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CONTROVERSIES IN PATHOLOGY

The concept of mesothelioma *in situ*, with consideration of its potential impact on cytology diagnosis

Sonja Klebe[1], Yukio Nakatani[2], Katalin Dobra[3], Kelly J. Butnor[4], Anja C. Roden[5], Andrew G. Nicholson[6,7], Alberto M. Marchevsky[8], Aliya N. Husain[9], Amanda Segal[10], Ann E. Walts[8], Birgit Weynand[11], Claire W. Michael[12,13], Sanja Dacic[14], David Godbolt[15], Richard Attanoos[16], Eric Santoni-Rugiu[17,18], Françoise Galateau-Salle[19], Kenzo Hiroshima[20,21], Andre L. Moreira[22], Juliet Burn[23], Kazuki Nabeshima[24], Allen R. Gibbs[25], Andrew Churg[26], Leslie A. Litzky[27], Luka Brcic[28], Ming Sound Tsao[29,30], Mari Mino-Kenudson[31,32], Sara B. Rørvig[17], Henry D. Tazelaar[5], Thomas Krausz[9], Yu Zhi Zhang[6,7], Lucian R. Chirieac[32,33], Mary B. Beasley[34], Anders Hjerpe[35,36]

[1]Department of Anatomical Pathology, Flinders University and SA Pathology, Adelaide, SA, Australia; [2]Department of Pathology, Chiba University Hospital, Chiba, Japan; [3]Department of Laboratory Medicine, Division of Pathology, Karolinska University Hospital, Stockholm, Sweden; [4]Department of Pathology and Laboratory Medicine, University of Vermont Medical Center, Burlington, VT, USA; [5]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; [6]Royal Brompton and Harefield NHS Foundation Trust, London, UK; [7]National Heart and Lung Institute, Imperial College London, UK; [8]Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [9]Department of Pathology, University of Chicago, Chicago, IL, USA; [10]PathWest, Queen Elizabeth II Medical Centre, Perth, WA, Australia; [11]Department of Pathology, University Hospital Leuven, Leuven, Belgium; [12]Department of Pathology, University Hospitals Cleveland Medical Center, Cleveland, OH, USA; [13]Department of Pathology, Case Western Reserve University, University Hospitals Cleveland Medical Center, Cleveland, OH, USA; [14]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [15]Department of Pathology, The Prince Charles Hospital, Brisbane, Qld, Australia; [16]Department of Cellular Pathology, University Hospital of Wales, Cardiff, Wales, UK; [17]Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [18]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark; [19]Department of Biopathology, Centre Léon Bérard, Lyon, France; [20]Department of Pathology, Yachiyo Medical Center, Tokyo Women's Medical University, Yachiyo, Japan; [21]Department of Biochemistry and Genetics, Chiba University Graduate School of Medicine, Chiba, Japan; [22]Department of Pathology, NYU Langone Health, New York, NY, USA; [23]Douglass Hanly Moir Pathology, Sydney, NSW, Australia; [24]Department of Pathology, Fukuoka University School of Medicine and Hospital, Fukuoka, Japan; [25]Department of Cellular Pathology, Cardiff and Vale University Health Board, Cardiff, Wales, UK; [26]Department of Pathology, Vancouver General Hospital and University of British Columbia, Vancouver, BC, Canada; [27]Department of Pathology, Hospital of the University of Pennsylvania, Philadelphia, PA, USA; [28]Diagnostic and Research Institute of Pathology, Medical University of Graz, Graz, Austria; [29]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; [30]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [31]Department of Pathology, Massachusetts General Hospital, Boston, MA, USA; [32]Harvard Medical School, Boston, MA, USA; [33]Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA; [34]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [35]Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; [36]Division of Clinical Pathology/Cytology, Karolinska University Laboratory, Karolinska University Hospital, Stockholm, Sweden

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Summary

Diffuse malignant mesothelioma (MM) is an incurable tumour of the serosal membranes, which is often caused by exposure to asbestos and commonly diagnosed at advanced stage. Malignant mesothelioma *in situ* (MMIS) is now included as diagnostic category by the World Health Organization (WHO). However, our international survey of 34 pulmonary pathologists with an interest in MM diagnosis highlights inconsistency regarding how the diagnosis is being made by experts, despite published guidelines. Whilst the WHO restricts the diagnosis to surgical samples, the very concept has implication for cytological diagnosis, which is already regarded as controversial in itself by some. MMIS is currently only applicable as precursor to MM with an epithelioid component, and raises the possibility for different molecular pathways for different histological MM subtypes. The clinical implications of MMIS at this stage are uncertain, but aggressive therapies are being initiated in some instances. Based on the results of the survey we here present a critical appraisal of the concept, its clinical and conceptual implications and provide practice suggestions for diagnosis. A low threshold for ancillary testing is suggested. The designations of 'malignant mesothelioma, cannot exclude MMIS' or 'atypical mesothelial proliferation with molecular indicators of malignancy, so-called MMIS' could be used on cytology samples, adding 'no evidence of invasion in sample provided' for surgical samples. Clinical and radiological correlation are integral to diagnosis and best done at multidisciplinary meetings. Finally, collaborative studies are required to improve our understanding of MMIS.

Key words: Diffuse malignant mesothelioma; mesothelioma *in situ*; WHO; cytology diagnosis; early diagnosis.

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INTRODUCTION

Diffuse malignant mesothelioma (MM) is an incurable tumour of the serosal membranes, which is often caused by exposure to asbestos. It most commonly affects the pleura and peritoneum, but the pericardium and tunica vaginalis testis (as a continuation of the peritoneum) can also be involved. Regarded as rare, approximately 30,000 were diagnosed worldwide in 2018 and incidence or case numbers in many countries remain stable or are increasing.[1,2] There is a long latency period between exposure to asbestos and development of disease. Clinically silent in the early stages, MM is often diagnosed at an advanced stage and to date, treatments have only had a modest impact on survival times.[2] MM is subtyped into epithelioid, biphasic and sarcomatoid subtypes. The correct designation of histological subtype affects survival significantly and impacts which treatment options are available. Morphology of MM is protean and pathological diagnosis requires a panel of immunohistochemical (IHC) markers that allow definitive diagnosis in most cases, but occasionally, diagnosis even on large surgical samples can be difficult because MM shows extremely variable morphology. For example, it can contain mucin vacuoles or crystalloids, heterologous elements including bone, mimic synovial sarcoma or exhibit small cell morphology.[3] MM may aberrantly express markers or lose expression even of cytokeratins.[4,5] Correlation with clinical and imaging findings is often suggested, but these may show overlap with other malignancies, including pseudomesotheliomatous carcinoma and various sarcomas.

Many patients present with recurrent pleural effusions, and there is the possibility of diagnosis of MM with an epithelioid component by cytology in isolation, which is embraced by some groups but regarded more cautiously or rejected by others.[4,6–8] However, there are ample data to indicate that in skilled hands, cytological diagnosis of MM is reliable.[9,10] In addition to these difficulties, benign asbestos related effusions are recognised. However, it is not uncommon for patients to present with recurrent pleural effusions, with no definite diagnosis being made, only to eventually be diagnosed with advanced disease. Some of these instances relate to sarcomatoid MM, which do not usually shed malignant cells into the effusion.

Guidelines have been publicised for both histological and cytological diagnosis, including guidelines that take into account the clinical context,[6,7,11,12] but many cases will be submitted for expert opinion. Owing to the rarity of the disease, diagnostic experience may be limited, and because of the clinical implications due to poor prognosis with few treatment options, as well as medicolegal implications, the diagnostic stakes for the reporting pathologist are often perceived as high.

Adding to this already complex landscape, the upcoming World Health Organization (WHO) classification will include the diagnosis of MMIS[13] (and https://bboss.iarc.fr/submission.php?subchapid=114chapid=114).

This is an important concept, since it suggests that pre-invasive disease precedes the invasive (and essentially treatment resistant) manifestation of MM. Such a diagnosis may provide opportunities for earlier diagnosis and treatment, and ultimately, better clinical outcomes.

In the upcoming WHO classification, MMIS will be defined as ' … a pre-invasive single layer surface proliferation of neoplastic mesothelial cells'. This diagnosis requires multidisciplinary information and discussion. Essential criteria for diagnosis include:

1. Pleural effusion (non-resolving).
2. No thoracoscopic or imaging evidence of tumour.
3. Single layer of atypical mesothelial cells on pleural surface.
4. Loss of BAP1 and/or MTAP by IHC and/or CDKN2A (p16) homozygous deletion by fluorescence *in situ* hybridisation (FISH).
5. Multidisciplinary discussion of diagnosis.
6. No histological features of invasive growth.

Some publications suggest that it is also essential that no invasive MM develops for at least one year after biopsy.[14]

What is the rationale, and value of such a diagnosis? Will pathologists be confident in making a prospective diagnosis based on morphology, multidisciplinary information and ancillary studies? The WHO specifically states that MMIS cannot be diagnosed on cytology, but cytology diagnosis of diffuse (invasive) MM is standard practice in many centres, and how could MMIS be differentiated from diffuse MM with an epithelioid component on cytology, given that loss of BAP1 and/or CDKN2A is used for cytological diagnosis of

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diffuse MM? Should a cytology report state that MMIS cannot be excluded, to prevent aggressive therapy? (There are case reports that describe positive cytology in MMIS cases.[15]) Or is aggressive therapy for MMIS acceptable, or even desirable? How can the diagnosis of MMIS be made in practice if the very definition of the entity, according to some experts, requires one year of follow up? Who takes responsibility for the clinico-radiological correlation? Should the possibility of diagnosing MMIS affect the approach to recurrent effusions? What is the implication for sarcomatoid MM, which does not appear to be preceded by MMIS (after all, sarcomatoid MM accounts for >10% of MMs). In particular, can a more detailed understanding help us better characterise the molecular pathways leading to diffuse MM? And what is the approach taken by experts around the world?

MATERIALS AND METHODS

A survey was designed to ascertain current clinical practice regarding cytology diagnosis of MM and experience with the concept of MMIS. The questions are provided verbatim in the figures. The survey was distributed by SurveyMonkey. Specialist pulmonary pathologists from 28 institutions from 11 countries with a declared interest in MM diagnosis, contributed answers and comments to the survey, and contributed to the writing of this paper. The results, and questions that are raised, are discussed below.

RESULTS

The pathologists involved in this survey included dedicated cytopathologists, surgical pathologists and pathologists with mixed cytology and surgical practice. Some pathologists kept personal databases of cases, whereas others searched departmental records. With regards to clinical follow-up, this was also variable, with some pathologists actively following patients and others relying on clinical databases. The diagnosis of MMIS had been made between 0 and >20 times by individual pathologists. Overall, diagnosis of MMIS as defined by the WHO was very rare, with two cases in a database of 4677 specimens and seven cases in a database of 3214 cases. However, these diagnoses have only been made in the last 2–4 years, and these databases spanned approximately 40 years of practice each.

A full questionnaire is included in the Supplementary data (Appendix A). Progression of disease was defined by clinical progression and/or tissue biopsy.

Cytological diagnosis of mesothelioma

A total of 80% of pathologists were comfortable making a diagnosis of MM (not specifically MMIS) on cytology. Only 35% were prepared to make a diagnosis based on malignant morphology in isolation, once mesothelial phenotype had been established, and those pathologists emphasised that in those cases no further studies (i.e., BAP1, CDKN2A) were indicated. Also, 65% would accept a diagnosis based on atypical morphology, ancillary pathology studies and radiological evidence of invasion, with 53% accepting atypical morphology and loss of BAP1, MTAP or CDKN2A deletion for diagnosis, without the need for radiological correlation. Of the pathologists surveyed, 24% were prepared to make a diagnosis of MM on cytology based on abnormal BAP1, MTAP or molecular result, regardless of cellular morphology or radiological appearances (e.g., evidence of invasion into lung, chest wall or ribs, diffuse pleural thickening with involvement of interlobar fissures, and or nodularity, mass lesion). In addition, 6% of pathologists were prepared to diagnose MM in a cytology sample with atypical morphology, normal BAP1, MTAP and molecular markers but positive IHC markers, such as desmin or EMA, emphasising in comments that reliance on BAP1 and MTAP would miss cases (Table 1).

We also asked what would prompt a pathologist to perform further IHC for BAP or MTAP or molecular studies on a pleural effusion sample which was not cytologically malignant, and 65% of pathologists would perform such studies if there was a high clinical suspicion, 35% if the effusion was recurrent and 12% if they were aware of a history of asbestos exposure. However, 6% would not perform BAP1, MTAP or molecular tests unless there was a compelling cytomorphological reason (Table 2).

Mesothelioma *in situ*: diagnosis

All pathologists surveyed were aware of MMIS as diagnostic entity, and 71% had made or suggested the diagnosis, most commonly on a video-assisted thoracoscopic surgery (VATS) biopsy, decortication or surgical extrapleural pneumonectomy (68%, 29% and 12%, respectively) but only 10% had made or suggested the diagnosis on cytology (Fig. 1). Only 9% of respondents would accept the diagnosis only if the mesothelium was flat, whereas 65% accepted flat or papillary mesothelium and 6% would only accept complex papillary mesothelium, with comments indicating that the differential diagnosis of atypical mesothelial proliferation suggested that proliferation was of particular relevance. Cytological atypia was required by 29%, but some commented that reactive mesothelium may be very atypical and that atypia was of no significance at this site. Support for the diagnosis by appropriate IHC/molecular marker results (CDKN2A loss, negative MTAP/BAP1) was required by 82%. Interestingly, only 47% required knowledge of the concurrent radiology result (Table 3).

Mesothelioma *in situ*: treatment

Whilst most respondents had only seen clinical follow up as a result of a diagnosis of MMIS, 47% of participants had seen patients who had received active treatment including systemic or intrapleural chemotherapy (15%), surgical therapy (decortication 26% and extrapleural pneumonectomy 6%) (Table 4). It could not be ascertained if further therapy options had been offered to those who did not receive any, but comments indicated that at least some of these patients had been discussed at multidisciplinary team (MDT) meetings. Progression of *in situ* disease had been observed by 44% of respondents, and 30% of reported cases that had progressed after 1–2 years, 10% reported cases that had progressed over 2–4 years, but 15% of respondents had seen progression between 6–12 months (Fig. 2), and 35% had seen disease progression after more than 4 years.

DISCUSSION

This study revealed that even amongst experts, despite guidelines to which many of the authors herein contributed, there is no consensus regarding the diagnostic criteria for

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Table 1 Results for Question 5: Tick all options which you would accept for definite diagnosis of mesothelioma on effusion cytology samples, assuming mesothelial phenotype has been established

Answer choice	%
Atypical morphology AND molecular (Loss of BAP1, MTAP of CDKN2A by FISH (or other) AND Clinical/radiological evidence of invasion	64.71%
Malignant morphology in isolation	35.29%
Atypical morphology AND molecular (Loss of BAP1, MTAP of CDKN2A by FISH (or other)	52.94%
Atypical morphology AND Clinical/radiological evidence of invasion	26.47%
Loss of BAP1, MTAP of CDKN2A by FISH (or other) regardless of morphology or clinical/radiological evidence of invasion	23.53%
Atypical morphology AND IHC markers (desmin, p53, EMA, etc) negative molecular (BAP1, MTAP, CDKN2A, etc)	8.82%
Atypical morphology AND serum biomarkers	0.00%

Table 2 Results for Question 6: Under what circumstances should cytology samples/biopsy samples that do not show obvious microscopic findings of malignancy be subjected to additional studies (BAP1, MTAP, CDKN2A FISH, IHC, other)? Tick all that apply

Answer choice	%
History of asbestos exposure- any effusion	11.76%
History of asbestos exposure- recurrent effusion	20.59%
Any recurrent effusion	35.29%
Any effusion	2.94%
Clinical request/indication of suspicion of malignancy	64.71%
If concerning features are seen in the sample	64.71%
Never	5.88%
Other (please specify)	20.59%

MM on cytology, and particularly regarding the diagnosis of MMIS. We have included the criteria for cytological diagnosis here because it is clear that early MM and MMIS cannot be distinguished on cytology in isolation, and whilst the WHO indicates that MMIS diagnosis should only be made on surgical/biopsy samples, the very concept of MMIS has implications for cytology diagnosis. In light of this, and recognising that radiological information may not always be available to the pathologist, a designation on cytology of 'malignant mesothelioma, cannot exclude MMIS' could be used. Such a category would also suggest that it is the role of the clinician or MDT meeting to correlate the clinical and radiological information.

The majority of pathologists are comfortable making a cytology diagnosis which is reflected in the published guidelines of the cytology society[6,8] and many published audits.[9] Of the more than 400 reported cases where MM was diagnosed on cytology, all cases but one were shown to have an invasive MM within 12 months.[10] Thus, the cytological diagnosis of MM may only rarely include MMIS, and one argument suggests that reliance on cytology (and acceptance that any molecularly malignant mesothelial tumour requires aggressive therapy) could spare patients the potential complications of a biopsy procedure.

However, this approach is not universally accepted, and this is reflected in current clinical guidelines, which state ' … effusion cytology for definitive diagnosis of MPM remains a controversial topic and is still generally not recommended … if effusion cytology is frankly malignant, the diagnosis may be strongly suggested but confirmation by biopsy, if possible, is recommended … '[12] and 'Do not rely on cytology alone to make a diagnosis of MPM unless biopsy is not possible or not required to determine treatment due to patient's wishes or poor performance status … '.[11] In practical terms, though,

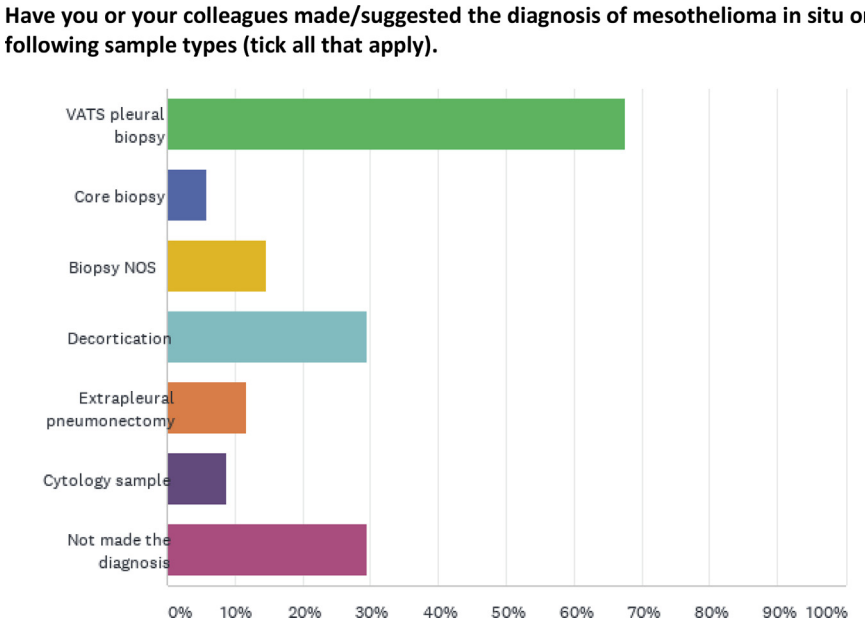

Fig. 1 A survey sent to 34 pulmonary pathologists asked if they or colleagues in their institution had made/suggested the diagnosis of mesothelioma *in situ* on different types of sample.

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Table 3 Results for Question 4: What diagnostic criteria do you use for diagnosis of mesothelioma *in situ* in a biopsy? Tick all that apply

Answer choice	%
Must be flat mesothelium	8.82%
Must be cytologically atypical	29.41%
Must be complex papillary	5.88%
May be flat OR papillary	64.71%
May be cytologically atypical or bland	52.94%
Must have loss of BAP1 OR MTAP OR homozygous deletion of CDKN2A by FISH	82.35%
Other molecular marker- please specify	2.94%
Must be cytologically bland	0.00%
IHC for EMA and/or desmin	11.76%
IHC for CD146, Imp3 and Glut-1	0.00%
Other IHC marker	2.94%
Must have negative radiology	47.06%
Serum biomarkers	0.00%
Other	35.29%

cytology diagnosis of MM has been shown to be reliable in experienced hands and is relied upon in clinical practice in many centres.[9,10] The clinical guidelines and WHO definition of MMIS emphasise the importance of clinico-pathological correlation, but many participants also emphasised that pathologists should exercise caution assuming responsibility for comprehensive clinico-radiological correlation, and that this was the role of an MDT or tumour board meeting.

Some recent publications emphasise a requirement for flat morphology,[16] whereas the earliest concepts suggested the diagnosis in atypical mesothelial proliferations with papillary features.[17] Others accept flat and papillary architecture.[18] This difference is reflected in the clinical practice of the participants. A differential diagnosis of well-differentiated papillary mesothelioma was not felt to pose significant diagnostic difficulties by most pathologists who specifically mentioned that as a potential differential diagnosis, but some did raise that possibility and suggested designation of any papillary lesion as 'atypical'. Participants in this survey placed the greatest reliance on the BAP1/MTAP IHC and molecular studies. It then follows that morphology plays a lesser role, and consequently most pathologists accept either morphology. However, whist this was the approach taken by the majority, it is not universally accepted, and some pathologists are disturbed by the reliance placed on IHC for BAP1, MTAP or molecular studies, especially when MMIS is potentially being diagnosed when morphology is essentially normal. This is based on our understanding of *in situ* malignancies in all other body sites that have at least some recognisable morphological abnormalities, and alternative terminology was suggested by some contributors, such as 'BAP1 loss or p16-deleted mesothelium' to capture these findings, short of making a firm diagnosis of MM.

This is a practically important consideration, because, like any ancillary test, the quality of BAP1/MTAP/FISH studies is variable, different antibody clones have not been harmonised, not all laboratories have these tests available, and interpretation can vary, especially if there is a background reactive mesothelial population with loss of labelling of either BAP1 or MTAP only observed on a proportion of cells.[18,19] Some researchers have found BAP1 and MTAP to be very reliable,[20–22] but major quality assurance programs have not published performance data (https://www.nordiqc.org/recommended.php). Interestingly, whilst the performance of MTAP and BAP1 has been reported as reliable in the literature,[22,23] several participants of this study commented on difficulties with optimisation of MTAP and BAP1 IHC, and reported batch inconsistencies of these antibodies. In addition, the different antibody clones available have not been harmonised. Furthermore, such reliance on molecular markers places great pressure on pathologists to carry out ancillary testing on morphologically unremarkable cytology and surgical samples. A recent study suggests that this may be justified, since testing allows earlier detection of MM in effusions otherwise regarded as benign based on morphology alone.[24] Most pathologists consider it appropriate to carry out ancillary studies simply based on the fact that an effusion was recurrent, but this information may not always be available. The ancillary testing does help to identify some cases carrying molecular alterations earlier, and may contribute to earlier diagnosis of MM in some cases,[24] but the clinical implications are not entirely certain (see below). Does failure to perform ancillary testing in a morphologically unremarkable biopsy expose a pathologist to a potential claim of negligence? And if morphologically normal mesothelium is tested, what are the implications for any other specimen that contains mesothelium? Should ancillary testing be performed, just to be sure?

Furthermore, even if all specimens containing mesothelium were to be tested, there are MM cases that express both of these markers, as highlighted by genetic studies showing that not all cases have homozygous deletion of the 9p21 band or loss of BAP1 expression.[25–27]

The designation of atypical mesothelial lesions may be very difficult, and a suggested algorithm for the designation of surgical and cytological specimens is presented in Fig. 3 and 4.

The diagnosis, and indeed the very concept of MMIS, has important implications for treatment. Some authors require one year of follow-up without invasive tumour developing, in addition to radiology not showing tumour at the time of diagnosis, before the diagnosis of MMIS can be made.[14] However, our survey shows that the diagnosis is suggested by pathologists in clinical practice in the absence of clinical information,[28] and in the experience of the participants of this study, treatment may begin immediately.

Clinicians accept that these cells are malignant at a molecular level, and treat accordingly, i.e., some of the patients were treated with chemotherapy or surgery before waiting for one year. Whilst some participants were concerned about aggressive therapy in the light of (probably) slowly progressive disease and advanced age, others considered that the diagnosis meant that malignant cells (at least at a molecular level) had been identified and that the diagnosis should lead to therapy. Many participants also indicated that early invasive disease could not be definitively excluded in a biopsy (or cytology) even with radiological correlation, and given that limitation, delaying therapy may disadvantage a patient. It was universally recognised that our understanding of the condition is limited, and that therapy may modify the tumour. This means that it is not possible to ascertain if an individual patient truly has MMIS as defined by the WHO, or has an early stage (invasive/diffuse) MM. Given that many patients are elderly and that median time to progression in the published literature is 60 months,[14] and 40% of participants of this survey have seen progression take 4 years or more, treatment benefits must be weighed against risks of procedures such as extrapleural pneumonectomy with significant mortality and morbidity. Chemotherapy was a popular option, but may need to be carefully considered, especially in

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Table 4 Results for Question 7: What treatment are you aware of that has been performed for mesothelioma *in situ*? Tick all that apply

Answer choices	%
I have not seen any cases	23.53%
I have not heard of any treatment being performed beyond clinical follow up	52.94%
Chemotherapy (systemic)	5.88%
Chemotherapy (intrapleural)	2.94%
Chemotherapy (NOS)	5.88%
Surgery- extrapleural pneumonectomy	5.88%
Surgery- decortication	26.47%
Surgery- NOS	2.94%
Radiation	0.00%
Other	5.88%

those cases that were diagnosed on flat, not obviously proliferative mesothelium. The clinical decision making in each of these cases included a complex combination of local preferences and perceptions of disease, patient performance status and preference as well as funding schemes, and is beyond the scope of this article. If the possibility of MMIS is flagged, could this help our clinical colleagues to 'first, do no harm'?

Does the concept of MMIS advance our molecular understanding on the pathogenesis of MM? In the past, a subserosal multipotential fibroblastoid cell (SMFC) was invoked as the stem cell for mesothelial renewal after serosal injury, and as the progenitor cell for the development of MM.[29,30] This theory suggested origin of MM from such SMFCs and would explain the biphasic differentiation characteristic of approximately 30% of MMs.[3,31] However, this also suggests that MM is invasive *ab initio*, with no *in situ*

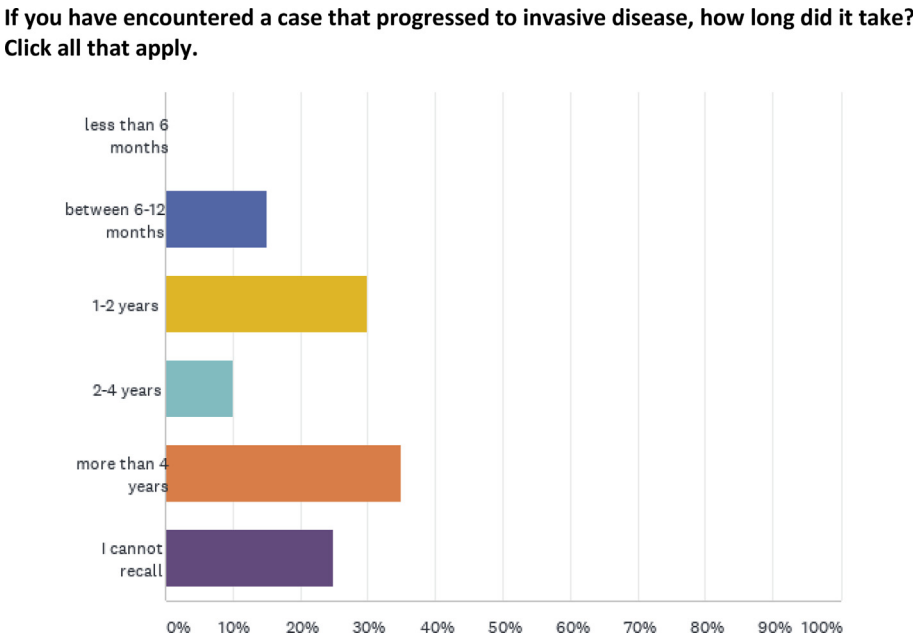

Fig. 2 A survey sent to 34 pulmonary pathologists asked if pathologists who made the diagnosis of mesothelioma *in situ* had encountered a case that progressed to invasive disease, and if so, how long it took. Only 20 of the participants had seen such a case, and five could not recall the time to progression.

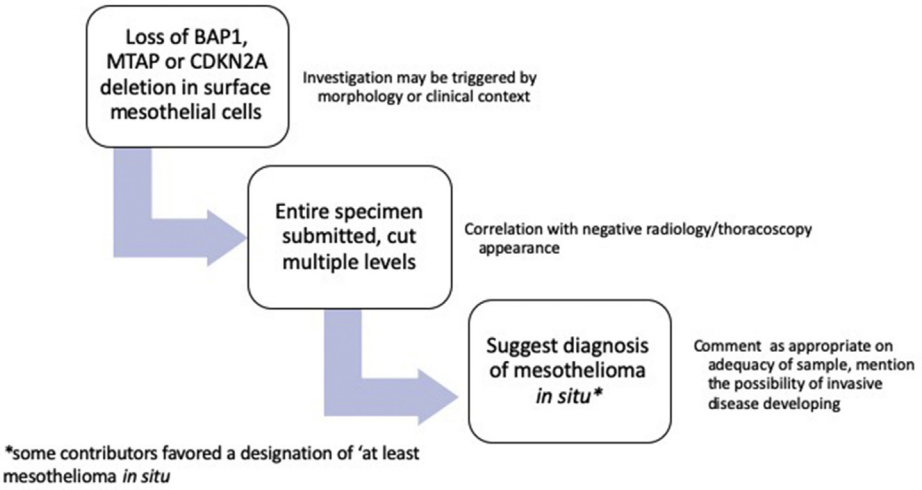

Fig. 3 Suggested algorithm for biopsy diagnosis of mesothelioma *in situ*.

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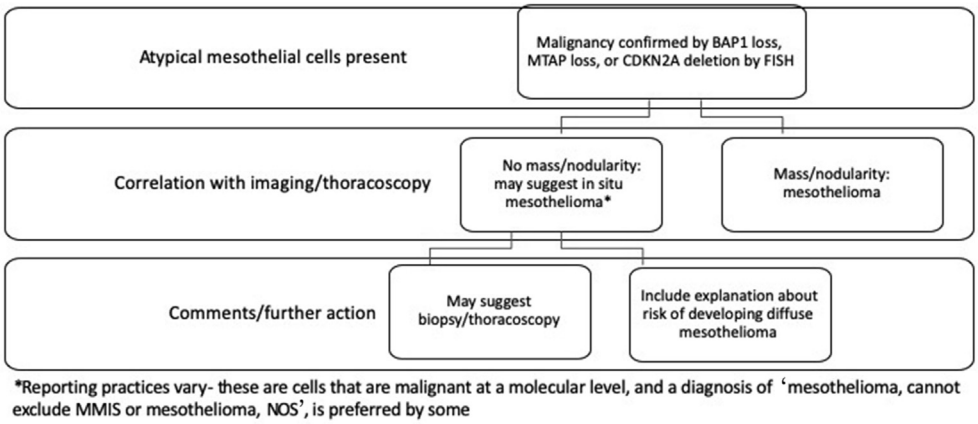

Fig. 4 Suggested algorithm for cytology diagnosis of atypical mesothelial proliferations.

phase of development. Based on experimental models of mesothelial healing following injury without disruption of the submesothelial basal lamina,[32–34] and on observations in early-stage MMs of epithelial type, Whitaker *et al.*[35] proposed the mesothelium itself as the progenitor cell for MM, advancing the concept of MMIS.[34] These authors[35,36] defined MMIS as the replacement of benign surface mesothelium by mesothelial cells with markers of malignancy, and BAP1, MTAP and homologous loss of CDKN2A have now been recognised as potentially suitable markers.

However, not all MMs have those changes, and to date the concept of MMIS has only been applied to MM with an epithelioid component.[27] Furthermore, a recent publication indicated that there was progression to invasive disease after median 60 months in 7/10 patients (70%), but that invasive/diffuse MM did not develop in the remaining three patients at 12, 57, and 120 months follow-up.[14] This could indicate that progression is very slow, or that malignant transformation is not inevitable, even though BAP1 loss is understood as indicating malignancy[37] or suggesting progression to malignant disease.[38] This is impressively demonstrated in this survey, with 40% of participants having observed cases that took more than 4 years to progress (or had not progressed during that time). Analogous to atypical adenomatous hyperplasia (AAH) of the lung, not all AAHs or even adenocarcinomas *in situ* (AIS) appear to progress to invasive adenocarcinoma when followed radiographically. Our current understanding suggests that a predominance of MMIS in biopsy may suggest early disease, with a better prognosis compared to diffuse MM,[18] but we do not know how to predict if or when MMIS progresses to invasive disease. This uncertainty could be reflected in a designation of 'atypical mesothelial proliferation with molecular markers of malignancy, indistinguishable from MMIS', as favoured by some of the participants of this study. Regardless of the designation used, further study is urgently needed to deepen our understanding of early MM, and ultimately, improve clinical outcomes.

CONCLUSION

MMIS was suggested as a concept in the 1990s and is now being included in the WHO classification as a diagnostic category. Despite published criteria for diagnosis, clinical practice varies amongst experts in the field, and the understanding of clinical significance is incomplete. Treatment approaches vary widely, and a concerted collaborative effort will be required to gather data and inform future practice.

Practice points

1. Low threshold for ancillary testing (BAP1 loss as assessed by IHC, CDKN2A deletion as assessed by FISH testing, or other testing methods) on cytology and histology samples.
2. On cytology, samples that fulfil published criteria for mesothelioma but lack clinico-radiological correlation could be designated 'malignant mesothelioma, cannot exclude MMIS' (recognising that this may prompt a phone call from a clinician not familiar with the concept).
3. Alternatively, a designation of 'atypical mesothelial proliferation with molecular indicators of malignancy' could be used +/− adding 'no evidence of invasion in sample provided' (in surgical samples).
4. Clinical and radiological correlation are best done at MDT.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2020.12.005.

Address for correspondence: Sonja Klebe, Department of Anatomical Pathology, Flinders University and SA Pathology, SA, 5042, Australia. E-mail: sonja.klebe@sa.gov.au

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