate claudin-4 into the different panels of markers that are currently used in the differential diagnosis of mesothelioma.

The ASCP is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. The ASCP designates this journal-based CME activity for a maximum of 1 *AMA PRA Category 1 Credit*™ per article. Physicians should claim only the credit commensurate with the extent of their participation in the activity. This activity qualifies as an American Board of Pathology Maintenance of Certification Part II Self-Assessment Module.

The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Questions appear on p 692. Exam is located at www.ascp.org/ajcpcme.

CME/SAM

Abstract

Claudin-4 (CL-4) is a tight junction–associated protein that is expressed in most epithelial cells but absent in mesothelial cells. The purpose of this study is to evaluate the utility of CL-4 immunostaining for assisting in the differential diagnosis of mesothelioma. Sixty mesotheliomas (40 epithelioid, 10 biphasic, and 10 sarcomatoid), 185 carcinomas of different origins that can potentially be confused with mesotheliomas, 37 soft-tissue sarcomas, and 5 melanomas were investigated for CL-4 expression. All 60 mesotheliomas were CL-4 negative. In contrast, 169 (91%) of 185 carcinomas expressed this marker. Five of 8 desmoplastic small round cell tumors and the epithelial component of all 5 biphasic synovial sarcomas were CL-4 positive, whereas none of the remaining soft-tissue sarcomas or melanomas expressed this marker. It is concluded that CL-4 is a highly specific and sensitive immunohistochemical marker for assisting in distinguishing epithelioid mesotheliomas from metastatic carcinomas to the serosal membranes.

A well-known characteristic of mesotheliomas is their ability to present a broad range of cytomorphologic features and grow in a variety of histologic patterns. Because of this, mesotheliomas can be difficult to diagnose on routine histologic preparations as they can be confused with a wide array of tumors that can metastasize to the serosal membranes. Because an absolutely specific and sensitive marker for mesotheliomas has not yet been identified, the differential diagnosis of these tumors largely depends on the use of immunohistochemical panels composed of positive mesothelioma markers (ie, those that are frequently expressed in mesotheliomas but not in carcinomas) and positive carcinoma markers (ie, those that are commonly expressed in carcinomas but not in mesotheliomas). The composition of the recommended panels, however, is constantly subject to change as a result of the identification of new markers that can be used in the differential diagnosis of these tumors and the continual publication of new information on the value of the individual markers.

Claudin-4 (CL-4), a transmembrane protein located in the tight junctions (TJs), is widely expressed in most epithelial cells but absent in mesothelial cells.[1] In 2006, Soini et al[2] investigated the potential utility of CL-4 immunostaining in distinguishing epithelioid mesotheliomas from carcinomas metastatic to the serosal membranes. In that study, 7 (29%) of 24 epithelioid, 1 (25%) of 4 sarcomatoid, and none of 7 biphasic mesotheliomas were found to be CL-4 positive, whereas all 23 (100%) metastatic carcinomas expressed this marker. Because the degree of CL-4 positivity in epithelioid mesotheliomas was lower than that observed in adenocarcinomas, the authors concluded that CL-4 immunostaining may have some

utility as an additional marker for discriminating between metastatic adenocarcinomas and mesotheliomas. In a subsequent study, Facchetti et al[1] reported expression for this marker in 245 (88%) of 278 primary carcinomas of various sites and 57 (98%) of 58 serosal metastases, whereas all 60 epithelioid, 11 biphasic, and 9 sarcomatoid mesotheliomas included in the study were negative. On the basis of these results, the authors concluded that CL-4 should be considered a primary immunohistochemical marker for assisting in the differential diagnosis of epithelioid mesotheliomas. The purpose of this study is to resolve the discrepancy between the 2 previously mentioned investigations and to discuss the practical utility of CL-4 when compared with other broad-spectrum positive carcinoma markers that are, at present, considered useful for assisting in the differential diagnosis of mesotheliomas.

Materials and Methods

The material used in this study was obtained from the files of the Department of Pathology at the University of Texas M.D. Anderson Cancer Center ▮**Table 1**▮. Only cases with an unequivocal clinical and pathologic diagnosis of mesothelioma were selected for CL-4 immunostaining. The selection of the nonmesothelial tumors was based on their potential for being confused with the different morphologic subtypes of mesothelioma. Immunohistochemical studies for CL-4 were performed on 5-μ-thick, formalin-fixed, paraffin-embedded tissue sections using the polymeric biotin-free horseradish peroxidase method on a Leica BOND-MAX stainer (Leica Biosystems, Buffalo Grove, IL). The primary antibody used was the 3E2C1 anti–CL-4 mouse monoclonal antibody (Invitrogen, Camarillo, CA; 1:250 dilution). In brief, slides were deparaffinized and hydrated, followed by heat-induced antigen retrieval in which a citrate buffer solution (pH 6.0) was used. Incubation with the primary antibody was followed by development of the immunostaining with 3,3'-diaminobenzidine. The secondary antibody and detection was applied as per instructions from the manufacturer (Leica Biosystems). To evaluate the specificity of the immunoreaction, known positive and negative tissues were used as controls. The immunostaining was graded on a sliding scale of 1+ to 4+ according to the percentage of distinctly reactive cells (1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, >75%).

▮**Table 1**▮
Results of Claudin-4 Immunostaining

			Reactivity[a]			
	Total No.	No. (%) of Positive Cases	1+	2+	3+	4+
Mesothelioma						
Epithelioid	40	0 (0)	0	0	0	0
Biphasic	10	0 (0)	0	0	0	0
Sarcomatoid	10	0 (0)	0	0	0	0
Lung						
Adenocarcinoma	25	25 (100)	0	2	4	19
Squamous cell carcinoma	20	19 (95)	4	3	2	10
Sarcomatoid carcinoma (spindle cell component)	10	2 (20)	2	0	0	0
Pleomorphic carcinoma	3	2 (67)	0	0	1	1
Small cell carcinoma	3	3 (100)	1	0	1	1
Carcinoid tumor	2	2 (100)	0	0	0	2
Ovarian serous carcinoma	45	44 (98)	1	3	15	25
Breast ductal carcinoma	7	7 (100)	0	1	1	5
Pancreatic adenocarcinoma	5	5 (100)	0	0	2	3
Colon adenocarcinoma	6	6 (100)	0	0	0	6
Prostate adenocarcinoma	2	2 (100)	0	0	1	1
Renal cell carcinomas						
Clear cell	33	28 (85)	1	7	6	14
Papillary	10	10 (100)	1	0	2	7
Chromophobe	12	12 (100)	0	0	5	7
Bladder urothelial carcinoma	2	2 (100)	0	0	1	1
Solitary fibrous tumor of the pleura	6	0 (0)	0	0	0	0
Angiosarcoma	3	0 (0)	0	0	0	0
Malignant fibrous histiocytoma	6	0 (0)	0	0	0	0
Desmoplastic small round cell tumor	8	5 (63)	0	0	3	2
Biphasic synovial sarcoma	5	5 (100)	0	0	1	4
Monophasic synovial sarcoma	3	0 (0)	0	0	0	0
Leiomyosarcoma	3	0 (0)	0	0	0	0
Rhabdomyosarcoma	3	0 (0)	0	0	0	0
Melanoma	5	0 (0)	0	0	0	0

[a] Reactivity was defined as follows: 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, >75%.

DOI: 10.1309/AJCP0B3YJBXWXJII

Results

None of the 60 mesotheliomas stained for CL-4 were positive for this marker. Expression was demonstrated, however, in all adenocarcinomas of the lung, pancreas, breast, colon, and prostate and in 44 (98%) of 45 serous carcinomas of the ovary (15 primary, 30 metastatic to the peritoneum) investigated **Image 1A** and **Image 1B**. Twenty-eight (85%) of 33 clear cell, 10 (100%) of 10 papillary, and 12 (100%) of 12 chromophobe renal cell carcinomas were also found to be CL-4 positive, as were 19 (95%) of 20 squamous cell and all 3 small cell carcinomas of the lung **Image 1C** and **Image 1D**. In the vast majority of these epithelial tumors, the immunoreaction was strong (4+) and occurred along the cell membrane in either a continuous or a punctated staining pattern (Image 1C). Two (67%) of the 3 pleomorphic carcinomas, but only 2 (20%) of the 10 sarcomatoid carcinomas of the lung, were focally positive (1+) for CL-4 in the spindle cell sarcomatoid areas of the tumors, whereas the epithelial areas were consistently positive **Image 2A** and **Image 2B**. In all 5 biphasic synovial sarcomas investigated, the positivity for CL-4 was restricted to the epithelial areas of the tumor. The spindle cell component of these tumors and that of all 3 monophasic spindle cell synovial sarcomas were negative for this marker **Image 2C**. Five (63%) of the 8 desmoplastic

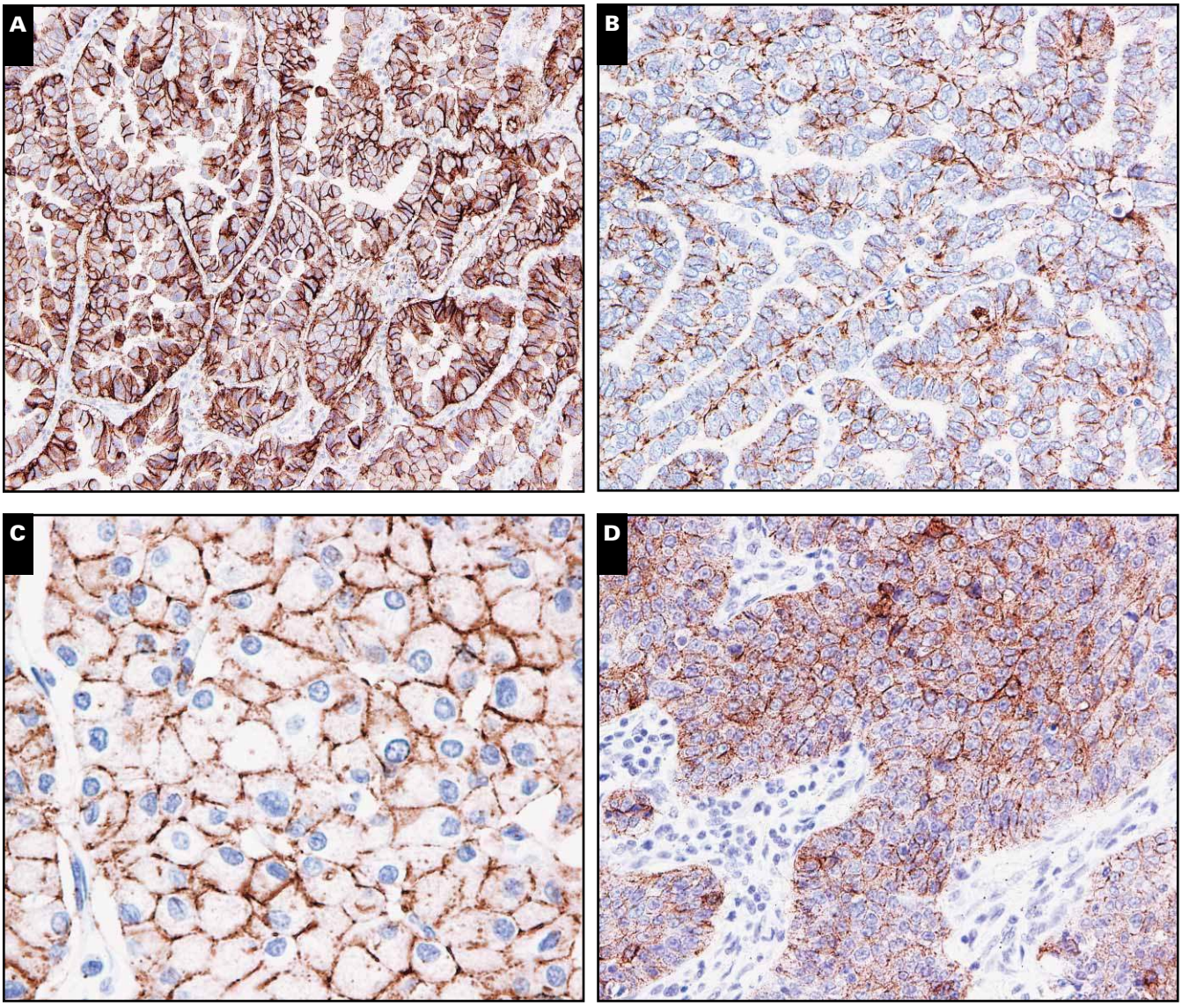


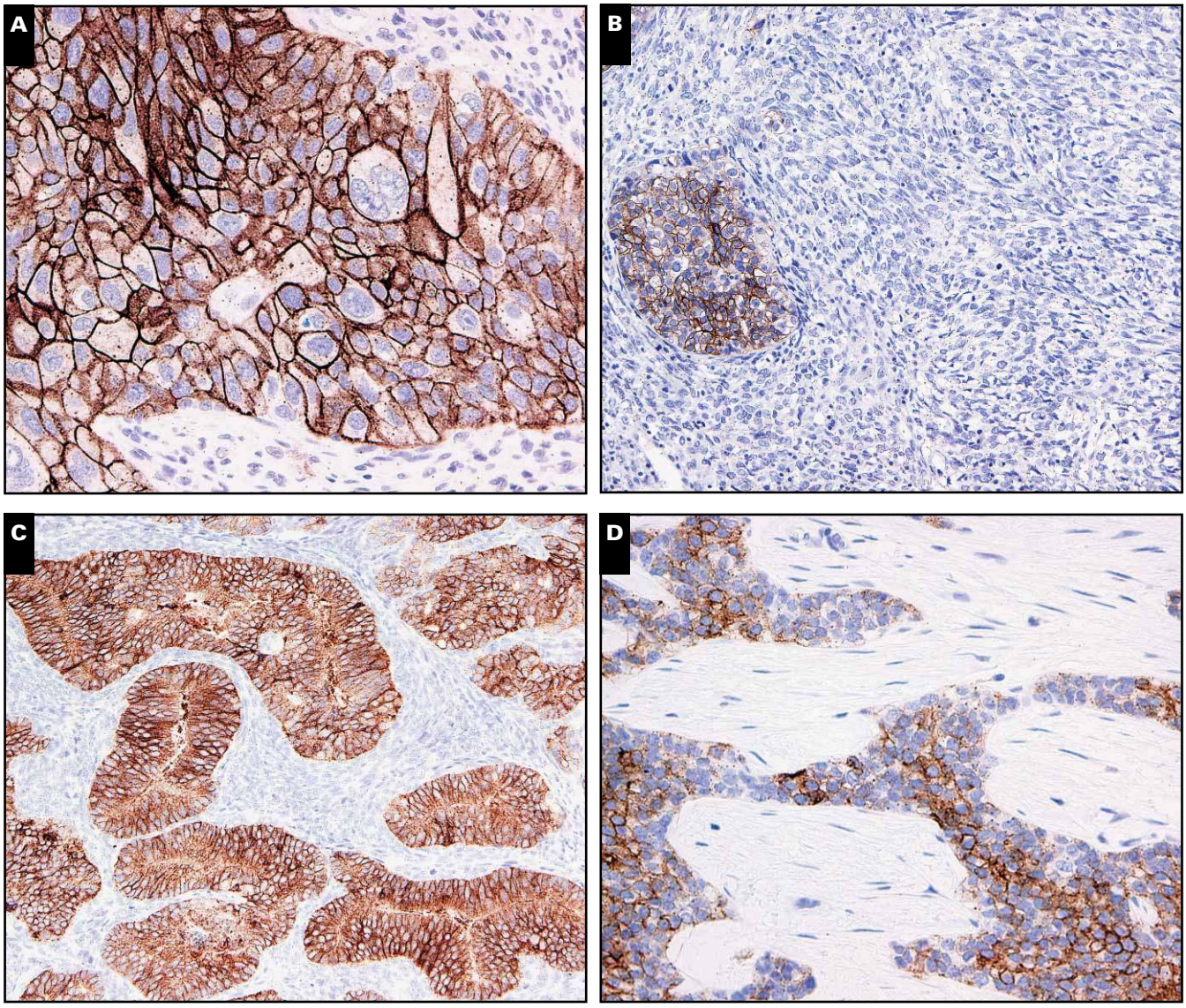
Image 1 **A**, Lung adenocarcinoma showing diffuse strong claudin-4 (CL-4) positivity along the cell membranes (×200). **B**, Ovarian serous carcinoma displaying membranous positivity for CL-4 (×200). **C**, Chromophobe renal cell carcinoma demonstrating a punctated staining pattern for CL-4 along the cell membrane (×400). **D**, Poorly differentiated squamous cell carcinoma of the lung exhibiting strong CL-4 expression (×200).

DOI: 10.1309/AJCP0B3YJBXWXJII

small round cell tumors investigated were found to express CL-4 ▮**Image 2D**▮, whereas all solitary tumors of the pleura, malignant fibrous histiocytomas, angiosarcomas, leiomyosarcomas, rhabdomyosarcomas, and melanomas were negative. The results of the immunostaining are summarized in Table 1.

Discussion

Claudins are a large family of transmembrane proteins essential for the formation and maintenance of TJs.[3] TJs or zonula occludens are the most apical component of the junctional complex present on the membranes of epithelial and endothelial cells.[4-6] TJs visualized by electron microscopy are regions where the outer leaflets of plasma membranes from adjacent cells appear to fuse together and the intercellular space disappears. These structures play a crucial role in the maintenance of cell polarity, cellular arrangement, adhesion, and paracellular transport.[7,8] To date, 24 human claudins that control the ability of TJs to regulate paracellular transport have been identified.[6] Most tissues express multiple claudins that can interact in both a homotypic and a heterotypic manner to form TJ strands. The exact combination of claudin proteins within a particular tissue is believed to determine the



▮**Image 2**▮ **A**, Pleomorphic lung carcinoma showing strong membranous staining for claudin-4 (CL-4) (×400). **B**, Sarcomatoid carcinoma showing strong reactivity for CL-4 in the better differentiated areas of the tumor (left), while the spindle tumor cells are negative (×200). **C**, Biphasic synovial sarcoma demonstrating strong CL-4 positivity in the epithelial areas of the tumor, while the spindle cell component is negative (×200). **D**, Desmoplastic small round cell tumor exhibiting CL-4 expression (×200).

DOI: 10.1309/AJCP0B3YJBXWXJII

selectivity and strength of the TJs. CL-4, also known as *Clostridium perfringens* enterotoxin receptor, is a 209–amino acid protein with a molecular weight of ~22 kDa that is encoded by a gene located on chromosome 7q11.23. CL-4 is widely expressed in most normal epithelial cells, including those of the lung, pancreas, breast, prostate, thyroid, thymus, and bladder.[1] It is expressed in collecting ducts and distal convoluted tubules in the kidney, as well as in the biliary duct epithelium in the liver. CL-4 is also expressed in follicular dendritic cells. It is not expressed in hepatocytes or mesothelial cells.[1,2,9]

Only a few studies have been published on CL-4 expression in mesotheliomas. The first of these investigations was by Soini et al,[2] who, using the 3E2C1 anti–CL-4 mouse monoclonal antibody, reported CL-4 expression in 29% of the epithelioid and 25% of the sarcomatoid mesotheliomas investigated. Although the authors indicated that CL-4 reactivity in mesotheliomas was significantly lower than that seen in the metastatic adenocarcinomas to the pleura included in the study, no quantitative grading of the reaction or illustrations of any of the positive mesothelioma cases were provided. The results of this study are in contrast to those obtained by Facchetti et al,[1] who, in a subsequent investigation using the same antibody employed by Soini et al,[2] were unable to demonstrate CL-4 expression in any of the 60 epithelioid or 11 sarcomatoid mesotheliomas included in their investigation. That none of the 60 mesotheliomas in the present study were CL-4 positive is an indication that this marker is not expressed in these tumors. The cause of the discrepancy between the results reported by both Facchetti et al and those obtained in the current investigation when compared with those reported by Soini et al is not clear, but it does not appear to be related to the antibody used because the same commercially obtained 3E2C1 anti–CL-4 mouse monoclonal antibody was employed in all 3 studies.

Over the past 3 decades, a large number of immunohistochemical markers that are frequently expressed in carcinomas, but not in mesotheliomas, have been identified. These markers are commonly referred to as positive carcinoma markers and can be subdivided into 2 major groups: broad-spectrum positive carcinoma markers, which are frequently expressed in a wide range of carcinomas, and organ-associated carcinoma markers that, because of their restricted expression, can help to establish the site of origin of a metastatic carcinoma.[10] Most of the positive carcinoma markers that have traditionally been used to assist in discriminating between metastatic carcinomas to the serosal membranes and epithelioid mesotheliomas belong to the first group and include MOC-31, Ber-EP4, tumor-associated glycoprotein-72 (TAG-72), carcinoembryonic antigen, BG-8, and CD15, which are the markers that are currently most commonly used in the differential diagnosis between these tumors. Because these markers are usually absent in sarcomatoid carcinomas, they have no utility in distinguishing this type of tumor from sarcomatoid mesotheliomas.

MOC-31 is a monoclonal antibody that reacts with a 40-kDa transmembrane protein known as epithelial cell adhesion molecule (Ep-CAM), which is normally expressed in the basolateral membrane of most epithelial tissues, including simple cuboidal and columnar, pseudostratified columnar, and transitional epithelium.[11,12] The vast majority of adenocarcinomas of the lung (86%-100%),[13-17] pancreas (100%),[18,19] breast (29%-100%),[13,14,18] and colon (100%)[13,14,18]; serous carcinomas of the ovary and peritoneum (97%-100%)[20-22]; squamous cell carcinomas of the lung (97%-100%)[13,23]; and urothelial carcinomas (10%-67%)[13,18] have been reported to be MOC-31 positive. This is in contrast to epithelioid mesotheliomas, in which 2% to 15% have been reported to be positive in small focal areas or in scattered neoplastic cells.[17,21,24,25] Because of its high sensitivity and specificity, MOC-31 is, in my experience, one of the best broad-spectrum positive carcinoma markers for assisting in discriminating between epithelioid pleural mesotheliomas and both lung adenocarcinomas and squamous cell carcinomas of the lung.[17,23] As only 50% of the renal cell carcinomas have been reported to react with this antibody, MOC-31 has limited utility in distinguishing these tumors from epithelioid mesotheliomas.[26]

Ber-EP4 is another mouse monoclonal antibody that, similar to MOC-31, also reacts with Ep-CAM. Published studies have shown that a high percentage of adenocarcinomas of the lung (91%-100%),[16,17,27-31] breast (81%-83%),[29,32] pancreas (80%-100%),[19,32] and colon (100%)[19,32]; serous carcinomas of the ovary and peritoneum (83%-100%)[21,22,29,33,34]; squamous cell carcinomas of the lung (74%-100%)[23,29,35]; and clear cell renal cell carcinomas (27%-42%)[26,36,37] react with the Ber-EP4 antibody. In contrast, only 13% to 26% of epithelioid mesotheliomas have been reported to be Ber-EP4 positive, usually in small areas of the tumor or in a few cells.[17,21,24,25] The results of these investigations indicate that Ber-EP4 immunostaining can be helpful in assisting in distinguishing epithelioid mesotheliomas from lung adenocarcinomas, serous carcinomas, and squamous cell carcinomas of the lung; however, it has little or no practical utility for assisting in discriminating between epithelioid mesotheliomas and renal cell carcinomas metastatic to the serosal membranes. In my experience, Ber-EP4 is less specific than MOC-31 as I have observed weak positivity for the latter marker in only 2% to 8% of epithelioid mesotheliomas, which is much lower than that seen with Ber-EP4.[13,17,23,26]

BG-8 is a mouse monoclonal antibody that recognizes the blood group Lewis^y. Published investigations have demonstrated that adenocarcinomas of the lung (58%-100%),[17,18,38-41] breast (71%-100%),[18,41,42] pancreas (73%),[18] prostate (80%-84%),[18,41] and colon (76%-90%)[18,41]; serous

DOI: 10.1309/AJCP0B3YJBXWXJII

carcinomas of the ovary and peritoneum (73%)[22]; and squamous cell carcinomas of the lung (80%-87%)[18,23,40,42] are frequently BG-8 positive and that, in most instances, the staining is strong and diffuse. This is in contrast to epithelioid mesotheliomas in which only a minority of cases (3%-9%)[17,38,41] have been reported to exhibit focal reactivity in small areas of the tumor or in sparse cells. Because of these differences in BG-8 reactivity, this marker can be helpful in assisting in distinguishing between epithelioid mesotheliomas and the previously mentioned tumors. BG-8 immunostaining, however, has no utility for assisting in distinguishing between epithelioid mesotheliomas and renal cell carcinomas as only a small percentage (4%-10%)[18,26] of the latter tumors have been reported to be positive for this marker.

TAG-72, also known as B72.3, is one of the earlier broad-spectrum positive carcinoma markers that was found to be useful in assisting in discriminating epithelioid mesotheliomas from metastatic carcinomas to the serosal membranes. Published studies indicate that expression of this marker can be demonstrated in the majority of adenocarcinomas of the lung (70%-100%),[17,27,30,40,43-50] breast (50%-80%),[42,51-53] pancreas (88%-100%),[54,55] and colon (68%-83%)[49,54]; serous carcinomas of the ovary and peritoneum (65%-98%)[20,22,33,56-60]; and squamous cell carcinomas of the lung (17%-84%).[40,61,62] Epithelioid mesotheliomas usually do not express TAG-72, and only 2% to 11% of these tumors have been reported to be positive in small areas or in sparse cells.[44,45,50,63,64]

CD15 (leu-M1) is another of the earliest broad-spectrum positive carcinoma markers that was found to be useful in assisting in the differential diagnosis between epithelioid mesotheliomas and carcinomas. According to published investigations, CD15 is frequently expressed in adenocarcinomas of the lung (~55%-85%),[15,17,27,28,44,45,48,50,65,66] breast (67%),[32] pancreas (80%),[67] and colon (100%)[32]; serous carcinomas of the ovary and peritoneum (30%-80%)[20-22,33,34,46,57-59,68]; and squamous cell carcinomas of the lung (13%-45%).[23,69,70] Even though some authors have reported CD15 expression in epithelioid mesotheliomas (2%-29%),[30,38,44,71,72] in my experience, as well as that of other investigators, this marker is not expressed in mesotheliomas.[17,28,31,45,46,63] Because a high percentage of clear cell (62%-71%)[26,37,73,74] and papillary (41%-100%)[26,37] renal cell carcinomas have been reported to express CD15, immunostaining for this marker could assist in distinguishing these tumors from epithelioid mesotheliomas.

Carcinoembryonic antigen (CEA) was the first immunohistochemical marker to become widely accepted as being useful in the differential diagnosis between epithelioid mesotheliomas and lung adenocarcinomas. Current information indicates that CEA is positive in the large majority of adenocarcinomas of the lung (70%-100%),[16,17,27,28,30,31,45,46,48,50,66,75] breast (80%),[18] pancreas (90%-100%),[18,19,54,76,77] and colon (100%)[18,19,32,54,76] and squamous cell carcinomas of the lung (77%-86%),[18,23] whereas epithelioid mesotheliomas are almost invariably negative for this marker.[17,50,78] Therefore, CEA immunostaining can assist in distinguishing epithelioid mesotheliomas from the previously mentioned tumors. In contrast, because CEA is expressed in only a small percentage of serous carcinomas of the ovary and peritoneum (4%-45%),[20,21,38,58] it has no practical utility for discriminating these tumors from mesotheliomas. In addition, because CEA expression is usually absent in renal cell carcinomas, immunostaining for this marker is not useful for distinguishing these tumors from mesotheliomas.[26]

That all adenocarcinomas of the lung, breast, pancreas, colon, and prostate, as well as the vast majority of the serous carcinomas of the ovary, renal cell carcinomas, and squamous cell carcinomas of the lung, in the present study were found to express CL-4, but none of the epithelioid mesotheliomas investigated were positive for this marker, is an indication that CL-4 is a highly specific and sensitive marker that can be used to discriminate between epithelioid mesotheliomas and metastatic carcinomas to the serosal membranes. In addition, when CL-4 is compared with the broad-spectrum positive carcinoma markers that have previously been recommended as being useful in this differential diagnosis, including MOC-31, Ber-EP4, BG-8, TAG-72, CD15, and CEA, both its specificity and/or sensitivity appear, in general, to be higher than all other markers. On the basis of these factors, CL-4 should be considered a primary marker to be included in the panels of immunohistochemical markers used for assisting in the differential diagnosis of epithelioid mesotheliomas. Because only 2 of the 10 sarcomatoid lung carcinomas investigated exhibited staining in small focal areas of the spindle cell component, CL-4 immunostaining does not appear to have significant value in distinguishing these tumors from sarcomatoid mesotheliomas. However, in those tumors that contain small epithelial areas that may not be apparent on routine histology, CL-4 can be helpful in their identification, as shown in Image 2B, and, therefore, may be useful in assisting in the differential diagnosis between these tumors and sarcomatoid mesotheliomas.

From the Department of Pathology, University of Texas M.D. Anderson Cancer Center, Houston.

Address correspondence to Dr Ordóñez: Dept. of Pathology, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030; e-mail: nordonez@mdanderson.org.

References

1. Facchetti F, Lonardi S, Gentili F, et al. Claudin 4 identifies a wide spectrum of epithelial neoplasms and represents a very useful marker for carcinoma versus mesothelioma diagnosis in pleural and peritoneal biopsies and effusions. *Virchows Arch*. 2007;451:669-680.

DOI: 10.1309/AJCP0B3YJBXWXJII

2. Soini Y, Kinnula V, Kahlos K, et al. Claudins in differential diagnosis between mesothelioma and metastatic adenocarcinoma of the pleura. *J Clin Pathol.* 2006;59:250-254.
3. González-Mariscal L, Betanzos A, Nava P, et al. Tight junction proteins. *Prog Biophys Mol Biol.* 2003;81:1-44.
4. Furuse M, Sasaki H, Fujimoto K, et al. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. *J Cell Biol.* 1998;143:391-401.
5. Morita K, Furuse M, Fujimoto K, et al. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc Natl Acad Sci U S A.* 1999;96:511-516.
6. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol.* 2001;2:285-293.
7. Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. *Annu Rev Physiol.* 2006;68:403-429.
8. Krause G, Winkler L, Mueller SL, et al. Structure and function of claudins. *Biochim Biophys Acta.* 2008;1778:631-645.
9. Lodi C, Szabo E, Holczbauer A, et al. Claudin-4 differentiates biliary tract cancers from hepatocellular carcinomas. *Mod Pathol.* 2006;19:460-469.
10. Ordóñez NG. Application of immunohistochemistry in the diagnosis of epithelioid mesothelioma: a review and update. *Hum Pathol.* 2013;44:1-19.
11. Litvinov SV, Velders MP, Bakker HAM, et al. Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. *J Cell Biol.* 1994;125:437-446.
12. Winter MJ, Nagtegaal ID, van Krieken JHJM, et al. The epithelial cell adhesion molecule (Ep-CAM) as a morphoregulatory molecule is a tool in surgical pathology. *Am J Pathol.* 2003;163:2139-2148.
13. Ordóñez NG. Value of the MOC-31 monoclonal antibody in differentiating epithelial pleural mesothelioma from lung adenocarcinoma. *Hum Pathol.* 1998;29:166-169.
14. Morgan RL, De Young BR, McGaughy VR, et al. MOC-31 aids in the differentiation between adenocarcinoma and reactive mesothelial cells. *Cancer.* 1999;87:390-394.
15. Oates J, Edwards C. HBME-1, MOC-31, WT1 and calretinin: an assessment of recently described markers for mesothelioma and adenocarcinoma. *Histopathology.* 2000;36:341-347.
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. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. *Am J Surg Pathol.* 2003;27:1031-1051.
18. Pan CC, Chen PC, Tsay SH, et al. Differential immunoprofiles of hepatocellular carcinoma, renal cell carcinoma, and adrenocortical carcinoma: a systemic immunohistochemical survey using tissue array technique. *Appl Immunohistochem Mol Morphol.* 2005;13:347-352.
19. Porcell AI, De Young BR, Proca DM, et al. Immunohistochemical analysis of hepatocellular and adenocarcinoma in the liver: MOC31 compares favorably with other putative markers. *Mod Pathol.* 2000;13:773-778.
20. Ordóñez NG. Role of immunohistochemistry in distinguishing epithelial peritoneal mesotheliomas from peritoneal and ovarian serous carcinomas. *Am J Surg Pathol.* 1998;22:1203-1214.
21. Barnetson RJ, Burnett RA, Downie I, et al. Immunohistochemical analysis of peritoneal mesothelioma and primary and secondary serous carcinoma of the peritoneum: antibodies to estrogen and progesterone receptors are useful. *Am J Clin Pathol.* 2006;125:67-76.
22. Ordóñez NG. The diagnostic utility of immunohistochemistry and electron microscopy in distinguishing between peritoneal mesotheliomas and serous carcinomas: a comparative study. *Mod Pathol.* 2006;19:34-48.
23. Ordóñez NG. The diagnostic utility of immunohistochemistry in distinguishing between epithelioid mesotheliomas and squamous carcinomas of the lung: a comparative study. *Mod Pathol.* 2006;19:417-428.
24. Delahaye M, Hoogsteden HC, van der Kwast TH. Immunocytochemistry of malignant mesothelioma: OV632 as a marker of malignant mesothelioma. *J Pathol.* 1991;165:137-143.
25. Edwards C, Oates J. OV 632 and MOC 31 in the diagnosis of mesothelioma and adenocarcinoma: an assessment of their use in formalin-fixed and paraffin wax embedded material. *J Clin Pathol.* 1995;48:626-630.
26. Ordóñez NG. The diagnostic utility of immunohistochemistry in distinguishing between mesothelioma and renal cell carcinoma: a comparative study. *Hum Pathol.* 2004;35:697-710.
27. Moch H, Oberholzer M, Dalquen P, et al. Diagnostic tools for differentiating between pleural mesothelioma and lung adenocarcinoma in paraffin embedded tissue, part I: immunohistochemical findings. *Virchows Arch A Pathol Anat Histopathol.* 1993;423:19-27.
28. Doglioni C, Dei Tos AP, Laurino L, et al. Calretinin: a novel immunocytochemical marker for mesothelioma. *Am J Surg Pathol.* 1996;20:1037-1046.
29. Ordóñez NG. Value of the Ber-EP4 antibody in differentiating epithelial pleural mesothelioma from adenocarcinoma: the M.D. Anderson experience and a critical review of the literature. *Am J Clin Pathol.* 1998;109:85-89.
30. 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;32:529-536.
31. 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;55:662-668.
32. Ma CK, Zarbo RJ, Frierson HF Jr, et al. Comparative immunohistochemical study of primary and metastatic carcinomas of the liver. *Am J Clin Pathol.* 1993;99:551-557.
33. Rothacker D, Möbius G. Varieties of serous surface papillary carcinoma of the peritoneum in northern Germany: a thirty-year autopsy study. *Int J Gynecol Pathol.* 1995;14:310-318.
34. Attanoos RL, Webb R, Dojcinov SD, et al. Value of mesothelial and epithelial antibodies in distinguishing diffuse peritoneal mesothelioma in females from serous papillary carcinoma of the ovary and peritoneum. *Histopathology.* 2002;40:237-244.
35. Pritchard SA, Howat AJ, Edwards JM. Immunohistochemical panel for distinction between squamous cell carcinoma, adenocarcinoma and mesothelioma. *Histopathology.* 2003;43:197-199.
36. Nolan LP, Heatley MK. The value of immunocytochemistry in distinguishing between clear cell carcinoma of the kidney and ovary. *Int J Gynecol Pathol.* 2001;20:155-159.

DOI: 10.1309/AJCP0B3YJBXWXJII

37. Pan CC, Chen PC, Ho DM. The diagnostic utility of MOC31, BerEP4, RCC marker and CD10 in the classification of renal cell carcinoma and renal oncocytoma: an immunohistochemical analysis of 328 cases. *Histopathology*. 2004;45:452-459.
38. 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;21:1409-1419.
39. Jordon D, Jagirdar J, Kaneko M. Blood group antigens Lewis^x and Lewis^y in the diagnostic discrimination of malignant mesothelioma versus adenocarcinoma. *Am J Pathol*. 1989;135:931-937.
40. Lyda MH, Weiss LM. Immunoreactivity for epithelial and neuroendocrine antibodies are useful in the differential diagnosis of lung carcinomas. *Hum Pathol*. 2000;31:980-987.
41. Ordóñez NG. Value of thyroid transcription factor-1, E-cadherin, BG8, WT1, and CD44S immunostaining in distinguishing epithelial pleural mesothelioma from pulmonary and nonpulmonary adenocarcinoma. *Am J Surg Pathol*. 2000;24:598-606.
42. Davidson B, Risberg B, Kristensen G, et al. Detection of cancer cells in effusions from patients diagnosed with gynaecological malignancies: evaluation of five epithelial markers. *Virchows Arch*. 1999;435:43-49.
43. 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;122:252-260.
44. Warnock ML, Stoloff A, Thor A. Differentiation of adenocarcinoma of the lung from mesothelioma: periodic acid-Schiff, monoclonal antibodies B72.3, and Leu M1. *Am J Pathol*. 1988;133:30-38.
45. Ordóñez NG. The immunohistochemical diagnosis of mesothelioma: differentiation of mesothelioma and lung adenocarcinoma. *Am J Surg Pathol*. 1989;13:276-291.
46. 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;21:759-766.
47. Wirth PR, Legier J, Wright GL Jr. Immunohistochemical evaluation of seven monoclonal antibodies for differentiation of pleural mesothelioma from lung adenocarcinoma. *Cancer*. 1991;67:655-662.
48. Brown RW, Clark GM, Tandon AK, et al. Multiple-marker immunohistochemical phenotypes distinguishing malignant pleural mesothelioma from pulmonary adenocarcinoma. *Hum Pathol*. 1993;24:347-354.
49. Brown RW, Campagna LB, Dunn JK, et al. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma: a diagnostic adjunct in the determination of primary site. *Am J Clin Pathol*. 1997;107:12-19.
50. 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;21:1399-1408.
51. Nuti M, Teramoto YA, Mariani-Costantini R, et al. A monoclonal antibody (B72.3) defines patterns of distribution of a novel tumor-associated antigen in human mammary carcinoma cell populations. *Int J Cancer*. 1982;29:539-545.
52. Galietta A, Pizzi C, Pettinato G, et al. Differential TAG-72 epitope expression in breast cancer and lymph node metastases: a marker of a more aggressive phenotype. *Oncol Rep*. 2002;9:135-140.
53. Honma N, Takubo K, Arai T, et al. Comparative study of monoclonal antibody B72.3 and gross cystic disease fluid protein-15 as markers of apocrine carcinoma of the breast. *APMIS*. 2006;114:712-719.
54. Hornick JL, Lauwers GY, Odze RD. Immunohistochemistry can help distinguish metastatic pancreatic adenocarcinomas from bile duct adenomas and hamartomas of the liver. *Am J Surg Pathol*. 2005;29:381-389.
55. Nawgiri RS, Nagle JA, Wilbur DC, et al. Cytomorphology and B72.3 labeling of benign and malignant ductal epithelium in pancreatic lesions compared to gastrointestinal epithelium. *Diagn Cytopathol*. 2007;35:300-305.
56. Thor A, Gorstein F, Ohuchi N, et al. Tumor-associated glycoprotein (TAG-72) in ovarian carcinomas defined by monoclonal antibody B72.3. *J Natl Cancer Inst*. 1986;76:995-1006.
57. Bollinger DJ, Wick MR, Dehner LP, et al. Peritoneal malignant mesothelioma versus serous papillary adenocarcinoma: a histochemical and immunohistochemical comparison. *Am J Surg Pathol*. 1989;13:659-670.
58. Wick MR, Mills SE, Dehner LP, et al. Serous papillary carcinomas arising from the peritoneum and ovaries: a clinicopathologic and immunohistochemical comparison. *Int J Gynecol Pathol*. 1989;8:179-188.
59. Khoury N, Raju U, Crissman JD, et al. A comparative immunohistochemical study of peritoneal and ovarian serous tumors and mesotheliomas. *Hum Pathol*. 1990;21:811-819.
60. Ponnusamy MP, Venkatraman G, Singh AP, et al. Expression of TAG-72 in ovarian cancer and its correlation with tumor stage and patient prognosis. *Cancer Lett*. 2007;251:247-257.
61. Loy TS, Darkow GV, Quesenberry JT. Immunostaining in the diagnosis of pulmonary neuroendocrine carcinomas: an immunohistochemical study with ultrastructural correlations. *Am J Surg Pathol*. 1995;19:173-182.
62. López-Ferrer A, Barranco C, de Bolós C. Differences in the O-glycosylation patterns between lung squamous cell carcinoma and adenocarcinoma. *Am J Clin Pathol*. 2002;118:749-755.
63. Gaffey MJ, Mills SE, Swanson PE, et al. Immunoreactivity for BER-EP4 in adenocarcinomas, adenomatoid tumors, and malignant mesotheliomas. *Am J Surg Pathol*. 1992;16:593-599.
64. González-Lois C, Ballestín C, Sotelo MT, et al. Combined use of novel epithelial (MOC-31) and mesothelial (HBME-1) immunohistochemical markers for optimal first line diagnostic distinction between mesothelioma and metastatic carcinoma in pleura. *Histopathology*. 2001;38:528-534.
65. Collins CL, Ordóñez NG, Schaefer R, et al. Thrombomodulin expression in malignant pleural mesothelioma and pulmonary adenocarcinoma. *Am J Pathol*. 1992;141:827-833.
66. Pan CC, Chen PC, Chou TY, et al. Expression of calretinin and other mesothelioma-related markers in thymic carcinoma and thymoma. *Hum Pathol*. 2003;34:1155-1162.
67. Genega EM, Hutchinson B, Reuter VE, et al. Immunophenotype of high-grade prostatic adenocarcinoma and urothelial carcinoma. *Mod Pathol*. 2000;13:1186-1191.
68. 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;31:1139-1148.

DOI: 10.1309/AJCP0B3YJBXWXJII

69. Spagnolo DV, Whitaker D, Carrello S, et al. The use of monoclonal antibody 44-3A6 in cell blocks in the diagnosis of lung carcinoma, carcinomas metastatic to lung and pleura, and pleural malignant mesothelioma. *Am J Clin Pathol.* 1991;95:322-329.
70. Kadota A, Masutani M, Takei M, et al. Evaluation of expression of CD15 and sCD15 in non–small cell lung cancer. *Int J Oncol.* 1999;15:1081-1089.
71. Brockstedt U, Gulyas M, Dobra K, et al. An optimized battery of eight antibodies that can distinguish most cases of epithelial mesothelioma from adenocarcinoma. *Am J Clin Pathol.* 2000;114:203-209.
72. Miettinen M, Limon J, Niezabitowski A, et al. Calretinin and other mesothelioma markers in synovial sarcoma: analysis of antigenic similarities and differences with malignant mesothelioma. *Am J Surg Pathol.* 2001;25:610-617.
73. Sim SJ, Ro JY, Ordóñez NG, et al. Metastatic renal cell carcinoma to the bladder: a clinicopathologic and immunohistochemical study. *Mod Pathol.* 1999;12:351-355.
74. Taki A, Nakatani Y, Misugi K, et al. Chromophobe renal cell carcinoma: an immunohistochemical study of 21 Japanese cases. *Mod Pathol.* 1999;12:310-317.
75. Dejmek A, Hjerpe A. Carcinoembryonic antigen-like reactivity in malignant mesothelioma: a comparison between different commercially available antibodies. *Cancer.* 1994;73:464-469.
76. Christensen WN, Boitnott JK, Kuhajda FP. Immunoperoxidase staining as a diagnostic aid for hepatocellular carcinoma. *Mod Pathol.* 1989;2:8-12.
77. Adsay NV, Merati K, Nassar H, et al. Pathogenesis of colloid (pure mucinous) carcinoma of exocrine organs: coupling of gel-forming mucin (MUC2) production with altered cell polarity and abnormal cell-stroma interaction may be the key factor in the morphogenesis and indolent behavior of colloid carcinoma in the breast and pancreas. *Am J Surg Pathol.* 2003;27:571-578.
78. 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;116:253-262.

DOI: 10.1309/AJCP0B3YJBXWXJII