Immunohistochemical Analysis Still Has a Limited Role in the Diagnosis of Malignant Mesothelioma

A Study of Thirteen Antibodies

Fiona Roberts, MRCPath, Christina M. Harper, MBChB, Ian Downie, MSc, and Rodney A. Burnett, FRCPath

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Abstract

To identify the most accurate and useful panel to diagnose mesothelioma, we immunostained sections from 112 mesotheliomas, 18 adenocarcinomas, and 11 reactive pleural specimens with 13 antibodies. Positive results for mesotheliomas, adenocarcinomas, and reactive pleura, respectively, were CAM5.2, 111, 18, and 11; vimentin, 30, 3, and 3; HBME-1, 75, 10, and 8; thrombomodulin, 31, 2, and 2; calretinin, 43, 6, and 11; and CD44H, 68, 10, and 4. Positive results for adenocarcinoma markers in mesotheliomas and adenocarcinomas, respectively, were carcinoembryonic antigen, 1 and 15; LeuM1, 7 and 9; and Ber-EP4, 5 and 12. All reactive pleura were negative. Positive results for markers to help distinguish mesothelioma from reactive pleura in mesotheliomas, adenocarcinomas, and reactive pleura, respectively, were epithelial membrane antigen, 76, 17, and 6; p53, 78, 16, and 9; P-170 glycoprotein, 37, 4, and 2; and platelet-derived growth factor receptor beta, 31, 1, and 2.

The differential diagnosis of mesothelioma from adenocarcinoma is based on negative markers. Individual mesothelial markers are of low sensitivity and specificity for mesothelioma. However, diagnostic accuracy is improved by the use of antibody panels. To date there are no antibodies that help distinguish mesothelioma from reactive pleura.

In the United Kingdom, the incidence of malignant mesothelioma is rising, and it has been suggested that it may not peak until 2020.[1] Between 1981 and 1989, the incidence of mesothelioma in Glasgow, Scotland, was 69 per million males per year.[2] In the Clydebank district of Glasgow, the incidence is 6 times higher than in the rest of Scotland.[3] This is related to the shipbuilding industry in which asbestos was used widely for lagging pipes and boilers.

The accurate diagnosis of malignant mesothelioma is important for clinical and medicolegal reasons. Early and precise diagnosis of biopsy samples may influence clinical management and avoid unnecessary invasive diagnostic procedures. Furthermore, from a legal viewpoint, compensation claims from workers occupationally exposed to asbestos demands an accurate diagnosis of malignant mesothelioma. That said, the interpretation of malignant tumors of the serosal surfaces remains a diagnostic challenge.

Most experts agree that immunohistochemical analysis is the most important ancillary technique used to differentiate malignant mesothelioma from adenocarcinoma, sarcoma, and reactive mesothelial proliferations. However, until recently, there were no immunohistochemical markers suitable for the positive diagnosis of mesothelioma. In the differential diagnosis of malignant mesothelioma and adenocarcinoma, the diagnostic application of immunohistochemical analysis relied on the confirmation of a tumor not being a mesothelioma and was based largely on exclusion. These adenocarcinoma-associated antibodies include carcinoembryonic antigen (CEA),[4-6] human epithelial antigen (Ber-EP4),[6-8] and LeuM1[6] and are used commonly in conjunction with mucin stains. A negative result for these antibodies and stains for epithelial mucin supports a diagnosis of malignant mesothelioma. In recent years, many new antibodies, some of which are putative, positive mesothelial markers, have been described. These include

human mesothelial antigen (HBME-1),[7,9] thrombomodulin,[10-12] calretinin,[13-17] and CD44H.[13,18] However, results have been conflicting, and no single immunohistochemical marker has been shown to be absolutely specific or sensitive for distinguishing mesothelioma from its mimics.

Immunohistochemical analysis also has been used to assist in the distinction of mesothelioma from reactive mesothelial proliferations. Several reports have described differential staining patterns with epithelial membrane antigen (EMA)[19,20] in reactive and malignant processes, as well as increased staining for p53[21,22] in malignant mesothelioma. Again, however, results are conflicting. Antibodies to the multidrug resistance gene product, P-170 glycoprotein,[23] and platelet derived growth factor receptor beta (PDGFR-beta)[24] also may be of use but have not been assessed fully.

The aim of the present study was to identify the most accurate and clinically useful immunohistochemical panel for the diagnosis of mesothelioma in formalin-fixed, paraffin-embedded biopsy material. For this purpose, we used a panel of 13 commercially available antibodies. The panel included antibodies generally considered most useful as markers for differentiating adenocarcinoma (CEA, Ber-EP4, and LeuM1), sarcoma (CAM5.2), and reactive mesothelial proliferations (EMA, p53, P-170 glycoprotein, and PDGFR-beta) from mesothelioma and putative selective markers for mesothelioma (antihuman mesothelial cell antigen [HBME-1], thrombomodulin, calretinin, and CD44H). In addition, vimentin, considered in some series to be highly sensitive and specific for mesothelioma,[8] also was included.

Materials and Methods

Tumor Samples

A total of 112 mesotheliomas were retrieved from the pathology records of the Western Infirmary (January 1994-December 1998) and Southern General Hospital (January 1980-December 1997), Glasgow. These comprised a mixture of needle biopsy specimens and thoracoscopic biopsy specimens, and all had been formalin-fixed and paraffin-embedded. The diagnosis of malignant mesothelioma in each case was supported by appropriate clinical findings (relevant history, chest radiograph and/or computed tomography scan appearances, and clinical course). Furthermore, the light microscopic features including negative mucin staining were considered consistent with those reported in standard texts.[25] There were 100 pleural tumors, 7 peritoneal tumors, 1 tumor of the tunica vaginalis testis, 2 chest wall nodules resulting from direct tumor spread, and 2 metastases, 1 to lymph node and 1 to scalp. Eighty-two mesotheliomas (73.2%) were the epithelioid subtype, 11 (9.8%) were sarcomatoid, 12 (10.7%) were biphasic, and 7 (6.2%) were desmoplastic.

In addition to the mesotheliomas, 18 adenocarcinomas and 11 reactive mesothelial proliferations were retrieved from the pathology records of the Western Infirmary (January 1994-December 1995). The adenocarcinomas were all pleural metastases from a variety of sites, most commonly lung. The reactive mesothelial proliferations were all pleural in origin. The diagnosis of reactive mesothelial proliferation was based on established histologic criteria. Specimens from patients with a history or suspicion of malignant neoplasm were not included. In 4 years of follow-up, no malignant neoplasms developed in patients whose specimens were included in the study. In a few cases, there was insufficient material to perform immunohistochemical analysis for all 13 antibodies. The total number of cases examined for each antibody is indicated in the "Results" section.

Immunohistochemical Analysis

Information about the antibodies selected is given in **Table 1**. For immunohistochemical analysis, 3-µm sections were immunostained using a standard avidin-biotin complex technique and 3,3-diaminobenzidene as the chromogen. Briefly, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol, and endogenous biotin activity was blocked using avidin and biotin. For antigen retrieval, slides were microwaved in a 1-mmol/L concentration of EDTA, pH 8.0, for 10 minutes or incubated with 0.1% trypsin, pH 7.8, for 10 minutes. The type of antigen retrieval for each antibody and dilutions of antibody used also are given in Table 1. Appropriate control material (according to manufacturer's instructions) was used for each run. For negative controls, the primary antibody was replaced with normal horse serum.

Interpretation of Results

The slides were evaluated semiquantitatively, and the percentage of positive tumor or reactive mesothelial cells was described as follows: 1, staining of 10% to 39% of the cells; 2, staining of 40% to 79% of the cells; or 3, staining of 80% to 100% of the cells. All cases showing staining of fewer than 10% of the tumor cells were regarded as negative. Weak staining or staining that was difficult to interpret was regarded as equivocal. The pattern of staining was recorded as membranous, cytoplasmic, or nuclear for each antibody. The sections were assessed independently by 2 observers (F.R. and C.M.H.). For cases in which there was substantial disagreement, both observers reassessed the slides and a consensus was reached.

Results

The results are summarized in **Table 2** through **Table 9**.

Table 1
Antibodies Used for Immunohistochemical Analysis

Antibody	Clone	Source	Dilution	Pretreatment
CAM5.2	—	Becton Dickinson, Oxford, England	1:10	Microwave
Vimentin	Vim 3B4	DAKO, Ely, Cambridge, England	1:400	Trypsin
Human mesothelial cell antigen	HBME-1	DAKO	1:100	Trypsin
Thrombomodulin	1009	DAKO	1:50	None
Calretinin	—	Chemicon, Wealdstone, Harrow, England	1:4,000	Microwave
CD44H	F10-44-2	Novocastra, Newcastle upon Tyne, England	1:75	Microwave
Carcinoembryonic antigen	12-140-10	Novocastra	1:50	Trypsin
LeuM1	CBD1	DAKO	1:20	Microwave
Human epithelial antigen	Ber-EP4	DAKO	1:100	Trypsin
Epithelial membrane antigen	E29	DAKO	1:50	None
p53	DO-7	DAKO	1:200	Microwave
P-170 glycoprotein	JSB-1	TCS Biological, Claydon, England	1:20	Microwave
PDGFR-beta	—	Genzyme, Cambridge, England	1:100	None

PDGFR, platelet-derived growth factor receptor.

Table 2
Immunohistochemical Staining of Mesotheliomas, Adenocarcinomas, and Reactive Mesothelial Proliferations

Tumor Type	CAM5.2	Vimentin	HBME-1	TM	Calretinin	CD44H	CEA	LeuM1	Ber-EP4	EMA	p53	P-170gp	PDGFR-beta
Mesotheliomas	111/112	30/108	75/111	31/112	43/108	68/112	1/112	7/111	5/112	76/112	78/112	37/105	31/109
Epithelioid	82/82	17/79	64/81	25/82	39/78	50/82	0/82	6/81	4/82	65/82	61/82	33/76	25/80
Sarcomatoid	10/11	5/11	2/11	2/11	2/11	10/11	0/11	0/11	0/11	0/11	6/11	1/11	1/11
Biphasic	12/12	4/11	6/12	3/12	1/12	6/12	1/12	1/12	1/12	9/12	7/12	3/12	5/11
Desmoplastic	7/7	4/7	3/7	1/7	1/7	2/7	0/7	0/7	0/7	2/7	4/7	0/7	0/7
Adenocarcinomas	18/18	3/18	10/18	2/18	6/17	10/18	15/18	9/18	12/18	7/18	16/17	4/18	2/18
Reactive mesothelial proliferations	11/11	3/11	8/11	2/11	11/11	4/11	0/11	0/11	0/11	6/11	9/11	2/11	2/11

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.

Table 3
Immunohistochemical Staining of All Mesotheliomas

Antibody	No. of Cases	Negative	3*	2*	1*	Equivocal
CAM5.2	112	1	110	1	0	0
Vimentin	108	75	13	11	6	3
HBME-1	111	36	54	14	7	0
TM	112	79	7	14	10	2
Calretinin	108	56	31	10	2	9
CD44H	112	41	34	21	13	3
CEA	112	110	0	1	0	1
LeuM1	111	103	3	2	2	1
Ber-EP4	112	107	1	4	0	0
EMA	112	35	53	16	7	1
p53	112	31	38	28	12	3
P-170gp	106	49	25	9	3	20
PDGFR-beta	109	65	15	12	4	13

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

CAM 5.2 and Vimentin

All but 1 mesothelioma (of the sarcomatoid subtype) and all adenocarcinomas and reactive mesothelial proliferations showed strong cytoplasmic staining for CAM5.2.

Vimentin was expressed in 30 (27.7%) of 108 mesotheliomas, 3 (17%) of 18 adenocarcinomas, and 3 (27%) of 11 reactive mesothelial proliferations. Of 30 positive mesotheliomas, 13 showed diffuse staining. The remaining 17 showed patchy staining. Fewer mesotheliomas of the epithelioid subtype (17/79 cases) were positive than were the subtypes that incorporated a spindle cell component (biphasic, 4/11; sarcomatoid, 5/11; desmoplastic, 4/7).

Table 4
Immunohistochemical Staining of 82 Epithelioid Mesotheliomas

Antibody	No. of Cases	Negative	3*	2*	1*	Equivocal
CAM5.2	82	0	82	0	0	0
Vimentin	79	60	6	6	5	2
HBME-1	81	17	48	11	5	0
TM	82	55	6	12	7	2
Calretinin	78	32	28	9	2	7
CD44H	82	30	24	17	9	2
CEA	82	81	0	0	0	1
LeuM1	81	75	2	2	2	0
Ber-EP4	82	78	1	3	0	0
EMA	82	17	46	14	5	0
p53	82	20	30	22	9	1
P-170gp	76	29	24	7	2	14
PDGFR-beta	80	43	12	10	3	12

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

Table 5
Immunohistochemical Staining of Biphasic Mesotheliomas

Antibody	No. of Cases	Negative	3*	2*	1*	Equivocal
CAM5.2	12	0	12	0	0	0
Vimentin	11	7	3	0	1	0
HBME-1	12	6	3	2	1	0
TM	12	9	0	1	2	0
Calretinin	12	10	1	0	0	1
CD44H	12	6	3	2	1	0
CEA	12	11	0	1	0	0
LeuM1	12	10	1	0	0	1
Ber-EP4	12	11	0	1	0	0
EMA	12	3	5	2	2	0
p53	12	4	3	4	0	1
P-170gp	12	6	1	1	1	3
PDGFR-beta	11	6	2	2	1	0

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

HBME-1, Thrombomodulin, Calretinin, and CD44H

Of 111 mesotheliomas, 75 (67.6%) showed positive membranous staining for HBME-1. These were predominantly of epithelioid type (64 cases) although staining was also identified in 2 sarcomatoid, 6 biphasic, and 3 desmoplastic subtypes. Membranous staining was also present in 10 (56%) of the 18 adenocarcinomas. There was no appreciable difference in the pattern of membranous staining between adenocarcinomas and mesotheliomas.

Of 112 mesotheliomas, 31 (27.7%) showed positive membranous staining for thrombomodulin. In the subtypes, staining was present in 25 epithelioid, 2 sarcomatoid, 3 biphasic, and 1 desmoplastic. In contrast only 2 (11%) of the adenocarcinomas showed patchy positive staining.

Of 108 mesotheliomas, 43 (39.8%) showed positive staining with calretinin. The pattern of staining was nuclear and to a lesser extent cytoplasmic. In the subtypes, staining was present in 39 epithelioid, 2 sarcomatoid, 1 biphasic, and 1 desmoplastic. Staining also was identified in 8 (47%) of 17 adenocarcinomas. There was no difference in the pattern of staining between mesotheliomas and adenocarcinomas.

Of 112 mesotheliomas, 68 (60.7%) showed positive membranous staining for CD44H. The majority of epithelioid mesotheliomas (50 cases) showed diffuse, membranous staining. However, staining also was present in 10 sarcomatoid, 6 biphasic, and 2 desmoplastic mesotheliomas. Ten (56%) of 18 adenocarcinomas showed diffuse membranous staining with this antibody.

Sixteen mesotheliomas, 15 epithelioid and 1 sarcomatoid, were positive for all 4 antibodies (HBME-1, thrombomodulin, calretinin, and CD44H). Fifteen mesotheliomas, 14 epithelioid and 1 biphasic, were positive for 3 antibodies. The most frequent antibody combination giving positive staining for 3 antibodies was HBME-1, calretinin, and CD44H (13 cases). Twenty-eight cases, 21 epithelioid, 4 biphasic, 1 sarcomatoid, and 2 desmoplastic, were positive for 2 antibodies. The most frequent antibody combination giving positive staining for 2 antibodies was HBME-1 and

Table 6
Immunohistochemical Staining of 11 Sarcomatoid Mesotheliomas

Antibody	Negative	3*	2*	1*	Equivocal
CAM5.2	1	10	0	0	0
Vimentin	5	2	3	0	1
HBME-1	9	2	0	0	0
TM	9	0	1	1	0
Calretinin	8	1	1	0	1
CD44H	1	6	1	3	0
CEA	11	0	0	0	0
LeuM1	11	0	0	0	0
Ber-EP4	11	0	0	0	0
EMA	10	0	0	0	1
p53	5	3	2	1	0
P-170gp	7	0	1	0	3
PDGFR-beta	9	1	0	0	1

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

Table 7
Immunohistochemical Staining of Seven Desmoplastic Mesotheliomas

Antibody	Negative	3*	2*	1*	Equivocal
CAM5.2	0	7	0	0	0
Vimentin	3	2	2	0	0
HBME-1	4	1	1	1	0
TM	6	1	0	0	0
Calretinin	6	1	0	0	0
CD44H	4	1	1	0	1
CEA	7	0	0	0	0
LeuM1	7	0	0	0	0
Ber-EP4	7	0	0	0	0
EMA	5	2	0	0	0
p53	2	2	0	2	1
P-170gp	7	0	0	0	0
PDGFR-beta	7	0	0	0	0

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

CD44H (13 cases). Forty-three cases stained with 1 antibody only, and 9 cases did not stain with any antibody.

None of the adenocarcinomas stained positively with all 4 antibodies. One case stained positively with 3 antibodies (HBME-1, calretinin, and CD44H). Seven cases stained with 2 antibodies, 5 with calretinin and CD44H, 1 with HBME-1 and CD44H, and 1 with thrombomodulin and CD44H. Seven cases stained with only 1 antibody (HBME-1 or CD44H), and 3 cases did not stain with any antibody.

The reactive mesothelial proliferations showed positive staining for HBME-1 in 7 cases, thrombomodulin in 2 cases, calretinin in 11 cases, and CD44H in 5 cases.

CEA, LeuM1, and Ber-EP4

Positive staining for CEA was observed in 1 mesothelioma of the biphasic subtype. Positive staining for LeuM1 and Ber-EP4 was observed in 7 (6.3%) and 5 (4.5%) of the 112 mesotheliomas, respectively. These were all of epithelioid subtype except for 1 biphasic mesothelioma. Staining was moderate to weak in the majority of cases, although 3 tumors showed strong membrane staining with LeuM1. No tumor stained with more than one of these antibodies.

Fifteen (83%) of 18 adenocarcinomas stained positively for CEA. Twelve of 15 adenocarcinomas showed diffuse cytoplasmic staining of tumor cells. In the remaining 3 cases, staining was patchy. Positive staining for LeuM1 and Ber-EP4 was observed in 9 (50%) and 12 (67%) of the adenocarcinomas, respectively. Strong, diffuse, membranous staining was present for LeuM1 and Ber-EP4 in 5 and 9 cases, respectively. In the remaining cases, the staining was patchy. The majority of adenocarcinomas stained positively for more than one antibody. Five cases stained for all 3 antibodies (CEA, LeuM1, and Ber-EP4), and 9 cases stained for 2 antibodies (4 with CEA and LeuM1 and 5 with CEA and Ber-EP4). One case stained with CEA only and 2 cases for Ber-EP4 only. One case was negative for all 3 antibodies.

The reactive mesothelial proliferations were negative for all 3 antibodies.

Table 8
Immunohistochemical Staining of Adenocarcinomas

Antibody	No. of Cases	Negative	3*	2*	1*	Equivocal
CAM5.2	18	0	18	0	0	0
Vimentin	18	15	1	1	1	0
HBME-1	18	8	4	3	3	0
TM	18	16	0	0	2	0
Calretinin	17	10	3	3	0	1
CD44H	18	8	4	5	1	0
CEA	18	3	12	2	1	0
LeuM1	18	9	5	2	2	0
Ber-EP4	18	6	9	2	1	0
EMA	18	1	13	4	0	0
p53	17	1	8	6	2	0
P-170gp	18	13	0	2	2	1
PDGFR-beta	18	15	0	2	0	1

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

Table 9
Immunohistochemical Staining of 11 Reactive Mesothelial Proliferations

Antibody	Negative	3*	2*	1*	Equivocal
CAM5.2	0	11	0	0	0
Vimentin	8	1	1	1	0
HBME-1	3	6	0	2	0
TM	9	1	0	1	0
Calretinin	0	5	4	2	0
CD44H	7	3	0	1	0
CEA	11	0	0	0	0
LeuM1	11	0	0	0	0
Ber-EP4	11	0	0	0	0
EMA	5	2	2	2	0
p53	2	4	2	3	0
P-170gp	9	0	2	0	0
PDGFR-beta	8	1	1	0	1

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; PDGFR, platelet-derived growth factor receptor; TM, thrombomodulin.
* 1, staining of 10%-39% of cells; 2, staining of 40%-79% of cells; 3, staining of 80%-100% of cells.

EMA, p53, P-170 Glycoprotein, and PDGFR-beta

Of 112 mesotheliomas, 76 (67.9%) stained positively for EMA. These included 65 epithelioid, 9 biphasic, and 2 desmoplastic mesotheliomas. Seventeen adenocarcinomas (94%) and 6 reactive mesothelial proliferations (55%) also stained with EMA. All positive mesotheliomas and reactive mesothelial proliferations showed membranous staining without cytoplasmic staining. The adenocarcinomas showed only membranous staining in 10 cases, only cytoplasmic staining in 5 cases, and both membranous and cytoplasmic staining in 2 cases.

The majority of these malignant tumors, 78 mesotheliomas (69.6%) and 16 adenocarcinomas (94%) showed moderate to widespread nuclear staining of tumor cells with p53. In addition, positive nuclear staining was present in 9 reactive mesothelial proliferations (82%).

Both observers experienced considerable difficulty in the interpretation of sections stained for P-170 glycoprotein and PDGFR-beta, resulting in a high number of equivocally stained sections. This was due to faint staining or difficulties in distinguishing membranous from cytoplasmic staining. Nevertheless, in sections in which staining was optimum, positive membrane staining for P-170 glycoprotein and cytoplasmic staining for PDGFR-beta was observed in 37 (34.9%) of 106 and 31 (28.4%) of 109 mesotheliomas, respectively. These were predominantly of the epithelioid and biphasic subtypes. Only 1 sarcomatoid mesothelioma was positive. All 7 desmoplastic mesotheliomas were negative for both antibodies. Positive staining for P-170 glycoprotein and PDGFR-beta was observed in 4 (22%) and 2 (11%) of 18 adenocarcinomas, respectively, and 2 (18%) and 2 (18%) of 11 reactive mesothelial proliferations, respectively.

Discussion

Immunohistochemical analysis has been used extensively to assist in the diagnosis of malignant mesothelioma.

To date, there is no single immunohistochemical marker both entirely specific and sensitive for distinguishing mesothelioma from its common mimics. Many laboratories perform immunohistochemical analysis using various combinations of monoclonal and polyclonal antibodies in an attempt to differentiate between mesothelioma and its mimics, particularly adenocarcinoma.[6,7,26] The majority of studies have dealt with only a few antibodies or relatively small numbers of cases. The results of these studies and those of the present study have generated disparate results. That said, most observers accept that the diagnosis of mesothelioma requires a panel of antibodies, although there is little agreement on the contents of this panel.

The present study was planned to ascertain the usefulness of commercially available, well-established antibodies in the diagnosis of malignant mesothelioma in our laboratory. As in the majority of studies, we found that all but 1 mesothelioma, all adenocarcinomas, and all reactive mesotheliomas showed strong and diffuse staining for CAM5.2, an antibody to low-molecular-weight cytokeratins. The CAM5.2-negative case was a poorly differentiated, sarcomatoid mesothelioma, which was negative for all markers except CD44H and vimentin. Other investigators have reported occasional cytokeratin-negative mesotheliomas.[27] Anticytokeratins generally are regarded as most useful for the distinction of mesothelioma from sarcoma, solitary fibrous tumor, and reactive pleural fibrosis.[6] These proliferations are usually negative for anticytokeratin, although occasional entrapped mesothelial cells may stain positively in reactive pleural fibrosis, and certain sarcomas including synovial sarcoma and leiomyosarcoma also can be positive.[28] Calretinin positivity also has been reported in synovial sarcoma.[29] Anticytokeratins do not contribute to the differential diagnosis between mesothelioma and adenocarcinoma. However, occasionally, epithelioid variants of sarcomas may involve serous cavities, and negative or weak staining for anticytokeratin will prompt the use of a wider immunohistochemical panel.[30]

In the present study, vimentin was expressed in 30 (27.7%) of 108 mesotheliomas. This is in contrast with other studies that suggest that more than 80% of mesotheliomas are positive for vimentin.[8,31] A small percentage of adenocarcinomas (17% [3/18]) also were positive for vimentin. Similar to other studies,[7] in our study vimentin does not seem to be substantially more specific and sensitive for mesothelioma than for adenocarcinoma. However, vimentin may have a useful role in the distinction of sarcomatoid mesothelioma from reactive pleural fibrosis and from sarcomas that usually show diffuse, strong positivity for vimentin and are negative for cytokeratin.[30]

The majority of immunohistochemical studies have concentrated on the distinction of epithelioid mesothelioma from adenocarcinoma. Until recently, these studies concentrated on so-called negative markers, ie, those that usually are expressed in adenocarcinomas but not in mesothelioma. The present study included monoclonal antibodies to CEA, LeuM1, and Ber-EP4. Of these 3 immunohistochemical markers, CEA has been the most extensively studied in this context.[4,6] The initial study by Wang et al[4] reported positive staining in 12 of 12 adenocarcinomas but none of 9 mesotheliomas. Subsequent studies confirmed this finding, although some authors have reported CEA positivity in up to 45% of mesotheliomas.[6] Some of these discrepancies may be attributable to the use of different anti-CEA antibodies.[6] In the present study, more than 80% of adenocarcinomas expressed CEA, and 1 biphasic mesothelioma showed patchy positive staining within the epithelial component but not the sarcomatous component. These findings are therefore in agreement with the majority of previous studies and support the view that CEA is one of the most useful immunohistochemical markers for differentiating between these 2 malignant neoplasms.

Ber-EP4, and LeuM1, monoclonal antibodies to epithelial glycoproteins, also have been studied extensively. Most reports have shown that LeuM1 is positive in 50% to 100% of adenocarcinomas and negative in mesothelioma.[13,14] However, in 1 study, LeuM1 positivity was seen in 8% (3/36) of epithelioid mesotheliomas.[5] Findings for Ber-EP4 have been more controversial, with reports of positive staining in 32% to 100% of adenocarcinomas and 0% to 88% of mesotheliomas.[6] This may be related, in part, to the cutoff level regarded as positive, which differs between studies.[32] Ordonez[32] suggested that discrepant staining of adenocarcinomas may be related to the site of origin of the metastatic tumor, since he found that 100% (20/20) of pulmonary adenocarcinomas were positive for Ber-EP4 in contrast with 84% (26/31) of adenocarcinomas of unknown origin. Another discrepancy may be related to the pattern of membrane staining regarded as positive by different investigators. For example, Riera et al[7] considered only lateral membrane staining to be positive, whereas other investigators regarded any membrane staining as positive. In practice, it is difficult to consistently qualify the pattern of membrane staining. In our study, LeuM1 and Ber-EP4 were positive in 50% (9/18) and 67% (12/18) of adenocarcinomas, respectively. Positive staining for LeuM1 and Ber-EP4 also was identified in a small percentage of epithelioid and biphasic mesotheliomas (8% [7/93] and 5% [5/94], respectively). However, staining was patchy in the majority of cases, and, in contrast with the adenocarcinomas in which 15 of 18 cases stained with at least 2 antibodies, no mesothelioma was positive for more than 1 of these antibodies. In our study, the combination of these 3 antibodies is highly specific for separating adenocarcinoma from mesothelioma and further promotes the argument for the use of antibody panels.

Antigens that are commonly expressed in mesothelioma but not in adenocarcinoma have been identified relatively recently. Similar to the negative markers of mesothelioma, a wide variety of opinions exists about the relative effectiveness of these putative positive markers. The present study included HBME-1, thrombomodulin, calretinin, and CD44H.

HBME-1 was one of the first commercially available antimesothelial antibodies that could be used successfully on formalin-fixed, paraffin-embedded specimens. This antibody is believed to react with an antigen present on the microvillous surface of mesothelial cells, producing thick, circumferential, membranous staining.[24] Adenocarcinomas show either cytoplasmic or brush-border–like staining. Initial investigators stated that HBME-1 was useful for differentiating mesothelioma from adenocarcinoma.[7,9] However, our study concurs with the opinion of the majority of investigators that HBME-1 alone is of little value in the differential diagnosis,[6] positively staining 79% of epithelioid mesotheliomas (64/81) and 56% of adenocarcinomas (10/18).

In 1992, Collins et al[10] were the first to report differential expression of thrombomodulin in mesotheliomas compared with adenocarcinomas. Since then, several other studies have concluded that thrombomodulin is a useful immunohistochemical marker for distinguishing between epithelioid mesothelioma and adenocarcinoma.[11,12] Most studies suggest that between 80% and 100% of mesotheliomas and 10% to 15% of adenocarcinomas express thrombomodulin.[11,12] A few studies have reported positive staining of 49% to 60% of mesotheliomas and staining in up to 60% of adenocarcinomas.[7,33] In our study, antithrombomodulin stained 30% of epithelioid mesotheliomas (25/82), and 11% of adenocarcinomas (2/18) also showed patchy positive staining. It is well recognized that thrombomodulin reactivity tends to be patchy in formalin-fixed, paraffin-embedded tissues, and false-negative results can occur in small biopsy specimens.[6] This may account for the lower specificity and, thus, reduced diagnostic usefulness seen in some studies, including our own.

Several investigators have proposed that calretinin is a useful marker for the diagnosis of malignant mesothelioma.[14-16] Doglioni et al[14] observed strong reactivity for calretinin in 100% (44/44) of mesotheliomas, including epithelioid and sarcomatoid subtypes, with only patchy staining in 9.5% (28/294) of adenocarcinomas. The staining pattern was both nuclear and cytoplasmic. Several other investigators reported staining in 100% of mesotheliomas with variable staining (10%-23%) of adenocarcinomas.[15,16] In contrast Riera et al[7] reported staining in 42% (24/57) of epithelioid mesotheliomas and 6.2% (13/211) of adenocarcinomas. Similar to CEA staining, this again may be a consequence of the different antibodies used. In a comparative study of 2 commercially available antibodies from Zymed, San Francisco, CA, and Chemicon, Temecula, CA, Ordonez[17] found that the Chemicon antibody was less sensitive than the Zymed antibody for mesothelioma. However, similarly small numbers of adenocarcinomas stained with both antibodies. As we used the Chemicon antibody in our study, this may explain why positive staining was obtained in only 39.8% of mesotheliomas. Surprisingly, however, 47% of adenocarcinomas (8/17) stained for calretinin. That said, our results are in agreement with a study by Oates and Edwards,[26] who found positive or equivocal staining in 70% (28/40) of antibodies using the Chemicon antibody. This may reflect differences in interpretation of nuclear and cytoplasmic staining, but it suggests that in our laboratory it would not be a useful discriminatory marker for mesothelioma and adenocarcinoma.

CD44H is a receptor for hyaluronic acid. It is involved in various physiologic functions, including cell-cell adhesion, cell-matrix interactions, and lymphocyte homing and activation. It also has a pathologic role in tumor metastasis. In 1997, Attanoos et al[18] reported CD44H reactivity in 75% (15/20) of pleural mesotheliomas and in reactive mesothelial proliferations but only patchy staining in 15% (3/20) of adenocarcinomas. However, since then, other investigators have reported staining in 63% to 90% of mesotheliomas and 34% to 43% of adenocarcinomas.[34,35] Similarly, in the present study, 60.7% of mesotheliomas and 56% (10/18) of adenocarcinomas were positive for CD44H.

In the present study, the relative sensitivity and specificity for each individual antibody is disappointing. The use of antibody combinations improves the diagnostic accuracy of these markers, but they still seem to be of relatively low sensitivity and specificity. Both negative and positive mesothelial markers have little place in the distinction of mesothelioma from reactive mesothelial proliferations.

The distinction of mesothelioma from reactive mesothelial proliferations has been studied less extensively than the distinction of mesothelioma from adenocarcinoma. Antibodies to EMA, p53, PDGFR-beta, and P-170 glycoprotein have been used in this respect. Most studies have reported strong membranous staining for EMA in 73% to 97% of mesotheliomas with only weak or equivocal membrane staining in 4% to 25% of reactive mesothelial proliferations.[19,20,35] In the present study, positive membranous staining was present in 67.8% of mesotheliomas and 55% of reactive mesothelial proliferations (6/11). Furthermore, although staining was diffuse in the majority of mesotheliomas, staining also was diffuse in 18% of reactive mesothelial proliferations (2/11).

It has been reported that immunoreactivity to p53 is useful for discriminating between neoplastic and reactive mesothelium.[21,22] Most studies have described positive nuclear staining in 44% to 55% of mesotheliomas with negative staining of reactive mesothelial hyperplasias.[21,22] An

article by Cury et al[20] reports staining for p53 in 97% (30/31) of mesotheliomas but also occasional nuclear positivity for p53 in 25% (5/20) of reactive mesothelial hyperplasias. They concluded that nuclear staining for p53 should be regarded as no more than suggestive of mesothelioma. In our study, staining for p53 was identified in 69.6% of mesotheliomas. However, p53 immunostaining also was positive in 82% (9/11) of reactive mesothelial hyperplasias with diffuse nuclear staining in 4 cases (36%). Immunostaining for p53 is markedly affected by tissue fixation and antigen retrieval,[36] and this may account for some of the discrepancies between findings in these studies.

Ramael et al[23,24] reported differing immunoreactivity for P-170 glycoprotein, the product of the multidrug resistance gene, and for PDGFR-beta in malignant mesothelioma and nonneoplastic mesothelium. They found 94% (31/33) of mesotheliomas stained for P-170 glycoprotein and 39% (13/33) stained for PDGFR-beta, whereas nonneoplastic mesothelium was negative for both antibodies. In our study, 34.9% and 28.4% of mesotheliomas stained for P-170 glycoprotein and PDGFR-beta, respectively. Of 11 reactive mesothelial proliferations, 18% (2) stained with each antibody. In the present study, none of these 4 antibodies seems to reliably distinguish between malignant and reactive processes in the pleura.

Immunohistochemical analysis remains important in the differential diagnosis of malignant mesothelioma. However, despite the growing number of antibodies available to assist with this difficult diagnosis, the diagnostic usefulness of these antibodies remains controversial. There are considerable variations between laboratories that can be attributed to multiple factors, including, for example, length of tissue fixation, biopsy specimen size, and method of antigen retrieval. This is one of the largest immunohistochemical studies performed to date on malignant mesothelioma. Our results emphasize the shortcomings of relying on individual antibodies and support the use of immunohistochemical panels. We recommend that an immunohistochemical panel include a cytokeratin cocktail and at least 2 positive markers of adenocarcinoma, depending on which is most effective in any individual laboratory. Positive mesothelial markers are of low specificity and sensitivity, but in combination they may provide useful additional evidence supporting a diagnosis of mesothelioma. There does not seem to be any immunohistochemical marker or panel of markers that reliably distinguishes reactive mesothelial proliferations from mesothelioma. New positive mesothelial markers, not included in this study, are continuing to emerge. These include antibodies to WT-1[26] and cytokeratin 5/6.[37] Recently developed antibodies that are selective for adenocarcinoma also are available, including MOC-1,[38] B72.3,[7] E-cadherin,[16] and BG-8.[7] It seems unlikely that any of these antibodies will be sufficiently sensitive and specific to be used in isolation. Therefore, continual update of immunohistochemical panels for the diagnosis of mesothelioma will be required for the foreseeable future.

From the Department of Pathology, University of Glasgow and Western Infirmary, Glasgow, Scotland.

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Address reprint requests to Dr Roberts: Dept of Pathology, Western Infirmary, Dumbarton Rd, Glasgow G11 6NT, Scotland.

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