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# Diagnostic capacity of BAP1 and MTAP in cytology from effusions and biopsy in mesothelioma

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## KEYWORDS

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BRCA1-Associated protein (BAP1);  
Methylthionadenosine phosphorylase (MTAP);  
Immunohistochemistry

**Introduction** Serous effusion is often the first sign of mesothelioma. Diagnosis based on cytologic material from the effusions remains controversial and complementary biopsy is usually required. However, obtaining representative tissue sample may be challenging, while obtaining cytologic material is a minimally invasive procedure, providing potential for an earlier diagnosis. Loss of BRCA1-associated protein (BAP1), combined with loss of methylthionadenosine phosphorylase (MTAP) detected by immunohistochemistry, have shown to be reliable markers in the diagnosis of mesothelioma on histologic sections. Here we evaluate the value of these biomarkers in cytologic specimens.

**Materials and methods** The BAP1 and MTAP expression in specimens of 162 mesothelioma patients (156 pleural, 6 peritoneal)—71 cytologic, 91 histologic (44 epithelioid, 31 biphasic, 16 sarcomatoid)—and 20 patients with reactive mesothelial proliferations were investigated.

**Results** The loss of BAP1 and/or MTAP was highly sensitive and specific in differentiating mesothelioma from reactive mesothelial proliferations, with no significant difference between pleural effusions and

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biopsies, specificity of 100% in both and a sensitivity of 78.9% and 80.2%, respectively ( $P = 0.3$ ). There was a 100% concordance of the expression of BAP1 and MTAP in cytologic and corresponding histopathologic samples. Loss of BAP1 and/or MTAP in histologic sections discriminated sarcomatoid, biphasic, and epithelioid mesothelioma from reactive mesothelial proliferations with a sensitivity of 81.2%, 83.9%, and 77.3% respectively.

**Conclusion** Loss of expression of BAP1 and/or MTAP differentiated mesothelioma from reactive mesothelial proliferations with excellent specificity and high sensitivity in cytologic samples, comparable to histopathologic sections.

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## Introduction

Mesothelioma is a relatively rare tumor with a poor prognosis, emerging from the mesothelial surface of pleura ( $\approx 80\%$ ), peritoneum ( $\approx 20\%$ ), or pericardium or tunica vaginalis ( $<1\%$ ).<sup>1</sup> The main carcinogen associated with mesothelioma is asbestos. Occupational, environmental, as well as domestic, exposure to asbestos are well-documented causes of mesothelioma.<sup>2,3</sup> The median lag-time from exposure to cancer development is 40 years. Due to its carcinogenic features asbestos has been banned in most European countries. However, large countries such as China, Russia, Brazil, and India still produce and/or use asbestos. Therefore, it is expected that the number of patients diagnosed with mesothelioma will increase worldwide over the next decades.<sup>1</sup> Analogously, the incidence of mesothelioma in countries such as Denmark with early bans on asbestos was predicted to decline. Unfortunately, data of the incidence in Denmark up to 2019 have not yet shown a declining tendency, but on the contrary a slight increase, with an incidence rate of 3.2/100,000 in 2019.<sup>4</sup> Survival after mesothelioma diagnosis is poor, with a median of 14 months with chemotherapy and 18 months with immunotherapy.<sup>5</sup>

The 3 main subtypes of mesothelioma are the epithelioid, sarcomatoid, and biphasic, which is a mixture of the 2 former. Differential diagnosis of mesothelioma can be difficult even for a trained pathologist because of its morphology mimicking benign and/or other malignant conditions, and the lack of supporting accurate biomarkers of sufficient sensitivity and specificity. The current recommendation from the International Mesothelioma Interest Group (IMIG) involves several histologic features, such as stromal invasion and necrosis, in combination with molecular assay and/or immunohistochemistry (IHC). The recommended IHC should be done with at least 2 positive and 2 negative markers, with a sensitivity and/or specificity greater than 80%.<sup>6</sup>

Recurrent serous pleural and peritoneal effusion are often the first symptom of pleural and peritoneal mesothelioma, respectively, and may precede a tumor seen on a computed tomography scan.<sup>7</sup> Comorbidities and other clinical factors may be hurdles in obtaining representative tissue for

diagnosis. On the contrary, obtaining pleural effusion or ascites for cytology is a minimal invasive procedure that most patients can endure, and in addition may serve as a therapeutic/palliative intervention. Therefore, provided that a definitive diagnosis is set on cytologic material, earlier treatment may be initiated, and unnecessary delay avoided.

The diagnosis of mesothelioma on cytologic material, although widely accepted in many countries,<sup>8</sup> is still considered controversial in Denmark.<sup>9</sup> According to surgical pathologists, cytologic material has limited usefulness in a definitive diagnosis of mesothelioma, mainly due to overlap of cytologic features with reactive and malignant epithelioid mesothelial cells. Therefore, in Denmark, complementary biopsy is usually necessary.<sup>6</sup>

The usefulness of various IHC biomarkers in histologic tissue and in cytology effusions was investigated in differentiating between malignant mesothelial proliferations and benign proliferation. One of the IHC markers is BRCA-1 associated protein (BAP1), which is a tumor suppressor protein encoded by the BAP1 gene located on chromosome 3p21.<sup>10</sup> Somatic BAP1 loss by IHC is observed in up to 60% of pleural mesothelioma.<sup>11-15</sup> The mutation/inactivation of BAP1 has been described over the last decade in various cancers, for instance cutaneous and ovular melanoma, renal cell carcinoma, meningioma, and cholangiocarcinoma.<sup>16</sup> The IMIG currently recommends BAP1 as a useful supplementary biomarker, in histologic as well as in cytologic material, since loss of BAP1 is found only in mesotheliomas and not in benign conditions, and thus has a high specificity in differentiating between those conditions.<sup>6</sup>

Another specific marker for mesothelioma is the deletion of the tumor suppressor cyclin-dependent kinase inhibitor 2A (CDKN2A) (p16), located in the 9p21 chromosomal region. This deletion is observed in more than 70% of mesotheliomas detected by fluorescent in situ hybridization (FISH).<sup>17-24</sup> A co-deleted gene, methylthioadenosine phosphorylase (MTAP),<sup>25-29</sup> is also located in the 9p21 chromosomal region and is correlated to the deletion status of 9p21.<sup>17,26,30</sup> Due to the high cost and technical equipment needed, IHC is thus a preferred technique if the results are comparable to FISH. MTAP IHC has therefore emerged as the most reliable surrogate marker for CDKN2A

deletion.<sup>17,26,30</sup> The expression of MTAP in histologic sections has been described in many studies,<sup>17,25,26,29,31</sup> although few have studied MTAP expression in effusion samples.<sup>28,29,32,33</sup> Regarding differentiating between malignant and benign mesothelial proliferations, the specificity of MTAP in histologic samples seems to be persistent and described of 100%, whereas the sensitivity ranks between 42% and 71%.<sup>27-29,32</sup> Thus, loss of BAP1, MTAP, or both detected by IHC, has been shown to be reliable markers in the diagnosis of pleural mesothelioma on histologic sections.<sup>26-28</sup>

This study aimed to determine whether loss of BAP1 and MTAP IHC expression in cytology effusion samples in mesothelioma patients is as reliable as in histology material in distinguishing mesothelioma from reactive mesothelial proliferations, potentially sparing the patient a more invasive procedure and preventing diagnostic delay.

## Methods

Histologic and cytologic material from the available cell blocks were obtained from a cohort of mesothelioma patients from Aalborg University Hospital, Denmark, treated in the period 1977-2013; this was used to construct a tissue microarray (TMA). The TMA was constructed using 2-mm diameter punches from the 3 most representative areas from each tumor or cell block. Due to the focal distribution of mesothelial proliferations, a normal paraffin block section was used from patients with confirmed reactive mesothelial proliferations. Normal breast and adrenal gland tissue were included as external controls.

IHC staining was performed on the formalin-fixed, paraffin-embedded, 3µm-thick tissue sections after heat-induced epitope retrieval, using ULTRA Cell Conditioning solution (CC1, Ventana Medical System, Inc., Oro Valley, AZ) for 44 minutes at 99°C followed by blocking of endogenous peroxidase. The sections were incubated with monoclonal antibody MTAB, 1:2000 dilution, retention time (RT) 32 min (Abcam, Cambridge, UK) and monoclonal antibody BAP1, 1:50 dilution; RT 32 min (Santa Cruz Biotechnology, Dallas, TX), respectively. Immunoreacting cells were visualised using OptiView DAB IHC Detection Kit (Ventana, Roche, Basel, Switzerland), followed by a haematoxylin counterstain. The immunostaining was carried out using Ventana Benchmark Ultra stainer (Roche, Basel, Switzerland).

The mesothelioma re-evaluation and confirmation, as well as the interpretation of the IHC staining of the biopsy specimens, was performed by 2 independent pathologists (L.L., C.M.).

The 3 TMA punches from the same patient were evaluated altogether and assigned a single score of “preserved” or “lost” expression, where the latter involved completely lost expression or an expression with an apparently lower intensity than the controls. This applied in some cases where

either a general and diffuse background staining, or a rather weak cytoplasmic reaction, was observed.

In case of inconsistency, agreement was reached using a double-headed microscope.

Fisher’s exact test was used to assess the differences between the BAP1 and MTAP status in histopathologic and cytologic samples. The sensitivity, specificity, and positive and negative predictive value of loss expression of biomarkers in differentiating between benign and malign condition were calculated. Tests of statistical significance were 2-sided and *P* values < 0.05 were considered statistically significant. Statistical analyses were performed using STATA version 16 (StataCorp, College Station, TX). The study was approved by the North Denmark Region Committee on Health Research Ethics (reg. no N-20140032) and reported to the Danish Data Protection Agency (reg. no 2008-58-0028).

## Results

A total of 167 cases of mesothelioma were available for analyses. Five samples were excluded from the study either due to insufficient confirmation of mesothelioma diagnosis (*n* = 4), or insufficient amount of tissue (*n* = 1). Finally, the study included 162 cases of malignant mesothelioma (MM) (156 pleural, 6 peritoneal). The samples consisted of 71 cytologic and 91 histologic material, of which 44 were epithelioid, 31 biphasic, and 16 sarcomatoid subtypes (Table 1). Furthermore, 20 reactive mesothelial proliferations histologic samples of benign condition were included as the control group.

MTAP IHC expression in many cases revealed nuclear as well as cytoplasmic staining, while BAP1 only revealed nuclear staining. Loss of MTAP expression was defined in concordance with other authors,<sup>17,26,29,31,32</sup> as the complete loss or a weaker expression than the controls (internal and external), and the only pattern considered was the cytoplasmic.<sup>33,34</sup> Both MTAP and BAP1 showed a strong positive reaction in the luminal breast epithelial cells and a non-to-weak reaction in the adrenal gland tissue (external controls). Non-mesothelial cells (eg, inflammatory cells, fibroblast, pneumocytes and endothelial cells) seen immunoreactive to MTAP and BAP1 served as internal positive control.

**Table 1** Characteristics of samples of malignant mesothelioma and reactive mesothelial proliferations.

Malignant mesothelioma cases ( <i>n</i> = 162)	RMP ( <i>n</i> = 20)
Female ( <i>n</i> = 27)	Female ( <i>n</i> = 5)
Male ( <i>n</i> = 135)	Male ( <i>n</i> = 15)
Pleural cytology ( <i>n</i> = 71)	
Pleural histopathology ( <i>n</i> = 91)	
Epithelioid ( <i>n</i> = 44)	
Biphasic ( <i>n</i> = 31)	
Sarcomatoid ( <i>n</i> = 16)	

Abbreviations: RMP, reactive mesothelial proliferation.

**Table 2** Observation rate of expressions of BAP1 and/or MTAP in histopathologic, cytologic, and reactive mesothelial proliferations samples.

Marker status, n (%)	Histologic samples (n = 91)	Cytologic samples (n = 71)	All samples (n = 162)	RMP (n = 20)
<b>BAP1 status</b>				
BAP1 (-)	50 (54.9%)	50 (70.4%)	100 (61.7%)	0
BAP1 (+)	41 (45.1%)	21 (29.6%)	62 (38.3%)	20 (100%)
<b>MTAP status</b>				
MTAP (-)	50 (54.9%)	31 (43.7%)	81 (50%)	0
MTAP (+)	41 (45.1%)	40 (56.3%)	81 (50%)	20 (100%)
<b>BAP1 and MTAP status</b>				
BAP1 (-) and MTAP (-)	27 (29.7%)	25 (35.2%)	52 (32.1%)	0
BAP1 (-) and MTAP (+)	23 (25.3%)	25 (35.2%)	48 (29.6%)	0
BAP1 (+) and MTAP (-)	23 (25.3%)	6 (8.4%)	29 (17.9%)	0
BAP1 (+) and MTAP (+)	18 (19.8%)	15 (21.1%)	33 (20.4%)	20 (100%)
Loss of BAP1 and/or MTAP	73 (80.2%)	56 (78.9%)	129 (79.6%)	0
BAP1 and MTAP retained	18 (19.8%)	15 (21.1%)	33 (20.4%)	20 (100%)

Abbreviations: BAP1, BRCA1-associated protein; BAP1 (-), loss of BAP1 expression; BAP1 (+), expression of BAP1 retained; MTAP, methylthionadenosine phosphorylase; MTAP (-), loss of MTAP expression; MTAP (+), expression of MTAP retained; RMP, reactive mesothelial proliferation.

### BAP1 and MTAP status in reactive mesothelial proliferations samples compared to mesothelioma

In the control reactive mesothelial proliferations group, loss of BAP1 expression and MTAP expression was not observed (Table 2, Fig. 1). Loss of expression of BAP1 and/or MTAP showed a specificity of 100% in both cytologic and histopathologic sections and sensitivity of 78.9% and 80.2% differentiating between mesothelioma and reactive mesothelial proliferations, respectively. The positive predictive value and negative predictive value for BAP1 and MTAP showing loss of expression was 100% and 82.6% in cytologic specimens and 100% and 83.5% in histopathologic sections (Table 3).

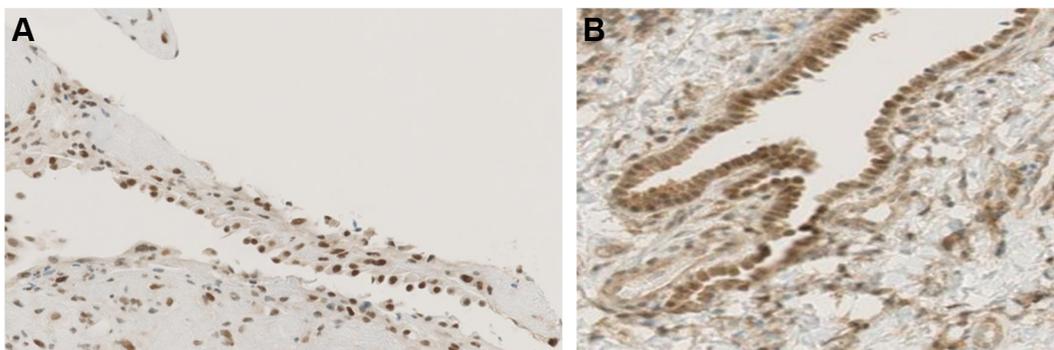
### BAP1 and MTAP status in histopathologic versus cytologic sections of mesothelioma

Loss of BAP1 expression was significantly more often observed in cytologic (n = 50 of 71, 70.4%) compared with

histopathologic specimens (n = 50 of 91, 54.9%) ( $P = 0.03$ ) (Fig. 2). There was no statistical difference in observation rate of loss of MTAP in cytologic samples (n = 31 of 71, 43.7%) compared to histopathologic sections (n = 50 of 91, 54.9%) ( $P = 0.1$ ) (Table 2, Fig. 3). Loss of expression of one or both markers was seen in 73 of 91 (80.2%) of histopathologic and in 56 of 71 (78.9%) of cytologic specimens ( $P = 0.3$ ) (Table 2).

The observation rate of loss of BAP1 (n = 50 of 91, 54.9%) and MTAP expression (n = 50 of 91, 54.9%) was not significantly different in histopathologic specimens ( $P = 0.5$ ). However, a trend of higher observation rate of loss of BAP1 (n = 50 of 71, 70.4%) compared with loss of MTAP expression (n = 31 of 71, 43.7%) was observed in cytologic samples ( $P = 0.08$ ).

Staining of histopathologic specimens showed that loss of one or both markers was observed in epithelioid mesothelioma in 34 of 44 (77.3%), in biphasic in 26 of 31 (83.9%), and in sarcomatoid mesothelioma in 13 of 16 (81.2%) without a significant difference within the subtypes ( $P = 0.8$ ). The sensitivity of loss of expression of BAP1



**Figure 1** Expression of BAP1 (A) and MTAP (B) in reactive mesothelial proliferations.

**Table 3** Prediction rate in malignant mesothelioma diagnosis of loss of expressions of BAP1 and/or MTAP in histopathologic, cytologic, and reactive mesothelial proliferations samples.

	Test outcome positive	Test outcome negative
	BAP1 and/or MTAP loss	BAP1 and/or MTAP retained
Histopathologic samples		
Condition confirmed (MM)	True positive (MM) 73/91 = 80.2%	False negative 18/91 = 19.8%
Condition not confirmed (RMP)	False positive 0/20 = 0%	True negative (RMP) 20/20 = 100%
Cytologic samples		
Condition confirmed (MM)	True positive (MM) 56/71 = 78.9%	False negative 15/71 = 21.1%
Condition not confirmed (RMP)	False positive 0/20 = 0%	True negative (RMP) 20/20 = 100%

Abbreviations: BAP1, BRCA1-associated protein; MTAP, methylthionadenosine phosphorylase; MM, malignant mesothelioma; RMP, reactive mesothelial proliferations.

and of or MTAP in mesothelioma diagnosis was comparable for the subtypes and highest for the biphasic (83.9%) and sarcomatoid (81.2%) subtype ( $P = 0.8$ ) (Table 4).

### BAP1 and MTAP agreement between cytologic and histopathologic samples from the same patient

Six patients had corresponding biopsy and cytology sample (5 with epithelioid and 1 with biphasic mesothelioma). There was a 100% concordance between the staining status, showing the same (lost or retained) expression of both BAP1 and MTAP in histopathologic and cytologic samples.

### Discussion

The study showed that loss of expression by IHC of either BAP1, MTAP, or both can be a reliable diagnostic biomarker for determining of mesothelioma diagnosis in cytology samples. Furthermore, the results represent a reliable indication of the markers' performance. Loss of BAP1 and/or MTAP differentiated mesothelioma from reactive mesothelial proliferations with a 100% specificity and sensitivity of 78.9% in effusion cytology and 80.2% in histopathologic sections and may therefore be used for the diagnosis of mesothelioma. Additionally, loss of BAP1 and/or MTAP in sarcomatoid and biphasic subtypes had a higher sensitivity than in epithelioid subtype.

The usefulness of BAP1 IHC expression in differentiating mesothelioma from reactive mesothelial proliferations in pleura effusions has been investigated by various groups. Previous studies showed sensitivity of BAP1 in effusions between 57% and 77%<sup>12,14,27,32,35-37</sup> not reaching the IMIG recommended threshold of sensitivity.

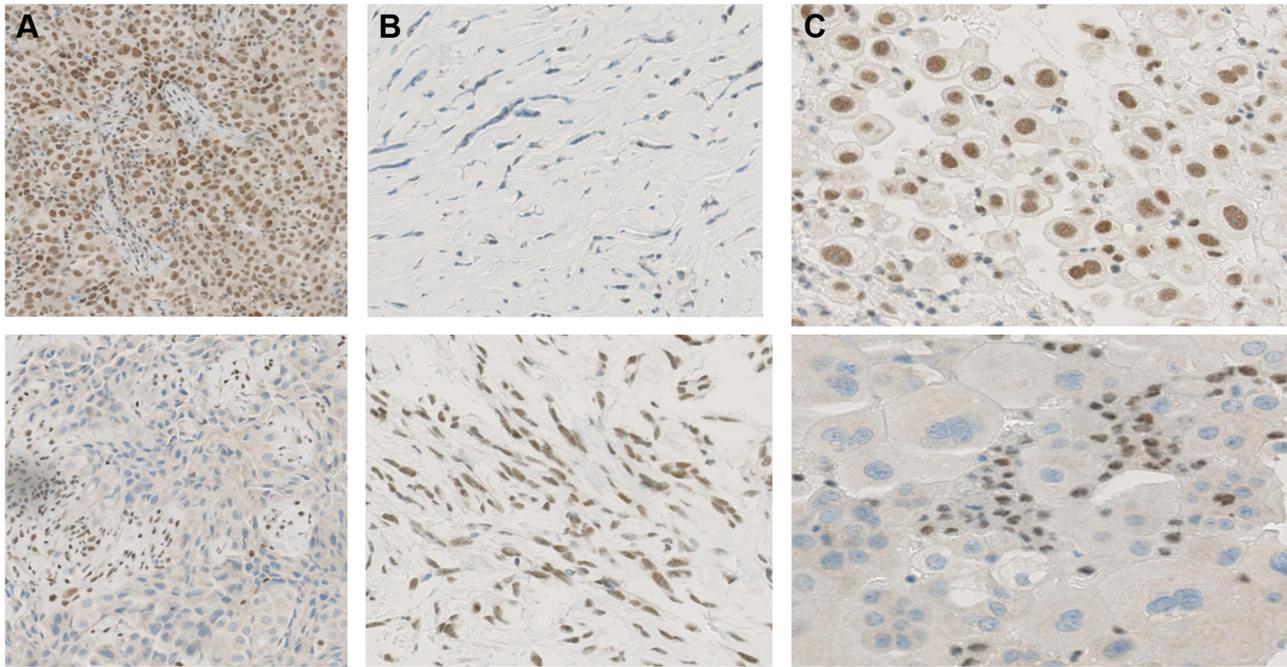
There are few reports of the utility of MTAP in effusion specimens. Zimling et al<sup>29</sup> observed a specificity of 90%

and sensitivity of 71% for MTAP IHC in a cohort of 14 mesothelioma effusions. Berg et al<sup>32</sup> reported a lower specificity of 33% regarding MTAP, in a cohort of 21 mesothelioma effusions. Finally, Kinoshita et al<sup>28</sup> observed MTAP IHC sensitivity of 42.2% in a cohort of 45 mesotheliomas. Our study shows results of relatively large cohort of 71 cytologic samples presenting a similar sensitivity of MTAP alone.

It seems that neither BAP1 nor MTAP alone can be a reliable diagnostic biomarker in mesothelioma. The only study so far, reporting a loss of one or both markers in cytologic specimens, revealed a specificity of 100% and a sensitivity of 77.8% in a group of 45 mesothelioma patients.<sup>28</sup> Our study confirms these findings in a larger cohort of 71 patients with cytologic specimens. We demonstrated similar sensitivity of 78.9% of one or both biomarkers in the cytologic specimens and comparable to the results of 80.2% in histopathologic sections. Therefore, we postulate that observation of loss of either BAP1 or MTAP in cytologic specimens could spare patients further invasive procedures given the 100% of diagnostic specificity.

Importantly, in histopathologic sections, the loss of expression of either BAP1 and/or MTAP was highly diagnostic for sarcomatoid and biphasic subtype showing sensitivity of 81.2% and 83.9%, respectively. Recent studies have shown that loss of BAP1, MTAP, or both detected by IHC in histologic sections could be reliable markers.<sup>26-28</sup> In line with these results, the current study confirms that the loss of BAP1 and/or MTAP improves the sensitivity in tissue sections compared with BAP1 or MTAP alone, or their combined loss in both. This also fulfils the internationally recommended requirement of a sensitivity of at least 80%.<sup>6</sup>

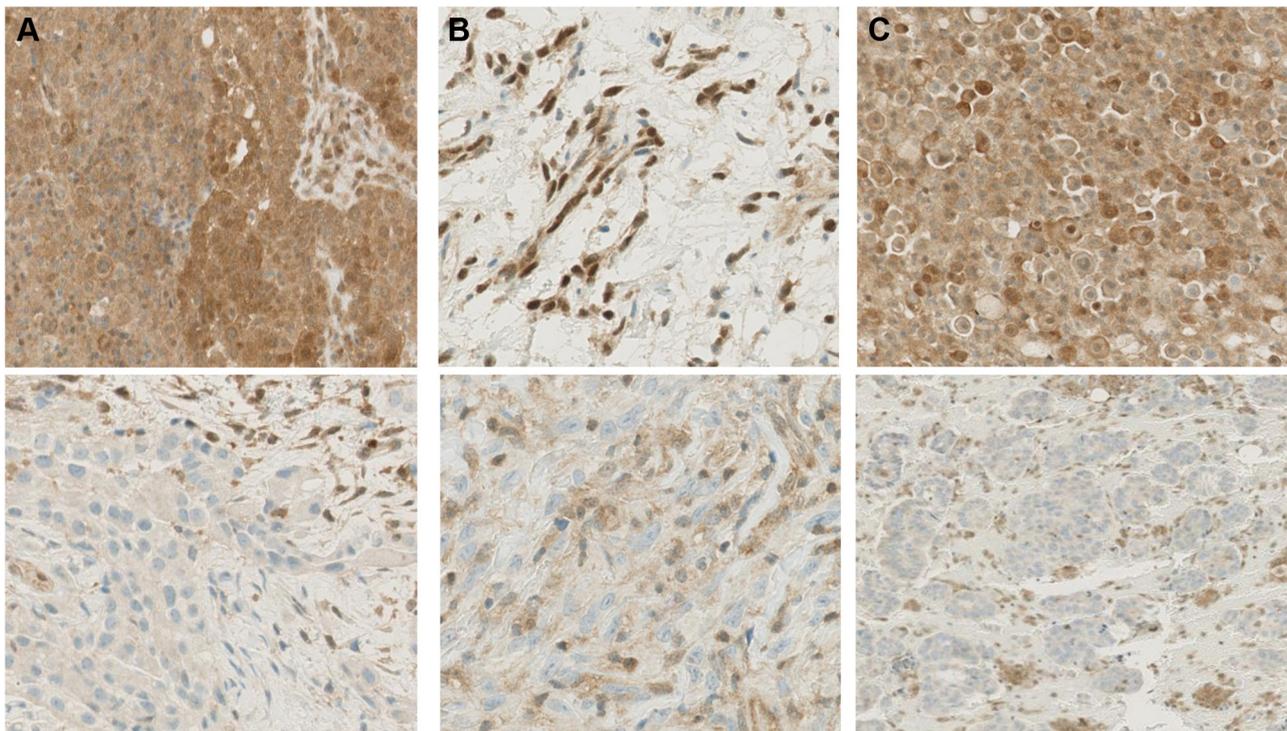
There are a few limitations of the study. Although this study is partially framed as a comparison of cytology effusion versus biopsy material, we recognize the fact that it is not completely accurate to directly compare proportions



**Figure 2** Expression of BAP1 in mesothelioma: epithelioid (A) and sarcomatoid (B) subtype in histologic sections and in cytology (C), respectively.

of IHC loss between groups of different patients. Nevertheless, although the number of the available paired material was limited, the concordance between the expression of the BAP1 and MTAP biomarkers was 100% between cytologic

and histopathologic sections. Moreover, the study should be considered as an indicative comparison. The relatively low number of patients and specimens could potentially confine the statistical analyses. However, the current study is to our



**Figure 3** Expression of MTAP in mesothelioma: epithelioid (A) and sarcomatoid (B) subtype in histologic sections and in cytology (C), respectively. In all figures, a vague tumoral cytoplasmic staining is present, but all substantially weaker than the positive internal control.

**Table 4** Observation rate of expressions of BAP1 and MTAP in histopathologic subtypes of malignant mesothelioma.

BAP1 and MTAP status, n (%)	Epithelioid (n = 44)	Biphasic (n = 31)	Sarcomatoid (n = 16)
BAP1 (–) and MTAP (–)	10 (22.7%)	13 (41.9%)	4 (25.0%)
BAP1 (–) and MTAP (+)	18 (40.9%)	4 (12.9%)	1 (6.2%)
BAP1 (+) and MTAP (–)	6 (13.6%)	9 (29.0%)	8 (50.0%)
BAP1 (+) and MTAP (+)	10 (22.7%)	5 (16.1%)	3 (18.7%)
Loss of BAP1 and/or MTAP	34/44 (77.3%)	26/31 (83.9%)	13/16 (81.2%)
BAP1 and MTAP retained	10/44 (22.7%)	5/31 (16.1%)	3/16 (18.7%)

Abbreviations: BAP1, BRCA1-associated protein; BAP1 (–), loss of BAP1 expression; BAP1 (+), expression of BAP1 retained; MTAP, methylthionadenosine phosphorylase; MTAP (–), loss of MTAP expression; MTAP (+), expression of MTAP retained.

knowledge one of the largest investigations of BAP1 and MTAP in cytologic specimens of mesothelioma patients. The 20 reactive mesothelial proliferations cases, which may be considered limited, were included for statistical reasons only. We did not consider it necessary to use more samples because no false positive case has ever been described in the literature and in our knowledge both markers demonstrate a 100% specificity. Also we can confirm the high specificity in our everyday practice and therefore it seemed redundant to include more cases.<sup>28,33</sup> The lack of p16 FISH in the validation of uncertain cases is an additional limitation. Nevertheless, previous studies have shown a very high concordance rate between FISH and MTAP,<sup>17,26,30</sup> minimizing the risk of uncertainty. The lack of sarcomatoid type mesothelioma in the cytology samples is due to its fibrous nature, which makes it almost impossible to shed cells into the pleural space. On the contrary, one should be aware of the pitfall of reactive mesothelial proliferations, often seen in pleural effusion in patients with the sarcomatoid subtype of mesothelioma. Furthermore, although the TMAs represented the tumor in the best possible way, due to tumor's heterogeneity in combination with the limited amount of tissue, the method could not fully guarantee evident representativeness of the tumor. However, the punched areas were carefully chosen and marked by an experienced pathologist from biopsies with confirmed diagnoses and tumor type. In the interpretation of the biomarkers' expression, one should be aware that a specimen's age may decrease the intensity of IHC reaction. However, this was not observed during our review and only usual variety and heterogeneity of staining was present. Some precautions should also be taken regarding the interpretation of MTAP, as it may show either nuclear and/or cytoplasmic reaction, with a broad spectrum of intensity, ranging between complete absent to strong. Therefore, it may be challenging for a less-experienced pathologist, especially when a fade or more intense background staining is present. Although our laboratory has a high level of IHC quality control, in some cases, regardless of the age of the specimen, a rather weak but present reaction was observed, significantly weaker than the control. Thus, loss of MTAP expression was defined in

concordance with other authors,<sup>17,26,29,31,32</sup> as the complete loss or a weaker expression than the controls (internal and external), and the only pattern considered was the cytoplasmic.<sup>33,34</sup> In contrast to MTAP, the interpretation of the BAP1 loss of expression is usually easier to the eye of the pathologist, mainly because a nuclear staining pattern as the BAP1 immunostaining is more distinct than a cytoplasmic one.

In conclusion, loss of expression of either BAP1 and/or MTAP IHC as biomarkers for differentiating mesothelioma from reactive mesothelial proliferations in cytology samples is as reliable method as in histopathologic sections, showing a specificity of 100%. However, retained expression of both BAP1 and MTAP in serous effusions should not be used to exclude mesothelioma diagnosis, due to its lower sensitivity, and the fact that the sarcomatoid subtype rarely sheds cells in the pleural space. Additionally, the loss of either BAP1 and/or MTAP is highly diagnostic for the biphasic and sarcomatoid subtype in histopathologic sections.

## Declarations of interest

None.

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## Authors contribution

Louise A. Lynggård: Data curation, formal analysis, investigation, methodology, writing—original draft, and writing-review and editing. Vasiliki Panou: Data curation, review and editing. Weronika M. Szejniuk: Data review, data analyses, writing-review and editing. Oluf Dimitri Røe:

Funding, methodology, writing-review and editing. Christos Meristoudis: Methodology, investigation, data curation, project administration, data curation, formal analysis, writing-review and editing. All authors have read and approved the final manuscript.

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