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Aberrant Immunohistochemical stains among 573 cases of Diffuse Mesothelioma

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ABSTRACT

Background: Diagnosis of malignant mesothelioma often requires differentiating it from other metastatic malignancies including breast cancer, lung cancer, ovarian cancer, colorectal cancer, etc. Immunohistochemical stains (IHC) are important means to assist in confirming mesothelioma and ruling out metastatic cancers. Although mesothelial specific markers have been used for diagnosis, aberrant immunostains were also observed in clinical practice which often confounded the diagnosis. In this study, we analyzed the positive rate of commonly used IHC markers in the diagnosis of mesothelioma among 573 patients.

Design: 427 cases of epithelioid mesothelioma, 87 cases of biphasic mesothelioma and 59 cases with sarcomatoid mesothelioma were retrieved from the pathology consultation files between 2020-2023. The positive rates of over 50 IHC markers including over 30 aberrant IHC markers were analyzed.

Results: The positive rates of mesothelial markers, such as calretinin, WT-1 and D2-40, and epithelial markers, such as cytokeratins, were highest in epithelioid type, intermediate in biphasic type and lowest in sarcomatoid type mesothelioma. The mesenchymal marker vimentin and additional marker GATA-3 were highly expressed in sarcomatoid mesothelioma. The highest loss rate of BAP-1 was in epithelioid type, while the highest loss rate of MTAP was in sarcomatoid type. The CDKN2A (p16) deletion was observed equally in both epithelioid and sarcomatoid/biphasic types. Over 30 aberrant markers were observed. For epithelioid type, commonly observed aberrant IHC markers included MOC-31, BerEP4, PAX-8, p63, CK20, NKX3.1 and ER. For biphasic type, significant aberrant IHC markers included MOC-31, Ber-EP4, PAX8, CK20 and p40. For sarcomatoid type, notable aberrant IHC markers included p63, SMA, ERG and CD31. Among these aberrant IHC markers, MOC-31, Ber-EP4 and p63 were more commonly observed in pleural epithelioid mesothelioma, whereas PAX-8 in peritoneal epithelioid mesothelioma.

Conclusions: Our data demonstrated that aberrant immunostains were common in all of the histological types of mesothelioma. Therefore, the diagnosis of mesothelioma should be based on correlation of clinical presentation, radiological findings, and selective panel of immunostains, and should not be distracted by aberrant immunostains.

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Introduction

Diffuse mesothelioma is a rare malignant neoplasm arising from the mesothelium which evolved from mesoderm [1, 2]. Mesothelium lines the body cavities including the pleura, peritoneum, pericardium and tunica vaginalis [3, 4]. Histologically, diffuse mesothelioma is categorized into three types, epithelioid, biphasic, and sarcomatoid [5, 6], each exhibiting distinct histologic features, immunohistochemical stain patterns and treatment responses [7,8]. Despite these differences, all three types of malignant mesothelioma had overall poor clinical outcome and survival.

The diagnosis of malignant mesothelioma often poses clinical and pathological challenges with differential diagnoses including benign mesothelial lesions and metastatic diseases. Accurate diagnosis of malignant mesothelioma requires clinical and pathological

Table 1. Demographic information among all histologic types of mesothelioma patients.

Number of patients (Male, Female)	Pleura	Peritoneum	Pleura/ Peritoneum	Tunica Vaginalis	Pleura/Tunica Vaginalis	Pericardium	Pleura/ Pericardium
Epithelioid	332 (229, 103)	81 (36, 45)	8 (4, 4)	3 (3, 0)	1 (1, 0)	1 (1, 0)	1 (1, 0)
Biphasic	78 (61, 17)	8 (4, 4)	1 (0, 1)				
Sarcomatoid	56 (47, 9)	2 (1, 1)	1 (1, 0)				

correlation as well as application of immunohistochemical stains (IHC) and molecular studies [9]. It is recommended to include a panel of IHC markers based on morphological features i.e. epithelioid vs sarcomatoid and location of the tumors i.e. pleural, peritoneal and testicular presentation [10, 11]. Mesothelioma is the only tumor that expresses mesothelial, epithelial and mesenchymal markers. Therefore, the use of a panel of IHC markers including these categories are recommended and routinely used [10]. Additional IHC markers may be included to differentiate mesothelioma from other local and metastatic neoplasms [12]. However, with the use of multiple IHC markers, aberrant expressions may be observed which can lead to inaccurate diagnosis of malignant mesothelioma [13]. Finally, to rule out benign reactive mesothelial hyperplasia and malignant mesothelioma, ancillary markers including BAP1, MTAP and CDKN2A play an important role because the homozygous deletion of CDKN2A (p16) and the loss of BAP1 and MTAP are often observed in malignant mesotheliomas [5, 14].

In order to determine the spectrum of aberrant IHC patterns in mesothelioma, we reviewed histology and immunostaining features in 573 cases of diffuse mesothelioma. Over 50 different IHC markers have been applied to these cases for the purpose of diagnosis and differential diagnosis. Our study demonstrated that it is common to see aberrant IHC stains in malignant mesothelioma, therefore, attention should be paid to recognize these aberrant IHC stains to avoid misleading diagnosis of other neoplasms rather than malignant mesothelioma.

Results

1. Demographics and pathology categories:

The patient demographics are summarized in Table 1. Among 573 cases of malignant mesothelioma, 389 were male and 184 were female. The ages at diagnosis ranged from 17 to 93 with a median age of 73 (Table 1). The overall median survival after the initial diagnosis were 4 months (0-40 months) for male, 6 months (0-49 months) for female, 5 months (0-49 months) for epithelioid type, 4 months (0-13 months) for biphasic type, 3 months (0-19 months) for sarcomatoid type, 5 months (0-40 months) for pleural mesothelioma, and 4 months (0-49 months) for peritoneal mesothelioma. Among 301 cases with available asbestos exposure histology, all of them demonstrated significant past asbestos exposure.

Among 573 cases of malignant mesothelioma, 427 cases were epithelioid type, 59 cases were sarcomatoid type and 87 cases were biphasic types (Table 1). Among 427 cases of epithelioid type mesothelioma, 332 cases involved pleura (age ranged 28-93 years old), 81 cases were peritoneum (age ranged 17-90 years old), 1 case were pericardium (24 years old), 3 cases were tunica vaginalis (age ranged 58-80 years old), 8 cases involved pleura and peritoneum (age ranged 49-74 years old), 1 case involved pleura and tunica vaginalis (68 years old), and 1 case involved pleura and pericardium (77 years old). Among 87 cases of biphasic type mesothelioma, 78 cases involved pleura (age ranged 44-91 years old), 8 cases involved peritoneum (age ranged 56-90 years old), and 1 case involved pleura and peritoneum (65 years old). Among 59 cases of sarcomatoid type mesothelioma, 56 cases involved pleura (age ranged 49-93 years old), 2 cases involved peritoneum (age 64 and 72 years old), and 1 case involved pleura and peritoneum (70 years old).

2. Expression patterns of conventional IHC markers

The conventional markers were defined as those IHC stains that were recommended for routine diagnostic purposes [10], including mesothelial markers (used in 85% cases), epithelial markers (in 55% cases) and mesenchymal markers (in 13% cases) (Table 2). For mesothelial markers, calretinin (used in 98.1% cases), WT-1 (in 90% cases) and D2-40 (in 67% cases) were commonly used for routine diagnostic pur-

Table 2. Expression pattern of conventional IHC markers.

Conventional markers		Epithelioid				Biphasic				Sarcomatoid			
		Pos	Neg	Total	%	Pos	Neg	Total	%	Pos	Neg	Total	%
Mesothelial markers	Calretinin	409	12	421	97.15%	76	10	86	88.37%	31	24	55	56.36%
	WT-1	353	26	379	93.14%	71	10	81	87.65%	41	13	54	75.93%
	D2-40	255	14	269	94.80%	49	11	60	81.67%	40	14	54	74.07%
	Mesothelin	34	1	35	97.14%	2	3	5	40.00%	2	3	5	40.00%
Epithelial markers	CKAE1/AE3	170	4	174	97.70%	43	3	46	93.48%	46	5	51	90.20%
	CK5/6	250	16	266	93.98%	40	20	60	66.67%	12	33	45	26.67%
	CK7	187	43	230	81.30%	36	6	42	85.71%	21	10	31	67.74%
	CK8/18	9	1	10	90.00%	4	2	6	66.67%	8	3	11	72.73%
	CAM5.2	44	4	48	91.67%	19	0	19	100.00%	16	3	19	84.21%
	EMA	69	10	79	87.34%	9	4	13	69.23%	3	10	13	23.08%
	OSCAR	7	1	8	87.50%	3	0	3	100.00%	6	2	8	75.00%
Mesenchymal markers	Vimentin	25	1	26	96.15%	12	0	12	100.00%	16	0	16	100.00%
	Desmin	6	50	56	10.71%	3	17	20	15.00%	0	18	18	0.00%
Additional markers	GATA-3	56	73	129	43.41%	21	4	25	84.00%	25	2	27	92.59%
	HBME-1	11	1	12	91.67%	3	1	4	75.00%	2	2	4	50.00%
	GLUT-1	10	0	10	100.00%					2	0	2	100.00%

Table 3. Expression pattern of ancillary IHC markers.

Newer markers	Epithelioid				Biphasic				Sarcomatoid			
	Pos	Neg	Total	Deletion (%)	Pos	Neg	Total	Deletion (%)	Pos	Neg	Total	Deletion (%)
BAP-1	73	131	204	64.22%	23	22	45	48.89%	36	6	42	14.29%
MTAP	29	10	39	25.64%	3	2	5	40.00%	3	4	7	57.14%
CDKN2A	22	40	62	64.52%	4	7	11	63.64%	10	13	23	56.52%

poses. For epithelial markers, CK5/6 (in 65% cases), CK7 (in 53% cases) and CKAE1/AE3 (in 47% cases) were commonly used. For mesenchymal markers, vimentin (in 9% cases) and desmin (in 16% cases) were commonly used.

The positive rate of each IHC stains was calculated as the percentage of positive cases among total stained cases. Among 427 epithelioid mesothelioma, mesothelial markers were expressed in greater than 90% cases, including calretinin (97%), WT-1 (93%), D2-40 (95%) and mesothelin (97%). Epithelial markers were expressed in >80% cases including CKAE1/AE3 (98%), CK5/6 (94%), CK8/18 (90%), CAM5.2 (92%), CK7 (81%), EMA (87%) and OSCAR (88%). For mesenchymal markers, vimentin expressed in 96% cases, while desmin only expressed in 11% cases. Additional IHC markers which are used in mesothelioma diagnosis were also analyzed, including GATA-3 (43%), HBME-1 (92%) and GLUT1 (100%).

Among 86 cases of biphasic mesothelioma, most of the mesothelial markers had a positive rate between 80% and 90%, including calretinin (88%), WT-1 (88%) and D2-40 (82%), except for mesothelin (40%). Epithelial markers had positive rates above 60% including CAM5.2 (100%), OSCAR (100%), CKAE1/AE3 (93%), CK7 (86%), EMA (69%), CK5/6 (67%) and CK8.18 (67%). For mesenchymal markers, vimentin expressed in 100% biphasic mesothelioma, while desmin only expressed in 15% cases. GATA3 is expressed in 84% of biphasic mesothelioma.

Among 59 cases of sarcomatoid mesothelioma, most of the mesothelial markers had positive rates between 50-80%, including calretinin (56%), WT-1 (76%), and D2-40 (74%), while mesothelin had merely 40%. For epithelial markers, most of them had positive rates between 60-90% including CKAE1/AE3 (90%), CAM5.2 (84%), OSCAR (75%), CK8/18 (73%) and CK7 (67%), while others were merely around 20% such as CK5/6 (27%) and EMA (23%). For mesenchymal markers, vimentin was expressed in 100% sarcomatoid mesothelioma (100%), while desmin did not show expression in any sarcomatoid mesothelioma. It was noted that GATA3, the additional marker used in the diagnosis, was more commonly expressed in sarcomatoid type (93%).

3. Expression patterns of ancillary markers

Several newer tests have been recommended to be used for mesothelioma diagnosis (Table 3), especially in those cases where conventional markers are inconclusive [15]. These ancillary markers include the loss of BAP-1 expression by IHC, the deletion of CDKN2A gene by FISH and the loss of MTAP expression by IHC, which is a surrogate for CDKN2A deletion. The genetic alterations of these 3 genes can also be detected by the next generation sequencing. For epithelioid mesothelioma, the rates of BAP-1 loss (64%) and CDKN2A deletion (65%) were similar, and the rate of MTAP loss was 26%. For biphasic mesothelioma, the rate of BAP-1 loss was 49%, the rate of MTAP loss was 40%, and the rate of CDKN2A deletion (64%) was similar in epithelioid mesothelioma. For sarcomatoid mesothelioma, the rate of BAP-1 loss was only 14%, the rate of MTAP loss was 57%, and the rate of CDKN2A deletion was 57%.

4. Expression of aberrant markers

The aberrant expression of various IHC markers has been observed in mesothelioma and may affect accurate diagnosis of mesothelioma [16]. Aberrant markers are defined as those markers usually are not observed in mesothelioma and not part of the penal in diagnosing mesothelioma [13]. We analyzed the expression rates of over 30 aberrant IHC markers and classified them into three categories based on the total number of positive cases and their positive rate (4%) (Table 4). The first category is those markers with 2 or more positive cases and more than 20 cases tested in total, therefore, may have more impact on diagnostic interpretation. The secondary category is those markers with 2 or more positive cases but less than 20 cases in total. The third category is those markers with the positive cases less than 2, thus less impact in diagnostic interpretation.

For epithelioid mesothelioma, the first category of aberrant markers included MOC-31 (14%) (Fig. 1A), Ber-EP4 (14%), PAX-8 (13%) (Fig. 1B), p63 (7%), CK20 (5%), ER (5%) and NKX3.1 (6%). The second category of aberrant markers included SMA (18%), CD10 (67%), CD56 (25%), CD68 (16%), CD99 (40%), CA-125 (56%) and RCC (60%). The third category of aberrant markers included Claudin-4 (3%) and CEA (2%).

For biphasic mesothelioma, the first category of aberrant markers included MOC-31 (12%), Ber-EP4 (6%), PAX-8 (9%), CK20 (10%) and p40 (7%). The secondary category of aberrant markers included p63 (15%), SMA (71%), CD31 (33%), CD56 (50%), CD99 (67%) and BCL-2 (50%). There was no marker that met the criteria of the third category.

Table 4. Expression patterns of aberrant IHC markers.

Aberrant Markers	Epithelioid				Biphasic				Sarcomatoid			
	Pos	Neg	Total	%	Pos	Neg	Total	%	Pos	Neg	Total	%
MOC-31	34	205	239	14.23%	6	43	49	12.24%	1	29	30	3.33%
Ber-EP4	31	198	229	13.54%	3	44	47	6.38%	0	21	21	0.00%
PAX-8	18	122	140	12.86%	2	20	22	9.09%	0	9	9	0.00%
CK20	10	179	189	5.29%	3	27	30	10.00%	0	16	16	0.00%
p63	4	55	59	6.78%	2	11	13	15.38%	2	17	19	10.53%
p40	1	129	130	0.77%	2	28	30	6.67%	0	26	26	0.00%
SMA	2	9	11	18.18%	5	2	7	71.43%	8	9	17	47.06%
S-100	1	67	68	1.47%	0	21	21	0.00%	1	30	31	3.23%
Claudin4	2	72	74	2.70%	1	20	21	4.76%	1	15	16	6.25%
ER	3	61	64	4.69%	0	11	11	0.00%	0	3	3	0.00%
CEA	2	124	126	1.59%	0	26	26	0.00%	0	14	14	0.00%
CDX2	0	125	125	0.00%	0	15	15	0.00%	1	9	10	10.00%
NapsinA	0	146	146	0.00%	1	31	32	3.13%	0	14	14	0.00%
SOX10	1	42	43	2.33%	0	14	14	0.00%	0	18	18	0.00%
TTF-1	1	350	351	0.28%	0	68	68	0.00%	1	40	41	2.44%
B72.3	1	59	60	1.67%	0	14	14	0.00%	0	5	5	0.00%
NKX3.1	2	34	36	5.56%	0	8	8	0.00%	0	3	3	0.00%
ERG	1	9	10	10.00%	1	6	7	14.29%	3	4	7	42.86%
CD31	0	11	11	0.00%	2	4	6	33.33%	2	5	7	28.57%
CD56	3	9	12	25.00%	2	2	4	50.00%	1	1	2	50.00%
CD68	3	16	19	15.79%	0	3	3	0.00%	1	3	4	25.00%
CD99	2	3	5	40.00%	2	1	3	66.67%	1	3	4	25.00%
CD117	0	8	8	0.00%	1	2	3	33.33%	1	7	8	12.50%
BCL-2	1	4	5	20.00%	3	3	6	50.00%	0	5	5	0.00%
STAT-6					1	7	8	12.50%	0	10	10	0.00%
CA-125	5	4	9	55.56%								
CD10	6	3	9	66.67%	1	1	2	50.00%	1	1	2	50.00%
GCDFP15	1	15	16	6.25%								
RCC	3	2	5	60.00%								
thyroglobulin	1	8	9	11.11%								
CD34					0	15	15	0.00%	1	23	24	4.17%

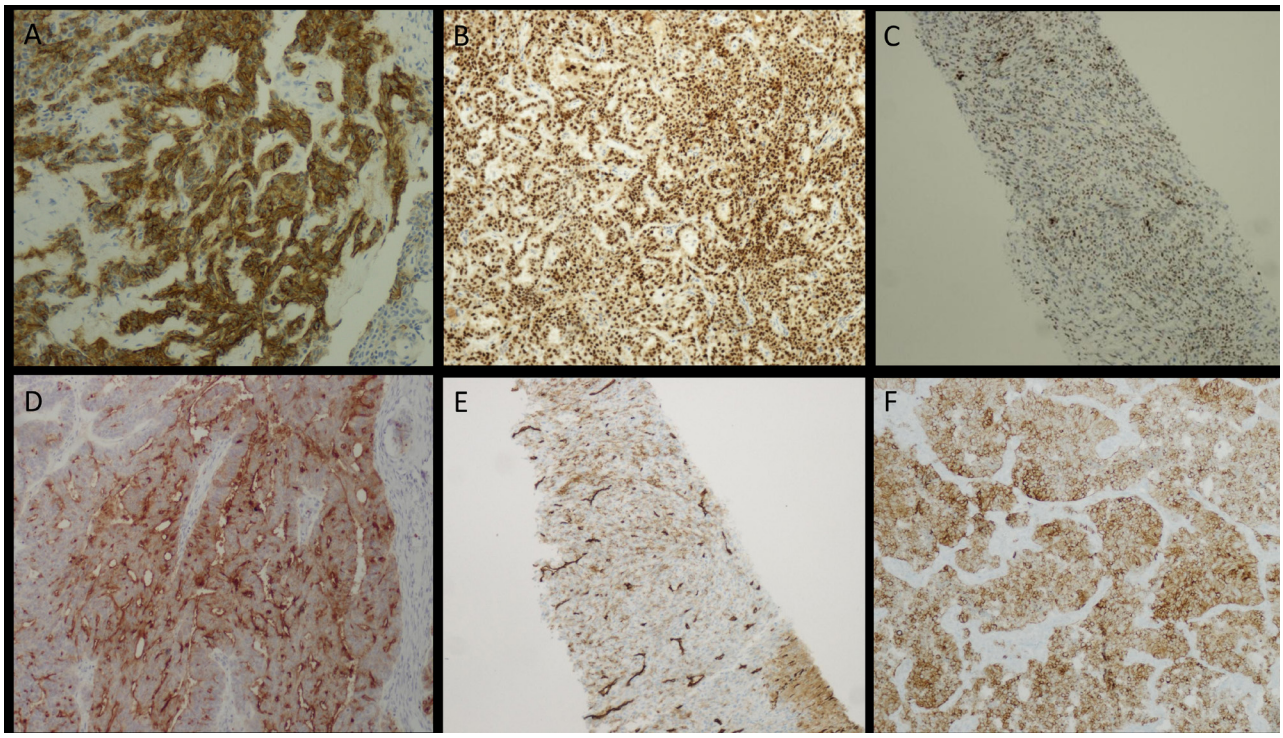

Figure 1. An example of immunohistochemistry for aberrant markers.
A, Aberrant MOC-31 expression (x200). **B**, Aberrant PAX-8 expression (x200). **C**, Aberrant ERG expression (x100). **D**, Aberrant CD10 expression (x200). **E**, Aberrant CD31 expression (x100). **F**, Aberrant CD56 expression (x200).

For sarcomatoid mesothelioma, being only 59 cases in total, no aberrant marker met the criteria for first and third categories. The second category of aberrant markers included p63 (11%), SMA (47%), ERG (43%) (Fig. 1C) and CD31 (29%).

5. Expression patterns of IHC markers in pleural and peritoneal epithelioid mesothelioma

We further analyzed the difference of IHC expression patterns in epithelioid mesothelioma between the two commonly involved locations - pleura and peritoneum (Table 5). These two locations also shared similar rates of expression of most epithelial markers except for CK7 (pleural: 77%; peritoneal: 95%), CAM5.2 (pleural: 89%; peritoneal: 100%) and EMA (pleural: 90%; peritoneal: 76%). For ancillary markers, the loss rates of BAP-1 were comparable between two locations, while the rates of homozygous deletion of CDKN2A was lower in peritoneal epithelioid mesothelioma (36%) than in pleural epithelioid mesothelioma (71%). Correspondingly, the loss rate of MTAP was lower in peritoneal (0%) compared to that in pleural (31%) epithelioid mesothelioma. For aberrant markers, the positive rate of CK20 and ER were comparable between the two locations, whereas MOC-31 (pleural: 16%; peritoneal: 12%), Ber-EP4 (pleural: 16%; peritoneal: 6%) and p63 (pleural: 8%; peritoneal: 0%) were found to have higher positive rates in pleural epithelioid mesothelioma. In contrast, PAX-8 (pleural: 9%; peritoneal: 21%) was found to have a higher positive rate in peritoneal epithelioid mesothelioma.

Discussion

The rarity of mesothelioma makes the study of the immunostain pattern difficult. Therefore, 573 cases with over 50 IHC markers in this study provide a comprehensive assessment immunophenotypical spectrum of mesothelioma. Our study showed the positive rates of conventional IHC panels were similar to those reported in previous studies [11, 17, 18] and summarized in WHO guidelines [15]. For conventional mesothelial markers, the overall positive rates were >90% for epithelioid, >80% for biphasic and 50-70% for sarcomatoid types, except for mesothelin, which was more often observed in epithelioid mesothelioma. For conventional epithelial markers, the overall positive rates were highly consistent with previous reports [10, 19, 20] with CKAE1/AE3 being the highest (>90%) in all 3 types of mesothelioma and CK5/6 being the lowest in sarcomatoid type.

Table 5. Expression patterns of IHC markers in pleural and peritoneal epithelioid mesothelioma

Epithelioid type			Pleural mesothelioma					Peritoneal mesothelioma				
			Pos	Neg	Total	Pos %	Neg %	Pos	Neg	Total	Pos %	Neg %
Conventional markers	Mesothelial markers	Calretinin	328	11	339	96.76%		85	1	86	98.84%	
		WT-1	278	24	302	92.05%		79	3	82	96.34%	
		D2-40	197	13	210	93.81%		60	2	62	96.77%	
		Mesothelin	27	1	28	96.43%		8	0	8	100.00%	
	Epithelial markers	CKAE1/AE3	126	3	129	97.67%		44	1	45	97.78%	
		CK5/6	195	13	208	93.75%		56	3	59	94.92%	
		CK7	132	40	172	76.74%		58	3	61	95.08%	
		CK8/18	7	1	8	87.50%						
		CAM5.2	31	4	35	88.57%		14	0	14	100.00%	
		EMA	57	6	63	90.48%		13	4	17	76.47%	
		OSCAR	3	1	4	75.00%		4	0	4	100.00%	
	Mesenchymal markers	Vimentin	20	0	20	100.00%		6	1	7	85.71%	
		Desmin	5	40	45	11.11%		1	10	11	9.09%	
	Additional markers	GATA-3	43	54	97	44.33%		14	20	34	41.18%	
		HBME-1	10	1	11	90.91%		1	0			
		GLUT-1	7	0	7	100.00%		3	0			
Newer markers		BAP-1	55	106	161		65.84%	19	33	52		63.46%
		MTAP	25	11	36		30.56%	7	0	7		0.00%
		CDKN2A	14	34	48		70.83%	9	5	14		35.71%
Aberrant Markers		MOC-31	31	160	191	16.23%		6	45	51	11.76%	
		Ber-EP4	29	153	182	15.93%		3	47	50	6.00%	
		PAX-8	8	79	87	9.20%		12	45	57	21.05%	
		CK20	8	131	139	5.76%		2	51	53	3.77%	
		p63	4	45	49	8.16%		0	10	10	0.00%	
		ER	2	36	38	5.26%		1	26	27	3.70%	

Among mesenchymal markers, vimentin was highly expressed in all 3 types of mesothelioma as previously reported [20], while desmin had a low positive rate as previously reported [21]. For GATA-3, >40% epithelioid type and >90% in sarcomatoid type expressed this marker, consistent with previous reports [18, 22, 23].

For ancillary markers, our study showed the homozygous deletion rates of CDKN2A in all 3 types were around 60%. The loss of BAP-1 expression was found in >60% epithelioid, 50% sarcomatoid, and <15% among sarcomatoid mesothelioma. These results indicated that loss of BAP-1 is not a sensitive marker for sarcomatoid mesothelioma, which was consistent with previous reports [11, 24-27].

Our study also showed that the expression patterns of most conventional IHC markers were similar between pleural and peritoneal mesothelioma. The homozygous deletion of CDKN2A was observed in around 30% peritoneal mesothelioma as compared to 70% pleural mesothelioma. On the other hand, MTAP showed no loss of expression in peritoneal mesothelioma but presented in 30% pleural mesothelioma. These results indicate that the homozygous deletion of CDKN2A and loss of MTAP expression are less useful markers in the diagnosis of peritoneal mesothelioma.

The notable significance of this study is the extensive survey of over 30 IHC markers that may aberrantly express in mesothelioma. Our study showed the aberrant expression patterns were different among histological types. Most of the markers aberrantly expressed in epithelioid mesothelioma, including Ber-EP4, MOC-31, PAX-8 and CK20, were rarely observed in sarcomatoid type. On the other hand, some markers including SMA were more frequently observed in sarcomatoid and biphasic types than epithelioid mesothelioma. Our study further showed that the aberrant expression patterns were different between pleural and peritoneal mesothelioma. In pleural epithelioid mesothelioma, in which the primary differential diagnosis is lung carcinoma, the aberrant expressions of MOC-31, BerEP4 and p63 were particularly high. While in peritoneal epithelioid mesothelioma, in which the top differential diagnosis is ovarian carcinoma in females, the aberrant expression of PAX-8 was remarkably high. Furthermore, some aberrant expressions such as CK20 [28] reported to be rare in previous studies were found to be higher in our large-scale study. It is worth to note that our study identified additional markers, including SMA, ERG, CD10, CD31, CD56, CD68, CD99, BCL-2, CA-125 and RCC, aberrantly expressed in mesothelioma, although with a limited number of cases. These results indicate that there will be more aberrant expressions seen in mesothelioma and the more immunostains performed, the more aberrant expressions will be observed.

In conclusion, our study of over 500 mesothelioma cases provides a comprehensive assessment of various IHC markers in this relatively rare disease. Due to a wide spectrum of tumor differentiation, aberrant IHC staining patterns are quite commonly seen [13]. Therefore, the diagnosis of mesothelioma should be based on correlation of clinical presentation, radiological findings, and selective panel of immunostains, and should not be distracted by aberrant IHC stains.

Material and Methods

A total of 573 cases of malignant mesothelioma were retrieved from the pathology consultation files between 2020-2023. These cases were submitted for legal consultation purposes to Dr. David Y Zhang who served as an asbestos expert witness. For each case, the histology slides and immunostained slides (if available) were reviewed and the diagnostic interpretation was made by Dr. Zhang. For those cases without the immunostained slides, interpretation of immunostains was based on pathology reports. In addition, the medical records and radiology reports were also reviewed for clinical and pathological correlation. Patients' age, gender, asbestos exposure history, tumor stage, treatment plan and survival time were extracted from the medical record and deposition transcripts. The study protocol was approved by Institutional Review Boards (IRB).

Data availability statement: N/A
Funding statement: No funding received for this project
Conflict of interest disclosure: Dr. David Zhang services as an expert for asbestos-related legal cases
Ethics approval statement: IRB approved by Diagnostics Investigational Review Board
Patient consent statement: N/A
Permission to reproduce material from other sources: N/A
Clinical trial registration: N/A

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