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Mesothelioma *in situ* of the peritoneum: report of three cases and review of the literature

Emily Symes,[1] Melissa Tjota,[1] Brittany Cody,[2] Hedy Kindler,[3] Owen Mitchell,[4] Hunter Witmer,[5] Kiran Turaga,[5,6] Jeffrey Mueller,[1] Thomas Krausz,[1] Aliya N. Husain[1] & Huihua Li[1,7]

[1]*Department of Pathology, University of Chicago, Chicago, IL,* [2]*Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA,* [3]*Section of Hematology/Oncology, Department of Medicine,* [4,5]*Department of Surgery, University of Chicago, Chicago, IL,* [6]*Department of Surgery, Yale Cancer Center, New Haven, CT and* [7]*Department of Pathology, Duke University Medical Center, Durham, NC, USA*

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Aim: Diagnosis of mesothelioma *in situ* (MIS) is historically controversial and, until recently, specific features defining the entity have not been well characterized. Most reported cases of MIS occurred in the pleura; peritoneal MIS is very rare. This study investigates the morphologic features and results of ancillary testing in peritoneal MIS.

Methods: We present three patients with peritoneal MIS, as defined by a single layer of mesothelial cells with loss of nuclear BRCA-1-associated protein-1 (BAP1) immunostaining and without evidence of invasive tumour by microscopic evaluation, imaging, or direct examination of the peritoneum. Histology and immunostains were reviewed by three expert thoracic pathologists with multidisciplinary input. Next-generation sequencing (NGS) was performed in all three cases. A literature review was conducted to characterize this rare precursor lesion.

Results: BAP1 was lost in all three lesions, while methylthioadenosine phosphorylase (MTAP) was retained in two (not performed in the third). NGS revealed *BAP1* pathogenic alterations in all three cases as well as mutations of *SMO*, *ERCC3*, *TET2*, and *U2AF1*. Progression to invasive mesothelioma occurred in one patient at 13 months postdiagnosis (case 1). One patient was diagnosed at age 24 and was later found to harbour a *BAP1* germline mutation (case 3).

Conclusion: This work describes the histologic features and clinicopathologic characteristics of peritoneal MIS in three cases, highlights *BAP1* somatic and germline mutations in peritoneal MIS, and strengthens the importance of ancillary studies (including immunohistochemical and molecular studies) in the diagnosis of MIS.

Keywords: BAP1, mesothelioma in situ, molecular study, peritoneum

Introduction

Mesothelioma *in situ* (MIS) is a preinvasive lesion recently accepted as a diagnostic entity with its addition to the *World Health Organization Classification of Thoracic Tumours* in 2021.[1-3] In the pleura, the defining features of MIS include a single layer of mesothelial cells with or without atypia that lacks stromal invasion and shows loss of BAP1 and/or MTAP by immunohistochemistry (IHC) and/or homozygous *CDKN2A* deletion by fluorescence *in situ* hybridization (FISH).[3] Loss of nuclear BAP1 immunostaining in neoplastic cells is 100% specific for

Address for correspondence: E Symes, Department of Pathology, The University of Chicago Medicine, 5841 S. Maryland Ave. MC3083, Chicago, IL 60637, USA.
e-mail: emily.symes@uchospitals.edu

mesothelioma, as compared to reactive mesothelial proliferations,[4] while the combination of BAP1 loss by IHC and deletion of *CDKN2A*/p1 by FISH is 58% sensitive for mesothelial malignancies compared to benign proliferations.[5] MIS is often accompanied by unresolving pleural effusions, and the absence of thoracoscopic/laparoscopic or radiographic evidence of tumour is required.[1] Lack of progression to invasive mesothelioma for at least 1 year has been used as an additional inclusion criterion in some case series.[4] This qualifier prevents misdiagnosis of suboptimal samples as MIS rather than invasive mesothelioma, which may potentially spread along serosal surfaces near the MIS. Multidisciplinary discussion should be conducted at diagnosis. These requirements also apply to peritoneal MIS; however, this entity has been less thoroughly studied.[4]

Progression to invasive mesothelioma is a well-documented feature of MIS but occurs over a relatively extended time period, varying from 9 to 92 (median 60) months.[4] Early diagnosis enables anticipatory screening and early intervention with more proactive follow-up and possible surgical excision.[1,4] Given the relative novelty of MIS, next-generation sequencing (NGS)-based molecular findings are not well described. Herein, we present three cases of peritoneal MIS and demonstrate the histologic features and clinicopathologic characteristics of MIS in these patients; additionally, we identify genetic alterations in MIS and in subsequent invasive mesothelioma. A literature review, including all previously published MIS cases (to our best knowledge), was conducted with the aim of characterizing this rare precursor lesion and describing relevant diagnostic considerations in a modern pathology practice.

Materials and Methods

After Institutional Review Board (IRB) approval, a total of three MIS cases out of approximately 500 cases of mesothelioma diagnosed between November 2016 and May 2021 at a single institution were assembled through a departmental database search. Histology and immunostains were reviewed by three expert thoracic pathologists with multidisciplinary input. Patients' clinical data and radiologic findings were extracted from electronic medical records.

Representative formalin-fixed, paraffin-embedded blocks were selected for NGS using the University of Chicago Medicine OncoPlus (UCM-OncoPlus) panel—a hybrid-capture panel targeting 1005 cancer-associated genes. DNA extraction, DNA quantification, library preparation, and NGS were performed as described previously.[6] Copy number variation was only assessed for *MTAP* and *CDKN2A*/*B*. Data analysis was executed on a high-performance computing system using an in-house-developed bioinformatics pipeline (Center for Research Informatics, University of Chicago, IL). Somatic variant calls were inspected using the Integrated Genomics Viewer (IGV; Broad Institute, MIT Harvard, Cambridge, MA). Splice site prediction models for intronic variants were investigated using Alamut Visual Plus (Sophia Genetics, Boston, MA). Germline testing was only performed in one of the three cases (case 3).

A PubMed search was conducted to find all previous MIS reports. To our best knowledge, twenty-one articles were included in the study. Important findings are summarized in Table 4.

Results

Clinicopathologic findings from each case of MIS are shown in Table 1. Case 1 is a 63-year-old male with a history of chronic hepatitis C virus infection and cirrhosis who presented with worsening abdominal distension and unintentional weight loss. Records of asbestos exposure were unavailable. The computed tomography (CT) scan showed marked abdominopelvic ascites with stranding along the peritoneal surface. Cytology specimens of ascites fluid were negative for malignancy, but subsequent omental biopsy and omentectomy showed multifocal papillary projections with paucicellular fibrovascular cores lined by a single layer of bland, monotonous cells. Several foci showed atypical cells with prominent nucleoli, which were present in clusters along the lesional surfaces, forming small nests within papillary excrescences, morphologically resembling well-differentiated papillary mesothelial tumour with core invasion (Figure 1). No areas of true invasion were identified. Given the cytologic atypia, further workup was performed. The neoplastic cells showed loss of BAP1 (Figure 1D) and 5-hmC by IHC. MTAP was retained, and atypical cells were positive for WT-1, calretinin, CK5/6, CAM 5.2, and AE1/AE3. Focal BerEP4 staining was present, and atypical cells were negative for MUC1, synaptophysin, chromogranin, CD56, CDX2, CK20, and PD-L1. Although imaging demonstrated no appreciable changes, progression to invasive mesothelioma was diagnosed 13 months later by peritoneal biopsies and excision specimens, at which time stromal invasion was readily appreciated.

Table 1. Demographics and pathologic findings in three cases of MIS

Case	Age (years)	Sex	Location	Histologic features	Asbestos exposure	IHC			Progression to mesothelioma	Survival (based on last clinical follow-up)
						BAP1	MTAP	5-hmC		
1	62	M	Omentum, peritoneum	Papillary projections with fibrovascular cores and nests of mesothelial cells	Unknown	Loss	Retained	Partial loss	13 mo	Alive (18 mo)
2	67	F	Peritoneum, omentum, ovaries	Ovarian mucinous cystadenoma (5 cm); Fibrosis underlying papillary projections with a superficial mesothelial cell layer	Denied	Loss	Retained	Retained	N/A	Alive (67 mo)
3	24	F	Omentum, peritoneum	Endometriotic nodules; Papillary projections with a superficial mesothelial cell layer	Denied	Loss	Not performed	Not performed	N/A	Alive (28 mo)

IHC, immunohistochemistry; MTAP, methylthioadenosine phosphorylase; 5-hmC, 5-hydroxymethylcytosine.

The epithelioid malignant cells demonstrated a predominantly solid to trabecular growth pattern with occasional mitotic figures and absence of necrosis. BAP1 was lost in tumour nuclei (Figure 2).

Case 2 is a 67-year-old female who presented with weight gain and abdominal pain with distension and no known asbestos exposure. Per outside report, the CT scan showed omental haziness, subtle peritoneal nodularity, moderate abdominopelvic ascites, and a 3 cm right adnexal cystic lesion. Paracentesis cytology showed rare, atypical mesothelial cell clusters, which were positive for calretinin, CK5/6, and PAX8 and were negative for MOC-31. The subsequent hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and peritoneal biopsies revealed an atypical mesothelial proliferation involving the omentum, multifocal peritoneum, and bilateral ovarian surfaces. An incidental, 5 cm ovarian mucinous cystadenoma was also present. No evidence of invasive disease was identified in any of the specimens. Atypical epithelioid cells were present superficially along with underlying fibrosis (Figure 3A–C). Further workup showed positivity for calretinin, CK5/6, WT1, weak PAX-8, and wildtype p53 in the atypical cells, and negativity for estrogen receptor (ER) and progesterone receptor. BAP1 expression was lost (Figure 3D), and both MTAP and 5-hmC were retained.

Case 3 is a 24-year-old female with a history of chronic pelvic pain and endometriosis. She had no known asbestos exposure. Imaging showed peritoneal thickening in the posterior pelvis without free fluid. Exploratory laparoscopy revealed diffuse omental vesicular lesions, which were subsequently biopsied. Review of this omental biopsy from an outside hospital showed colonization of decidualized, endometriotic stromal nodules by superficial papillary projections composed of mesothelial cells. These nodules were positive for ER, CD10, and WT1, supporting a diagnosis of endometriosis. Mesothelial cells were atypical and showed no evidence of invasion. Immunostains were positive for calretinin, WT1, and pancytokeratin and negative for PAX-8 with loss of nuclear BAP1 expression. Four months later, a diagnostic exploratory laparoscopy with omentectomy and left partial anterior pelvic peritonectomy showed multiple areas of mesothelial surface proliferation with decidualized endometriotic stromal nodules involving both the greater omentum and anterior pelvic peritoneum (Figure 4). Most of the proliferation was omental, and immunostaining showed loss of BAP1 nuclear expression with patchy loss in the peritoneum, possibly representing multifocal MIS. Evidence of invasive disease was not present.

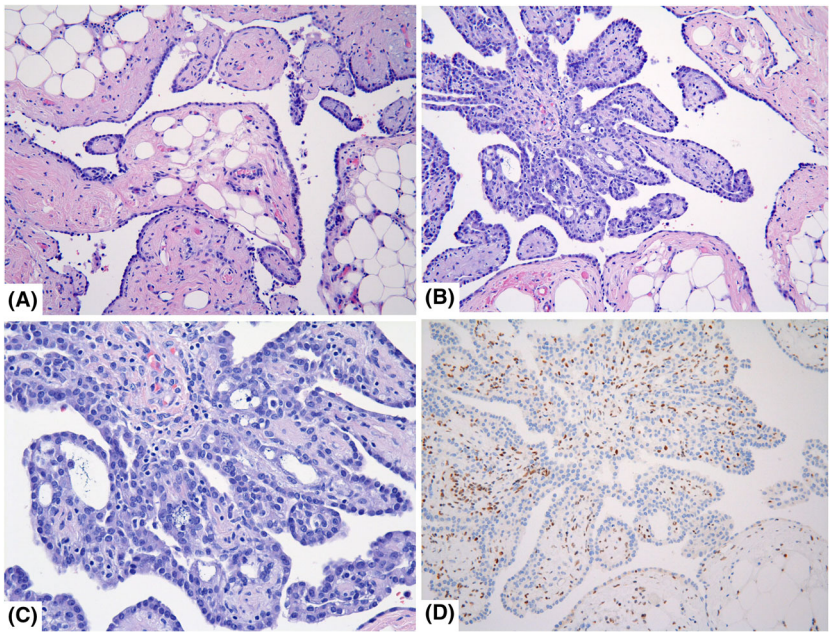

Figure 1. Case 1, hematoxylin and eosin (H&E) sections show papillary projections lined by a single layer of monotonous, atypical cells (A, B). These neoplastic mesothelial cells show hyperchromasia, focal cellular crowding, and prominent nucleoli (C). Complete loss of BAP1 immunostaining was identified in the lesional atypical cells, while BAP1 expression was retained in the stromal cells (D).

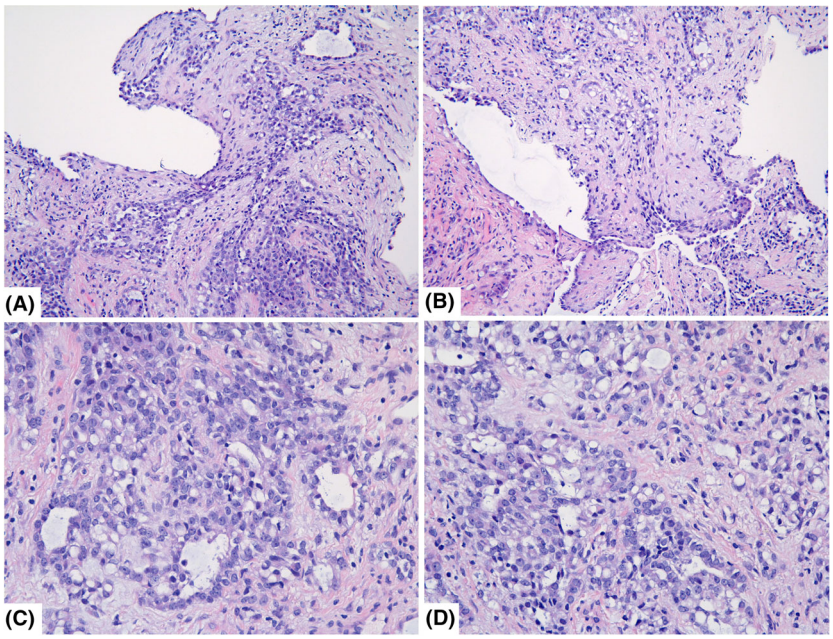

Figure 2. Case 1, MIS progressed to mesothelioma 13 months later. Epithelioid malignant cells demonstrate a solid to trabecular growth pattern with stromal invasion. Necrosis is absent, and mitotic figures are occasionally present (A–D).

To define any molecular variants, each case was sent for NGS. The molecular results are summarized in Table 2. In case 1, two *BAP1* gene rearrangements were detected with one common breakpoint in exon 3 of *BAP1* (NM_004656) and with distal connections in the 5′ UTR and intron 8 of *APEH* (NM_001640).

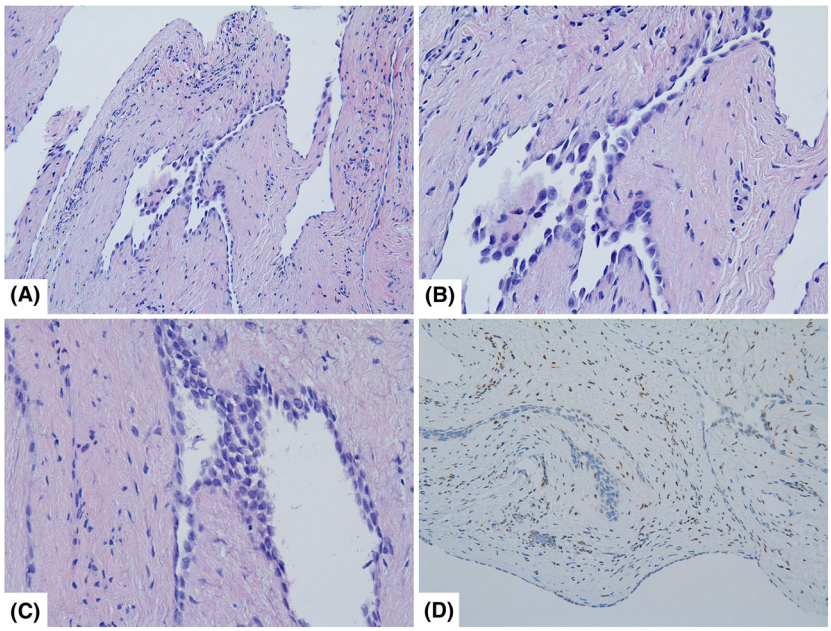

Figure 3. Case 2, atypical epithelioid mesothelial cells are present superficially, along with underlying fibrosis. The atypical cells are focally crowded with occasional prominent nucleoli (A–C). Nuclear BAP1 expression is lost in the atypical cells (D).

The exact nature of these gene rearrangements was uncertain, but could have been consistent with a large-scale inversion. This case also had a missense mutation in *SMO* (p.L412F), which is a well-known hotspot mutation site. In case 1, the patient progressed to invasive mesothelioma 13 months following the diagnosis of MIS. Molecular changes in the invasive disease showed identical mutations to the antecedent MIS:*BAP1* gene rearrangements and missense mutation in *SMO* (p.L412F). Case 2 had a frameshift mutation in *BAP1* (p.V530Cfs*41) and a nonsense mutation in *ERCC3* (p.R109*). Because chromosomal microarray was not performed, definitive loss of heterozygosity was not confirmed. Case 3 had mutations in three genes: *BAP1* (c.67+3_67+12del), *TET2* (p.E711Afs*11), and *U2AF1* (p.S34F). Given the patient's young age, genetic testing was conducted, and the results identified a *BAP1* germline mutation. Significant copy number alterations were not detected in any of these cases.

To supplement our three reported cases and characterize this rare precursor lesion, we reviewed all previously published literature on MIS (21 articles comprising 40 MIS cases) and summarized the major clinicopathologic features in Table 4. Of the 43 total cases (40 published cases plus three cases from our study), 30 cases arose from the pleura, while 11 cases (including our three cases) involved the peritoneum, one case occurred in the testis (processus vaginalis), and in one patient with *BAP1* germline mutation, MIS involved both the pleura and peritoneum.[7] Male gender was slightly more common than female (24 versus 18, one unknown); the age ranged from 24 to 95 years (median 68). IHC was not performed in every case, but the results included 31 cases (including our three cases) with BAP1 loss, six cases with both BAP1 and MTAP loss, and only one case with MTAP loss alone. NGS was performed in nine cases (including our three cases): all demonstrated *BAP1* alternations. Using adequate cell blocks and available IHC studies, four cases were diagnosed based on cytology specimens alone. Of these, three specimens showed BAP1 loss. Eighteen cases (including one case from our study) subsequently developed invasive mesothelioma in 8–96 months (median 58). Lastly, three cases (including one case from our study) harboured *BAP1* germline mutations, although few patients underwent germline testing. All *BAP1* germline mutated patients were female, and the ages at diagnosis were 24, 24, and 43 years. One patient (age 24) developed invasive disease within 10 months of the MIS diagnosis.

Discussion

MIS is a new entity in the most recent edition of the *World Health Organization Classification of Thoracic*

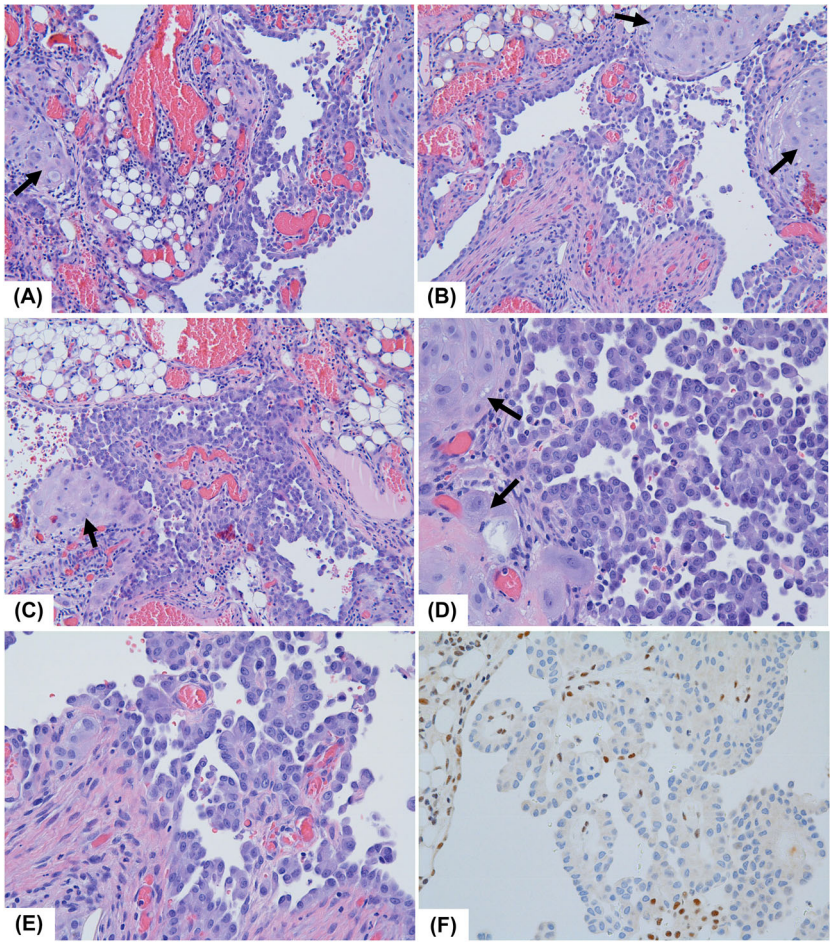

Figure 4. Case 3, papillary projections with surface mesothelial cells are accompanied by underlying decidualized endometriotic stromal nodules (indicated by arrows) (A–E). The mesothelial cells are negative for BAP1 expression (F).

TABLE 2. Somatic genetic alternations identified in three cases of MIS

Case	Gene	Coding effect	Nucleotide change	Amino acid alterations	Variant allele frequency (VAF; %)
1	*BAP1*	Rearrangement	Two breakpoints in exon 3 of *BAP1* (NM_004656) with distal connections in the 5′UTR and intron 8 of *APEH* (NM_001640)	N/A	N/A
	SMO	Missense	c.1234C>T	p.L412F	43
2	*BAP1*	Frameshift	c.1588del	p.V530Cfs*41	41
	ERCC3	Nonsense	c.325C>T	p.R109*	42
3	*BAP1*	Splicing	c.67+3_67+12del	Predictive protein truncation	16
	TET2	Frameshift	c.2130_2131del	p.E711Afs*11	11
	U2AF1	Missense	c.101C>T	p.S34F	12

Tumours (2021). Defining features include a single layer of mesothelial cells with or without atypia, no stromal invasion, and loss of BAP1 and/or MTAP by IHC and/or homozygous *CDKN2A* deletion by FISH.[3] In daily practice, the MIS diagnosis is quite challenging, and the entity can be overlooked or misdiagnosed, as the neoplastic mesothelial cells are arranged in a single layer without invasion and do not always demonstrate cytologic atypia. Herein, we present three cases of MIS (all in the peritoneum) with papillary proliferation and no stromal invasion. Immunostains showed nuclear BAP1 loss, and NGS confirmed *BAP1* and other genetic alterations in all three cases. Notably, there was one case (case 3, age 24) in which the patient had a *BAP1* germline mutation. Patients 1 and 2 were not tested for germline mutations.

The differential diagnosis for MIS includes well-differentiated papillary mesothelial tumour (WDPMT, formerly known as well-differentiated papillary mesothelioma) and mesothelioma with microinvasion. The morphologic features and ancillary studies that are helpful to distinguish MIS from other mimickers are summarized in Table 3. WDPMT consists of myxoid or fibrous papillae with a single surface layer of flattened to cuboidal mesothelial cells. The tumour is most often incidentally discovered in the peritoneum, but can also be seen in the pericardium, pleural cavity, or tunica vaginalis. No invasion of the underlying stroma is present. WDPMT shows contrasting genetic features from invasive mesothelioma or MIS[8,9] and demonstrates indolent behaviour. Molecular analyses of WDPMT are limited but show mutations of *EHD1*, *FBXO10*, *CHD5*, *MAGED1*, *ATM*, and *TP73*. Alterations in *TRAF7* or *CDC42* have also been identified.[9,10] Recent studies have suggested that papillary lesions, morphologically identical to WDPMT, truly represent MIS.[11,12] Unlike MIS, in WDPMT, BAP1 expression by IHC is retained, and no *CDKN2A* deletion is detected on FISH studies[11]; therefore, loss of BAP1 and/or MTAP by IHC and/or homozygous *CDKN2A* deletion by FISH differentiates papillary MIS from WDPMT. In our study, all three cases occurred in the peritoneum, and each demonstrated papillary projections with a superficial mesothelial cell layer, mimicking WDPMT. However, the surface neoplastic mesothelial cells showed some degree of cytologic atypia, including hyperchromasia, focal cellular crowding, and prominent nucleoli. Since WDPMT has indolent prognosis compared to MIS and mesothelioma, discrimination between these entities is paramount for appropriate treatment and surveillance. In cases with classical morphology of WDPMT, if the surface cells show cytologic atypia and/or if clinical history is unusual (i.e. recurrent unilateral pleural effusion, ascites), BAP1 and MTAP IHC or molecular studies should be considered to rule out MIS.

An additional diagnostic consideration is mesothelioma with microinvasion when neoplastic mesothelial cells infiltrate into the underlying fat and fibrous stroma. Invasion, when present, is the most reliable criterion for the diagnosis of mesothelioma. Cytokeratin or calretinin immunostains may be used to highlight infiltrating mesothelial cells that invade into underlying tissues when invasion is minimal or equivocal. In all three MIS cases reported here, no

Table 3. Morphologic assessment and ancillary studies for MIS, WDPMT, and mesothelioma

Features	Flat MIS	Papillary MIS mimicking WDPMT	WDPMT	Mesothelioma
Morphology	Single layer of bland mesothelial cells	Identical to WDPMT; Associated flat MIS is present	Single layer of bland mesothelial cells lining myxoid or fibrous papillary cores	Invasive mesothelial cells; May include papillary foci as a minor component
IHC	BAP1 and/or MTAP loss	BAP1 and/or MTAP loss	No BAP1 and/or MTAP loss	BAP1 and/or MTAP loss
FISH	Homozygous deletion of *CDKN2A*	Homozygous deletion of *CDKN2A*	No homozygous deletion of *CDKN2A*	Homozygous deletion of *CDKN2A*
Outcome	Majority later progress to mesothelioma	Majority later progress to mesothelioma; Could be relatively indolent	Considered benign; Indolent behaviour	Aggressive behaviour with short survival

BAP1, BRCA-1 associated protein-1; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MTAP, methylthioadenosine phosphorylase; MIS, mesothelioma *in situ*; WDPMT, well-differentiated papillary mesothelial tumour.

TABLE 4. Demographics and pathologic findings in all reported MIS cases (Cases 3–5 from our series)

Case	Site	Pattern	Age	Sex	Asb.	Symptoms	Imaging	Cyto. Dx	Cyto. IHC (BAP1/MTAP)
1	Perito	Papillary	24	F	N	Chronic pelvic pain	Inferior omental and peritoneal lesions	—	—
2	Perito	Papillary	68	F	—	Recurrent ascites	—	—	—
3	Perito	Papillary	63	M	—	Ascites	Stranding along peritoneum	Y	—
4	Perito	Papillary	67	F	N	Weight gain, Abdominal pain with distension	Peritoneal nodularity, Adnexal cystic lesion	Y	—
5	Perito	Papillary	24	F	N	Chronic pelvic pain	Peritoneal thickening	—	—
6	Perito	Papillary and Flat	68	F		Ascites	Peritoneal nodularity	—	—
7	Perito	Papillary and Flat	81	F	—	Abdominal discomfort, Ascites	Peritoneal tumour	—	—
8	Perito	Papillary and Flat	39	F	—	Umbilical mass	Umbilical mass, Normal serosal membranes	—	—
9	Perito	Papillary and Flat	68	F	—	Abdominal symptoms, Ascites	Peritoneal carcinomatosis	—	—
10	Perito	Papillary and Flat	31	F	—	Ascites	Peritoneal and omental nodularity	—	—
11	Perito	—	53	F	—	Ascites	No significant findings	—	—
12	Pleu	Papillary	77	M	—	Recurrent spontaneous pneumothorax	Nodular pleural thickening	—	—
13	Pleu	Papillary	63	M	Y	Recurrent effusion	No significant findings	Y	BAP1 loss

Surg. specimen type	Surg. IHC (BAP1/MTAP)	*CDKN2A* homo deletion by FISH	NGS	Prog. on imaging (mo)	Inv. Dx	Time to inv. (mo)	F/U (mo)	References
Omental biopsy	BAP1 loss, MTAP retained	—	Germline *BAP1* pathogenic variant (c.1588del (p.Val530 Cysfs*41))	—	Yes	10	Alive (10)	Fels Elliott *et al*.[16]
—	BAP1 loss	—	*BAP1* somatic splice site (intron 5-exon 6 boundary; A126_splice; allelic fraction 10%), *BAP1* copy number loss; No germline mut.	—	—	—	—	Dacic *et al*.[15]
Omental biopsy, Omentectomy	BAP1 loss, MTAP retained	—	Rearrangement with breakpoints in *BAP1* (exon 3; NM_004656) and distal connections in *APEH* (5′ UTR & intron 8; NM_001640)	No	Yes	13	Alive (18)	Our study
Peritoneal biopsies, Omentectomy	BAP1 loss, MTAP retained	—	*BAP1* frameshift (p.V530Cfs*41), *ERCC3* nonsense (p.R109*)	—	No	NA	Alive (67)	Our study
Partial peritonectomy, Omentectomy	BAP1 loss	—	*BAP1* (c.67+3_67+12del), *TET2* (p.E711Afs*11), *U2AF1* (p.S34F)	—	No	NA	Alive (28)	Our study
—	BAP1 loss, MTAP retained	—	—	—	No	NA	—	Galateau-Salle *et al*.[12]
—	BAP1 loss, MTAP retained	—	—	—	No	NA	Alive (24)	Galateau-Salle *et al*.[12]
—	BAP1 loss, MTAP retained	—	—	—	No	NA	—	Galateau-Salle *et al*.[12]
—	BAP1 loss, MTAP retained	—	—	—	No	NA	—	Galateau-Salle *et al*.[12]
—	BAP1 loss, MTAP retained	—	—	—	No	NA	LTFU	Galateau-Salle *et al*.[12]
Peritoneal biopsy	BAP1 loss, MTAP loss (partial)	N	—	—	Yes	12	—	Churg *et al*.,[4] Churg *et al*.[34]
Pleurectomy	BAP1 loss, MTAP loss	—	—	—	Yes	25	—	Fels Elliott *et al*.[24]
Pleural peel	BAP1 loss	—	Pleu fluid: *BAP1* loss (3p21), *SETD2* loss (3p21), *CDKN2A/B* loss (9p21), *PIK3R1* (p.T369I; VAF 17%)	No	No	NA	LTFU	Michael *et al*.[25]

TABLE 4. (*Continued*)

Case	Site	Pattern	Age	Sex	Asb.	Symptoms	Imaging	Cyto. Dx	Cyto. IHC (BAP1/MTAP)
14	Pleu	Papillary	59	M	Y	Recurrent effusion	No significant findings	Y	BAP1 loss
15	Pleu	Papillary	74	M	Y	Recurrent effusion	Plaques	Y	BAP1 loss
16	Pleu	Papillary	75	F	N	Recurrent effusion	No significant findings	Y	BAP1 loss
17	Pleu	Papillary	90	M	Y	Recurrent effusion	Plaques	Y	BAP1 retained
18	Pleu	Papillary	74	M	Y	Pleural effusion	Effusion	Y	BAP1 loss
19	Pleu	Papillary	67	M	—	S/p lung cancer resection	—	—	—
20	Pleu	Papillary and Flat	79	M	Y	Recurrent effusion	No significant findings	Y	BAP1 loss
21	Pleu	Papillary and Flat	67	M	—	Pleural effusion	Effusion	—	—
22	Pleu	Papillary and Flat	68	F	—	Pleural effusion	Effusion	—	—
23	Pleu	Papillary and Flat	66	M	—	Pleural effusion	Effusion	—	—
24	Pleu	Papillary and Flat	—	—	—	Recurrent effusion	Effusion	Y	—
25	Pleu	—	81	F	Y	Recurrent effusion	No significant findings	Y	BAP1 loss (partial)
26	Pleu	—	80	M	Y	Pleural effusion	Plaques	Y	BAP1 loss
27	Pleu	—	95	M	Y	Pleural effusion	Plaques	Y	—
28	Pleu	—	71	M	Y	Dyspnea	Effusion, Mild pleural thickening	Y	—
29	Pleu	—	74	M	Y	Chest pain, Dyspnea, Cough	Effusion, Pleural nodularity	Y	BAP1 loss
30	Pleu	—	73	M	Y	Chest pain, Cough	Effusion, Pleural thickening, Multiple plaques	—	—
31	Pleu	—	50	F	Y	Recurrent effusion, Cough, Chest tightness	Effusion	—	—

Surg. specimen type	Surg. IHC (BAP1/MTAP)	*CDKN2A* homo deletion by FISH	NGS	Prog. on imaging (mo)	Inv. Dx	Time to inv. (mo)	F/U (mo)	References
Biopsy	BAP1 loss	—	—	Yes (84)	Yes	Apprx. 96	DOD (120)	Michael *et al.*[25]
Pleurectomy, Decortication	BAP1 loss	—	—	No	No	NA	Alive, SD	Michael *et al.*[25]
—	NA	—	—	Yes (36)	No	NA	Alive	Michael *et al.*[25]
—	NA	Y	—	No	No	NA	Alive, SD	Michael *et al.*[25]
Biopsy	BAP1 loss, MTAP retained	N	—	Yes (44)	Yes	44	DOD (52)	Yabuuchi *et al.*[26]
Lung resection	BAP1 loss	N	LOH in *BAP1* locus (chromosome 3); No germline mutation	—	—	—	—	Dacic *et al.*[15]
Biopsies	BAP1 loss	—	—	No	No	NA	Alive, SD	Michael *et al.*[25]
—	BAP1 loss	—	—	—	Yes	45	DOD (45)	Galateau-Salle *et al.*[12]
—	BAP1 loss	—	—	—	Yes	69	DOD (69)	Galateau-Salle *et al.*[12]
—	BAP1 loss	—	—	—	Yes	94	DOD (94)	Galateau-Salle *et al.*[12]
Biopsy	BAP1 loss	—	—	Yes (58)	Yes	58	Alive (58)	Klebe *et al.*,[30] Klebe *et al.*,[1] Pulford *et al.*[31]
Biopsies, Partial pleurectomy	BAP1 loss (partial)	—	—	Yes (36)	Yes	Apprx. 72	DOD (72)	Michael *et al.*[25]
—	NA	—	—	No	No	NA	Alive, SD	Michael *et al.*[25]
Biopsy	BAP1 retained, MTAP loss	—	—	No	No	NA	Alive, SD	Michael *et al.*[25]
Biopsy, Pleurectomy	BAP1 retained	Y	—	No	No	NA	Alive	Ando *et al.*[27]
—	—	—	—	Yes (12)	Yes	12	Dead (26)	Almeida *et al.*[28]
Biopsy	BAP1 retained, MTAP loss	Y	—	Yes (25)	Yes	32	Alive (41)	Nishikubo *et al.*,[23] Minami *et al.*[29]
Biopsy	BAP1 loss (focal), MTAP retained	N	No *CDKN2A* homozygous deletion or *NF2* hemizygous loss; No germline mutation	Yes (84)	Yes	84	DOD (180)	Hidaka *et al.*[32]

TABLE 4. (*Continued*)

Case	Site	Pattern	Age	Sex	Asb.	Symptoms	Imaging	Cyto. Dx	Cyto. IHC (BAP1/MTAP)
32	Pleu	—	57	M	—	Cough, B Symptoms	—	Y	—
33	Pleu	—	70	F	—	Recurrent effusion	No significant findings	—	—
34	Pleu	—	71	F	—	Recurrent effusion	Smooth pleural thickening	—	—
35	Pleu	—	72	F	—	Recurrent effusion	Smooth pleural thickening	—	—
36	Pleu	—	68	M	—	Recurrent effusion	—	—	—
37	Pleu	—	69	M	—	Recurrent effusion	—	—	—
38	Pleu	—	79	M	—	Recurrent effusion	No significant findings	—	—
39	Pleu	—	67	M	—	S/p lung cancer resection	No significant findings	—	—
40	Pleu	—	68	M	—	Recurrent effusion	Few mm nodule on top of a pleural plaque	—	—
41	Pleu	—	76	M	—	S/p lung cancer resection	Few mm nodule on pleural surface	—	—
42	Perito, Pleu	Papillary and Flat	43	F	N	Pleural effusion	Pleural nodularity	Y	—
43	Processus vaginalis	Papillary	82	M	—	Inguinal mass	Spermatic cord swelling	—	—

Apprx., approximately; Asb., asbestos; BAP1, BRCA-1 associated protein-1; BP, base pair; Cyto., cytology; DOD, died of disease; Dx, diagnosis; F/U, follow up; FISH, fluorescence *in situ* hybridization; Homo, homozygous; Inv., invasive; LOH, loss of heterozygosity; LTFU, lost to follow-up; MTAP, methylthioadenosine phosphorylase; Mut., mutation; NA, not applicable; NGS, next-generation sequencing; NP, not performed; Perito, peritoneal; Pleu, pleural; Prog., progression; Rec., recurrence; SD, stable disease; Surg., surgical.

invasive component was identified in the first biopsy. In case 1, invasive mesothelioma was diagnosed in a later resection specimen (13 months later), it is impossible to determine if invasive mesothelioma was present at the time of MIS diagnosis due to sampling limitations. In cases 2 and 3, this should not be an issue, as exploratory laparoscopy with omentectomy and peritoneal excision revealed only MIS and no invasion. The median post-MIS follow-up time for all cases was 28 months (range 18–67; Table 1).

All three cases of MIS in this series showed an associated *BAP1* gene mutation. *BAP1*, located on chromosome 3p21.1, is an established tumour suppressor gene, which regulates the cell cycle, DNA damage repair, chromatin modification, programmed cell death, cellular differentiation, and immune responsiveness.[13,14] The BAP1 protein is a deubiquitinating enzyme produced by this gene.[14] Mutations in *BAP1* are associated with various aggressive malignancies, including uveal and cutaneous melanoma,

Surg. specimen type	Surg. IHC (BAP1/MTAP)	*CDKN2A* homo deletion by FISH	NGS	Prog. on imaging (mo)	Inv. Dx	Time to inv. (mo)	F/U (mo)	References
Biopsy	BAP1 loss	Y	—	Yes (8)	Yes	8		Haefliger *et al.*[22]
Biopsy	BAP1 loss, MTAP loss (partial)	Y	—	—	Yes	36	—	Churg *et al.*,[4] Churg *et al.*,[34] Churg *et al.*[35]
Biopsy	BAP1 loss, MTAP retained	Not performed	—	—	Yes	64	—	Churg *et al.*,[4] Churg *et al.*[34]
Biopsy	BAP1 loss, MTAP retained	N	—	—	Yes	92	—	Churg *et al.*,[4] Churg *et al.*[34]
Biopsy	BAP1 loss, MTAP retained	N	—	—	Yes	58	—	Churg *et al.*,[4] Churg *et al.*[34]
Biopsy	BAP1 loss	N	—	—	Yes	69	—	Churg *et al.*,[4] Churg *et al.*[34]
Biopsy	BAP1 loss	Not performed	—	—	Yes	60	—	Churg *et al.*,[4] Churg *et al.*[34]
Lung resection	BAP1 loss, MTAP loss (partial)	N	—	—	No	NA	Alive (12)	Churg *et al.*,[4] Churg *et al.*[34]
Biopsy	BAP1 loss, MTAP retained	N	—	—	No	NA	Alive (120)	Churg *et al.*,[4] Churg *et al.*[34]
Lung resection	BAP1 loss, MTAP loss	N	—	—	No	NA	Alive (57)	Churg *et al.*,[4] Churg *et al.*,[34] Pillappa *et al.*[36]
Biopsy	BAP1 loss, MTAP retained	—	Germline 2-BP *BAP1* frameshift deletion (c.458_ 459delCT)	No	No	NA	Alive	MacLean *et al.*[7]
Radical orchiectomy	BAP1 loss	—	—	—	—	—	Alive (24)	Kobayashi *et al.*[33]

mesothelioma, and renal cell carcinoma. Molecular analysis has rarely been performed on MIS, and Dacic *et al.* performed whole-exome sequencing on two MIS cases with copy number loss, loss of heterozygosity, and a small interstitial copy number loss with splice site deletion–insertion mutation, all in *BAP1*.[15] Additionally, recent case reports have shown a relationship between pleural and/or peritoneal MIS and germline *BAP1* mutations.[7,14,16] Germline *BAP1* mutations have previously been reported in MIS progressing to invasive mesothelioma, and *BAP1* mutation may be an early event in invasive mesothelioma tumorigenesis, including preinvasive disease.[4,15,16] In our case 3, the patient was age 24 at diagnosis and later confirmed to harbour a germline *BAP1* mutation after diagnosis of MIS. As of the last follow-up, 28 months after MIS diagnosis, there was no evidence of progression to mesothelioma, in contrast to a previously reported *BAP1* germline mutated patient who was diagnosed at the same age.

This patient, however, developed invasive mesothelioma after 10 months.[16] Interestingly, MIS accompanying endometriosis with a progestin effect was reported in the same patient,[16] similar to findings in our case 3. Endometriosis induced chronic inflammation of the peritoneal cavity, and the hormonal environment may promote mesothelial proliferation, both benign peritoneal inclusion cysts and WDPMT have been reported in association with endometriosis.[17] Further investigating the possible correlation between peritoneal MIS and endometriosis in young *BAP1* germline mutant female patients may be worthwhile. Future work should also compare the prognosis of MIS between germline *BAP1* mutated patients and those without germline *BAP1* mutations.

The specific alterations of *BAP1* in our cases of MIS of the peritoneum were not uniform. Case 1 showed gene rearrangements of exon 3, likely causing disruption and inactivation of *BAP1* and *APEH*, whereas case 2 demonstrated *BAP1* frameshift (p.V530Cfs*41) and *ERCC3* nonsense (p.R109*) mutations, both of which would be expected to reduce or abrogate the activity of protein products. Case 3 contained a deletion of the intron 1 donor site, which could result in protein truncation through defects in mRNA processing. *TET2* and *U2AF1* mutations are more commonly seen in haematologic disorders and can be identified in otherwise healthy older individuals as a feature of clonal haematopoiesis of indeterminate potential (CHIP).[18]

Analogous to these MIS cases, invasive mesothelioma has a known association with *BAP1* mutations, both recurrent somatic and/or germline, which are seen in 50%–70% of both pleural and peritoneal cases.[19] *BAP1* mutations in invasive disease vary and comprise frameshift, nonsense, splice site, and missense mutations. Additionally, structural rearrangements/deletions, inactivating rearrangements, and copy number loss are observed. Tumours often harbour more than one alteration in the *BAP1* gene. In our study, case 1 was the only case with progression and harboured an identical somatic *BAP1* mutation in both MIS and invasive mesothelioma. Missense mutation of *SMO*, as seen in the invasive mesothelioma for case 1, is present in 5%–10% of invasive mesothelioma cases and may confer a poor prognosis.[20,21] Haefliger *et al.* proposed that genomic transition from a diploid to an aneuploid state might play a role in progression from MIS to mesothelioma.[22] Other reports note that progression to mesothelioma from MIS occurred earlier in patients with *CDKN2A* homozygous deletion or MTAP loss, suggesting that *CDKN2A* homozygous deletion and MTAP loss could be poor prognostic factors.[23]

In summary, MIS is clinically suspected in patients presenting with nonresolving pleural effusion(s) or ascites in the setting of heavy asbestos exposure, with or without pleural plaques, after irradiation, and in patients with a familial predisposition. The diagnosis of MIS is multidisciplinary and should be made with consideration of clinical, imaging, and pathological features.[2] In pathology practice, ancillary studies including immunohistochemical and molecular studies play an indispensable role in the diagnosis of MIS.

Author contributions

ES collated data, performed the study, analysed data, and wrote the article. ANH and HL designed the study, performed portions of the analysis, and wrote the article. MT, BC, JM, and TK identified and contributed cases as well as reviewed pathology and edited the final version of the article. HK, OM, HW, and KT edited the final version of the article.

Conflict of interest

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, ES.

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