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RESEARCH ARTICLE

Immunohistochemical Study of 5-Methylthioadenosine Phosphorylase (MTAP) and Epithelial Membrane Antigen (EMA) in Differentiating Epithelioid Pleural Mesothelioma from Reactive Mesothelial Hyperplasia

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ABSTRACT

Introduction: The differentiation of mesothelioma from reactive mesothelial hyperplasia can be challenging, especially in effusion cytology or when tissue biopsies are not feasible. So immunohistochemical studies are considered substantial tools for establishing the appropriate diagnosis. **Aim**: The current study aimed to evaluate the immunohistochemical expression of MTAP as well as EMA in epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH) in both cell block preparations and tissue specimens, and to compare the diagnostic utility of MTAP and EMA in EPM. **Material and methods**: After the confirmation of the mesothelial lineage, immunohistochemical expression of MTAP and EMA antibodies in both tissue biopsies and cell blocks was evaluated. The samples were obtained from 30 cases of EPM and 30 cases of RMH. **Results**: MTAP differentiated EPM from RMH with 63.3% sensitivity (64.7% for tissue biopsies and 61.5% for cell blocks) and 100% specificity (100% for both tissue biopsies and cell blocks); the optimal cut-off value for MTAP expression that could best distinguish EPM from RMH was 52.5%. On the other hand, EMA differentiated EPM from RMH with 93.3% sensitivity (88.2% for tissue biopsies and 100% for cell blocks) and 66.7% specificity (58.3% for tissue biopsies and 72.2% for cell blocks). **Conclusion**: MTAP is a highly specific marker for distinguishing EPM from RMH, whereas EMA showed significant sensitivity for the differentiation of EPM and RMH. Moreover, cell block preparations could be a reliable surrogate for tissue biopsies to differentiate mesothelial lesions.

Keywords: Diagnosis, Immunohistochemistry, Mesothelial lesions

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INTRODUCTION

Mesothelioma is a highly fatal and aggressive tumor of serosal surfaces and is closely attributable to prior asbestos exposure. Its incidence has globally increased during the past few decades (Schürch et al., 2017). A spectrum of hyperplastic and neoplastic mesothelial lesions arises from the serosal linings. Mesothelial neoplasms range from benign localized tumors to aggressive diffuse malignancies that can destructively infiltrate surrounding tissues and can metastasize distally as well. The distinction between benign and malignant mesothelial proliferations is critical to patient care and prognosis, but is often morphologically challenging. Hence, different ancillary techniques, mainly immunohistochemistry, may be essential in such cases (Churg et al., 2016). A variety of immunohistochemical markers have been claimed to be beneficial in this context, yet have been reported with contradictory results in multiple studies. 5-Methylthioadenosine phosphorylase (MTAP) is a key enzyme in the salvage pathway of methionine amino acid. It is frequently deleted in human cancers because of its chromosomal juxtaposition to the tumor suppressor gene CDKN2A. CDKN2A is located on 9p21 chromosomal region, encoding the cell-cycle inhibitor $p16^{CDKN2A}$. It is a well-known tumor suppressor gene that has been found to exhibit somatic mutations in different cancers (Mavrakis et al., 2016).

Multiple studies had described that the lost immunohistochemical expression for the protein product of the 5-methylthioadenosine phosphorylase (MTAP) gene, was correlated with the deletion status of 9p21 by FISH technique in pleural mesothelioma (Kinoshita et al., 2018a); implying that immunohistochemical loss of MTAP expression can act as a potential surrogate for detection of homozygous deletion of CDKN2A (Hiroshima et al., 2021). MTAP is one of the new generation markers and its loss is claimed to be a highly specific marker of malignancy in mesothelial lesions compared with conventional markers, and it attains an acceptable diagnostic utility, with a considerable sensitivity (Chapel) et al*.,* 2020b).

Epithelial membrane antigen (EMA), *a high molecular weight transmembrane glycoprotein,* is a member of mucin family, including O-glycosylated proteins. It plays a major role in the formation of protective mucous barriers on epithelial surfaces as well as in intracellular signaling (Bruno et al., 2018). EMA is considered among the most valuable and widely used markers for distinguishing benign from malignant mesothelial effusion, with relatively good sensitivity and specificity among various reports (Lin et al., 2016). Yet, some authors claim that EMA is not a solely reliable marker for distinguishing atypical mesothelial proliferation from overt malignancy (Bruno et al., 2018) Thus, it should be used as a member of a panel for the diagnosis of individual cases of mesothelioma (Husain et al., 2018).

The current work aimed to evaluate immunohistochemical expression of 5-Methylthioadenosine Phosphorylase (MTAP) as well as Epithelial Membrane Antigen (EMA) in epithelioid pleural mesothelioma and reactive mesothelial hyperplasia in both cell block preparations and tissue specimens and to compare the diagnostic utility of both markers in mesothelioma.

MATERIAL AND METHODS

Included cases

The current study included 60 formalin-fixed paraffin-embedded diagnostic specimens which were 30 specimens of pleural mesothelioma: 17 tissue biopsies and 13 cell blocks prepared from pleural effusion.

All of the 30 specimens were obtained from histopathologically and/or immunohisto-chemically confirmed cases of mesothelioma, all of pleural origin and epithelioid subtype; epithelioid pleural mesothelioma (EPM). The control group consisted of 30 specimens of reactive mesothelial hyperplasia: 12 tissue biopsies (decortication biopsies) and 18 cell blocks prepared from pleural effusion. All of the 30 specimens were obtained from histopathologically and/or immunohisto-chemically confirmed cases of pleural reactive mesothelial hyperplasia (RMH). Cases included pleural specimens obtained from cases diagnosed with cardiovascular diseases, pneumonia, lung malignancies, and traumatic chest injuries.

Inclusion criteria

- Histopathologically and/or immunohistochemically confirmed cases of epithelioid pleural mesothelioma (EPM).
- Histopathologically and/or immunohistochemically confirmed cases of pleural reactive mesothelial hyperplasia (RMH).
- Cases with complete clinico-pathological data regarding age, sex, and associated mesothelial lesion.
- Diagnostic specimens of good quality of the paraffin block and sufficient tissue for immunostaining.

Exclusion criteria

- Histopathologically and/or immunohisto-chemically confirmed cases of metastatic adenocarcinoma to the pleura.
- Cases with incomplete history and clinico-pathological data.
- Diagnostic specimens with poor quality of the paraffin block or insufficient tissue for immunostaining.

The design of the current work was a case-control selection type of cross-sectional study. The study was approved by the research ethics committee of Tanta University (Approval code: 34391/1/21). Specimens were collected retrospectively from Pathology Department, Faculty of Medicine, Tanta University, and from some private laboratories during the period from February 2021 till April 2022.

Methods

The paraffin blocks were re-sectioned and stained by Hematoxylin and Eosin for routine examination and confirmation of the diagnosis. The mesothelial origin of each specimen was confirmed using Immunohistochemical assays. Calretinin was identified as a positive mesothelial marker, whereas TTF-1 and CEA were identified as negative mesothelial markers (Husain et al., 2013). Histological mesothelioma diagnosis and classification were established following the World Health Organization guidelines and classification (2021).

Immunohistochemistry

MTAP and EMA immunohistochemical staining were performed on formalin-fixed paraffin-embedded sections, which were cut at 3 µm, then collected on positively charged slides. Slides were transferred to the Autostainer Link 48 instrument (Dako, Agilent Technologies Inc, Santa Clara, USA). The autostainer applies the polymer chain two-step indirect technique for staining.

Primary antibodies

MTAP: rabbit IgG polyclonal antibodies; ABclonal, Massachusetts, USA, with dilution 1:100. EMA: rabbit IgG polyclonal antibodies; ABclonal, Massachusetts, USA, with dilution 1:100. Primary antibodies were used after heat-induced epitope retrieval, employing High pH EnVision™ FLEX Target Retrieval Solution (Dako, Agilent 99 Technologies Inc, Santa Clara, USA) at pH 9.0 at 95ºC for 30 minutes, and were blocked and visualized using The Dako EnVision™ FLEX Detection system (Kinoshita$_{(a)}$ et al., 2018).

Secondary antibodies

Polyclonal Rabbit Anti-FITC/HRP, Rabbit F(Ab'), Blot/ISH, solid-phase absorbed and affinity-isolated secondary antibody conjugated with (horseradish peroxidase) HRP, 0.5 mL. For MTAP, sections from human tonsils served as a positive control (Zimling et al., 2012). For EMA, a section of colorectal adenocarcinoma served as a positive control (Cho et al., 2009). MTAP immunohistochemical expression was defined as lost/decreased when the intensity of cytoplasmic staining is lower than that of the cytoplasmic staining of the internal positive control. Nuclear staining was neglected (Yoshimura et al., 2019). Non-mesothelial immunoreactive inflammatory cells, including histiocytes, lymphocytes as well as fibroblasts and endothelial cells served as an internal positive control. For each specimen, at least 500 mesothelial cells were evaluated (Kinoshita et al., 2018a). The optimal diagnostic cut-off points to distinguish EPM from RMH, representing the percentage of MTAP-lost/decreased expression in tumor cells, was calculated using the Receiver operator characteristic (ROC) curve.

EMA immunohistochemical expression was defined as positive when demonstrating brownish cytoplasmic staining that often-displayed membranous accentuation. The scoring of EMA was graded on a semi-quantitative basis using the percentage and the intensity of the stained cells; percentage scores (0: no stained cells (0), 1: less than 10% of cells were stained, 2: 10%-50% of cells were stained, 3: more than 50% of cells were stained), and Intensity score: (0: no staining, 1: mild staining, 2: moderate staining, 3: intense staining). The final scoring of the marker was calculated by adding percentage and intensity score; cases with a final score equal to or more than 4 were identified as positive (Nautiyal et al., 2017).

Statistical analysis

Qualitative data were described using numbers and percentages. Quantitative data were described using range (minimum and maximum), mean, median, and standard deviation (SD). Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV), and accuracy were used to assess the diagnostic utility of the two tested markers with the histopathological evaluation considered the gold standard. EPM cases with lost/decreased cytoplasmic MTAP expression are defined as true positive, whereas RMH cases with retained cytoplasmic MTAP expression are defined as true negative. Chi-square (X^2) test was used for categorical variables, to compare different groups, and Fisher's exact test was employed for the correction of chi-square. The receiver operator characteristic (ROC) curve was used in conjunction with Youden's (*J*) index to select the optimal cut-off point for MTAP by assessing the diagnostic values of different

percentages of MTAP expression. The area under the curve (AUC) is calculated through the ROC curve analysis and measures the diagnostic test's accuracy. The significance of the obtained results was judged at the 5% level ($p<0.05$). Statistical analysis of the data was done using the statistical package for the social sciences (SPSS) software version 23.0.

RESULTS

Clinico-pathological data

Regarding EPM cases, ages ranged between 60 and 83 years, with mean age 70.7. Considering the 30 cases diagnosed with RMH, ages ranged between 45 and 75, with mean age 56.07; a statistically significant difference was found between the two study groups EPM and RMH (p-value=0.00). The included tissue biopsies from cases diagnosed with EPM included the following architectural patterns/cytological features: 9 cases (53%) of tubulopapillary EPM, 4 cases (23.5%) of solid EPM. 1 case (5.9%) of clear cell EPM, and 3 cases of mixed tubulopapillary/micropapillary EPM (17.6%).

Immunohistochemical results

Selection of the cut-off value for MTAP expression: ROC curve analysis was performed to identify the optimal cut-off value for MTAP expression that could best distinguish EPM from RMH. The optimal cut-off point was generated by SPSS software (Graph 1) and was set at 52.5%. At this point, Youden's index showed the maximum value (J= 0.633). Accordingly, cases were diagnosed as EPM if equal to or greater than 52.5% of the tumor showed loss/decreased cytoplasmic staining of MTAP (nuclear staining is neglected) (Table 1).

MTAP immunohistochemical expression: MTAP showed lost/decreased cytoplasmic staining in 19 cases (63.3%) out of the 30 cases of EPM, which included 11 tissue biopsies (Figures 1A and 1B) and 8 cell blocks (Figure 2A). The remaining 11 cases (36.7%) retained the MTAP expression (Table 2). On the contrary, all RMH cases (100%) retained the MTAP expression (Figures 1E and 2B) (Tables 2).

Table 2 shows that there was no statistically significant difference in MTAP expression as regard the type of the specimens (tissue biopsies or cell blocks) obtained in EPM or RMH (P1= 0.858 and P2= 0.4).

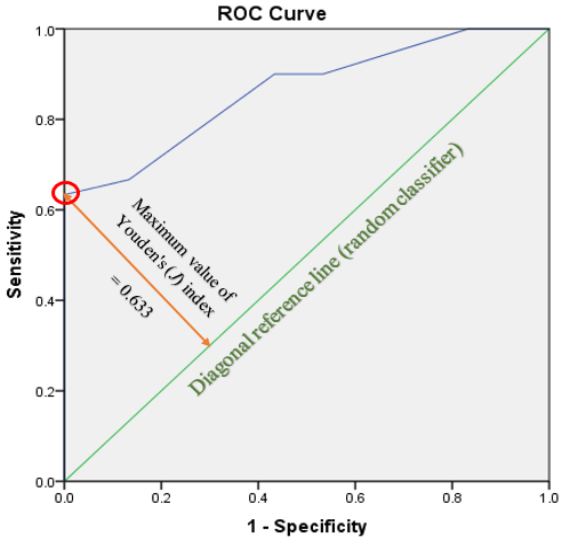
EMA immunohistochemical expression: Among the 30 EPM cases, EMA showed positive staining in 28 cases (93.3%); 15 tissue biopsies (Figures 1C and 1D) and 13 cell blocks (Figure 2C). Meanwhile, the remaining 2 cases (6.7%) which were in the form of tissue biopsies only, showed negative staining for EMA (Table 3). Regarding RMH cases, 10 cases (33.3%) including 5 tissue biopsies and 5 cell blocks showed positive staining for EMA. Meanwhile, the remaining cases (66.7%), including 7 tissue biopsies (Figure 1F) and 13 cell blocks (Figure 2D) did not show an expression for EMA (Table 3). Table 3 shows that there was no statistically significant difference in EMA expression as regard the type of the specimens (tissue biopsies or cell block) obtained in EPM or RMH (P1= 0.492 and P2= 0.461).

Table 4 illustrates the diagnostic utility of MTAP and EMA in differentiation between epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH) in both tissue biopsies and cell blocks.

DISCUSSION

The key feature for the diagnosis of mesothelioma is the demonstration of either stromal or sub-serosal fat invasion by malignant mesothelial cells. However, not all cases are illegible for tissue biopsy to establish such a diagnosis. Besides, serosal effusion is the earliest and most common clinical sign reported in many patients (Kinoshita et al., 2018a).

The current study was carried out on 60 cases of mesothelial lesions, which were divided equally into two groups: epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH). Thirty cases of epithelioid pleural mesothelioma were included; many publications related to the current work included a variable number of cases, up to 99 as in Chapel et al. (2020a). Such studies included either only cell blocks (Kinoshita et al., 2018a; Berg et al., 2020), or only tissue biopsies (Yoshimura et al., 2019), or both (Chapel et al., 2020a).



Graph 1. ROC curve analysis for evaluating the diagnostic value of MTAP in distinguishing epithelioid pleural mesothelioma (EPM) from reactive mesothelial hyperplasia (RMH). The red circle highlights the optimal cut-off point according to Youden's index.

Table 1. Coordinates of the ROC curve for calculation of MTAP cut-off point

*Cut-off points (%)	Sensitivity	1 – Specificity
19.0000	1.000	1.000
22.5000	1.000	.867
27.5000	1.000	.833
32.5000	.900	.533
37.5000	.900	.433
42.5000	.667	.133
52.5000	**.633**	**.000**
65.0000	.467	.000
75.0000	.133	.000
81.0000	.000	.000

*Cases were diagnosed as EPM if greater than or equal to (…%) of the tumour showed lost/decreased cytoplasmic staining of MTAP (Nuclear staining is neglected)

The current study incorporated both tissue biopsies and cell blocks to be more comprehensive, covering the cytological and histological aspects of mesothelial lesions.

The current study included only mesothelioma of pleural origin. Mesothelioma of peritoneal, pericardial, and para-testicular origin were not included in the current study owing to their rarity. Regarding the age of the two studied groups, the mean age of patients diagnosed with epithelioid pleural mesothelioma was about 70.7 years. This was close to the mean age in the study of Kinoshita et al. (2018a), which was 69.7 years. However, it was slightly less than the mean ages in the work of Hida et al. (2017) and Yoshimura et al. (2019) which were 63.8 years and 65.3 years, respectively. These findings are consistent with the fact that pleural mesothelioma is typically a disease of the elderly, with a median age at presentation is 74 years for pleural mesothelioma (Thomas et al., 2015). However, the current study revealed that the mean age for the diagnosis of reactive mesothelial hyperplasia was about 56.07 years. This was found to be much lower than the mean age of the cases included in the study of Kinoshita et al. (2018b) which was 70.5 years, and conversely, higher than the mean ages of the cases included in the work of Hida et al. (2017) and Yoshimura et al. (2019) which were 34.4 years, and 32.5 years, respectively. Reactive pleural mesothelial hyperplasia can be associated with many pathological conditions, which could be seen in all age groups, justifying the variability in the mean ages among publications.

The current study found a statistically significant difference in the mean ages between the two study groups (EPM and RMH). Variability in the mean age between the two studied groups could be explained as follows: the reactive mesothelial hyperplasia was associated with inflammatory, cardiovascular and traumatic conditions, which can be encountered in young and old ages as well. Meanwhile, pleural mesothelioma is typically diagnosed in the elderly (Thomas et al., 2015).

Malignant mesothelial cells may not exhibit obvious morphological features of malignancy, and occasionally, the evident histological pattern of invasion may not be apparent, particularly in small tissue biopsies. On the other hand, benign proliferating mesothelial cells may demonstrate atypical morphological and sometimes deceiving architectural features of malignancy. Thus, the differentiation of mesothelioma from reactive mesothelial proliferation can be challenging in small tissue biopsies and even more in cytological specimens (Berg et al., 2020). Thus, ancillary techniques are of great importance for discrimination between malignant and benign mesothelial growth, especially when there are no overt malignant criteria or when small tissue biopsies or cytological specimen is the only available specimens (Davidson et al., 2018).

Table 2. The distribution of MTAP expression status among tissue biopsies and cell blocks in cases of epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH)

Diagnosis			MTAP expression		Total	Statistical test value	P-value
			Retained	Lost/decreased			
EPM N=30	Type of Specimen	Tissue biopsies N=17 100%	6 35.3%	11 64.7%	17 100 %	X^2= 0.032	0.858 ns
		Cell blocks N=13 100%	5 38.5%	8 61.5%	13 100%		
RMH N=30	Type of Specimen	Tissue biopsies N=12 100%	12 100%	0 0.%	12 100%	Fisher Exact Test=1.885	0.4 ns
		Cell blocks N=18 100%	18 100%	0 0%	18 100%		

ns: non-significant difference. EPM: epithelioid pleural mesothelioma, RMH: reactive mesothelial hyperplasia, N: number

Table 3. The distribution of EMA expression status among tissue biopsies and cell blocks in cases of epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH)

Diagnosis			EMA expression		Total	Fisher Exact Test	P-value
			Negative	Positive			
EPM N=30	Type of Specimen	Tissue biopsies N=17 100%	2 11.8%	15 88.2%	17 100%	2.381	0.492 ns
		Cell blocks N=13 100%	0 0%	13 100%	13 100%		
RMH N=30	Type of Specimen	Tissue biopsies N=12 100%	7 58.3%	5 41.7%	12 100%	0.62	0.461 ns
		Cell blocks N=18 100%	13 72.2%	5 27.8%	18 100%		

ns: non-significant difference, EPM: epithelioid pleural mesothelioma, RMH: reactive mesothelial hyperplasia, N: number

Table 4. Comparison of the diagnostic utility of MTAP and EMA in differentiation between epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH) in both tissue biopsies and cell blocks

MTAP	**Sensitivity 63.3%**	**Specificity 100%**	**PPV 100%**	**NPV 73.2%**	**Accuracy 81.7%**
Tissue biopsies	64.7%	100%	100%	66.7%	79.3%
Cell blocks	61.5%	100%	100%	78.3%	83.9%
EMA	**Sensitivity 93.3%**	**Specificity 66.7%**	**PPV 73.7%**	**NPV 90.9%**	**Accuracy 80%**
Tissue biopsies	88.2%	58.3%	75%	77.8%	75.9%
Cell blocks	100%	72.2%	72.2%	100%	83.9%

PPV: positive predictive value, NPV: negative predictive value

Numerous immunohistochemical markers have been suggested for distinguishing malignant mesothelial cells. While the early generation of these markers has revealed considerable sensitivity, yet unsatisfactory specificity, most recent studies do not recommend these markers for routine diagnostic use and emphasize the significance of marker's specificity, taking into account the implications of the grave diagnosis of mesothelioma (Chapel et al., 2020b). It should be asserted that all of the previously discussed studies evaluating the MTAP expression were conducted on cases diagnosed with mesothelioma of pleural origin only.

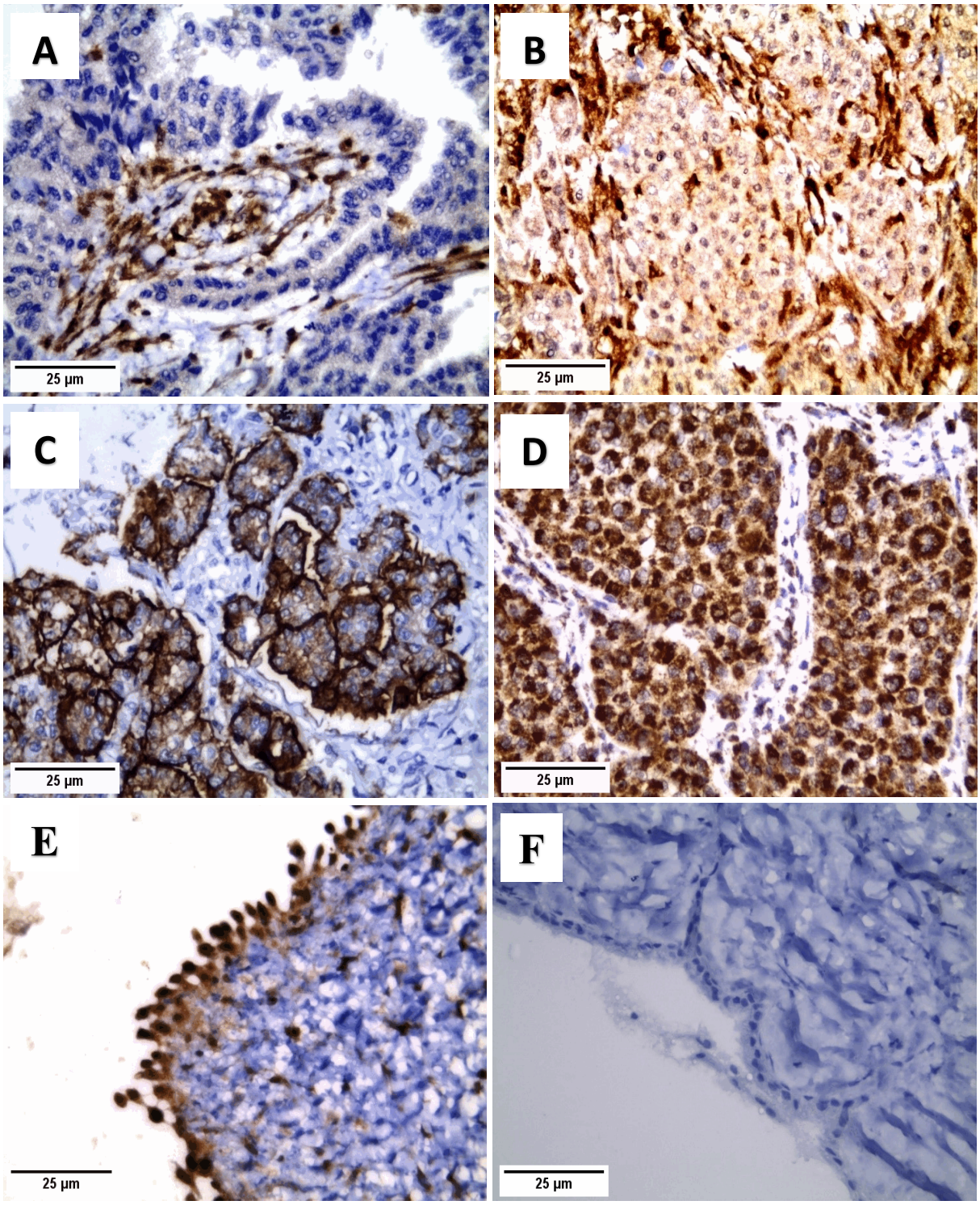


Figure 1. Immunohistochemical expression of MTAP and EMA antibodies in tissue biopsies in cases of epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH): (A) A case of EPM with tubulopapillary architecture showing loss of MTAP expression. Note the MTAP-positive endothelial cells and fibroblasts (x400). (B) A case of EPM with solid architecture showing decreased cytoplasmic expression of MTAP with intensity of staining less than that of the internal positive control. Note the MTAP-positive endothelial cells and fibroblasts (x400). (C) A case of EPM with mixed tubulopapillary/micropapillary architecture showing positive cytoplasmic staining for EMA with membranous accentuation (x400). (D) A case of EPM with solid architecture showing positive cytoplasmic staining for EMA with membranous accentuation (x400). (E) A case of RMH showing mesothelial cells with retained cytoplasmic staining of MTAP (nuclear staining is neglected) (x400). (F) A case of RMH showing negative staining for EMA (x400).

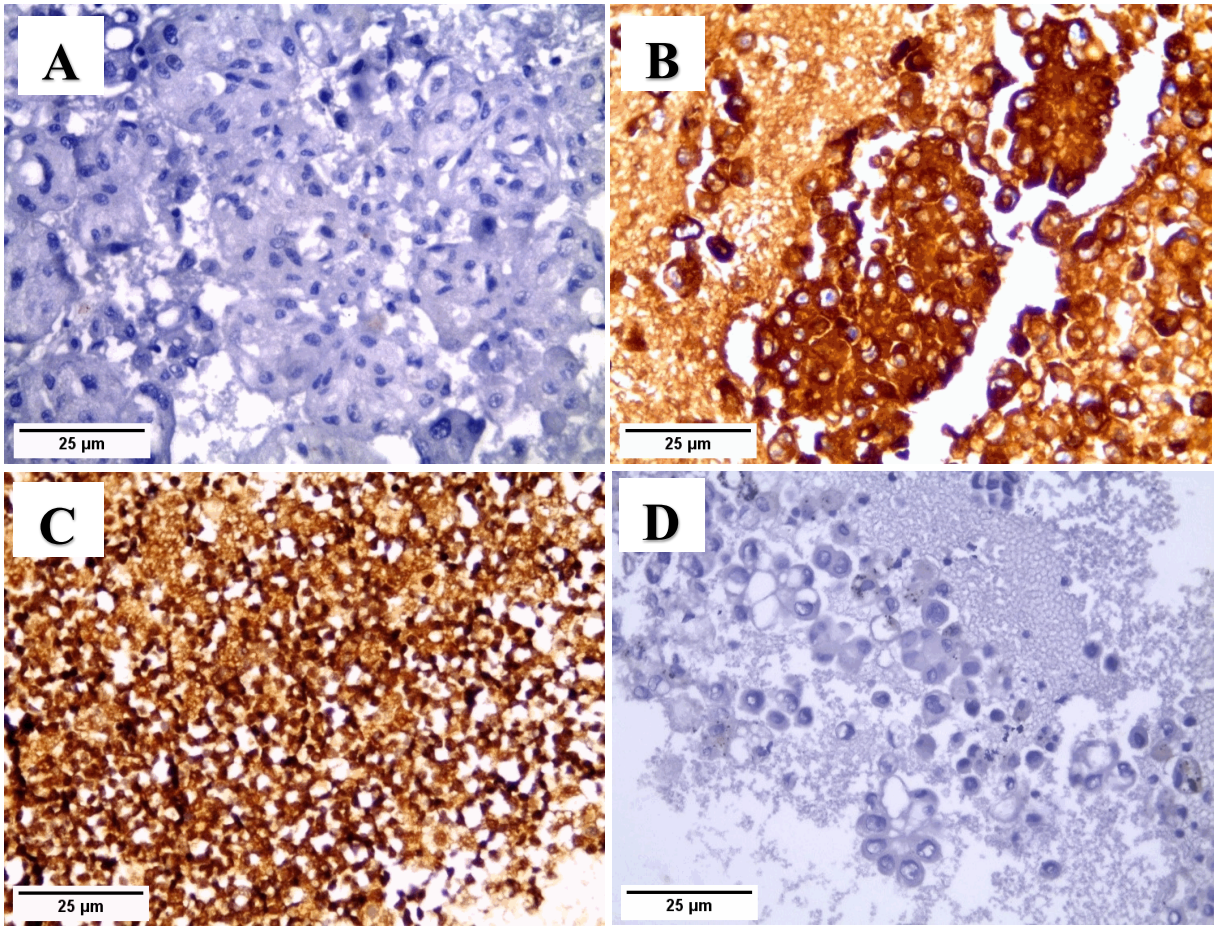


Figure 2. Immunohistochemical expression of MTAP and EMA antibodies in cell blocks in cases of epithelioid pleural mesothelioma (EPM) and reactive mesothelial hyperplasia (RMH): (A) A case of EPM showing loss of MTAP expression (x400). (B) A case of EPM showing positive staining for EMA with membranous accentuation (x400). (C) A case of RMH showing retained cytoplasmic staining of MTAP (nuclear staining is neglected) (x400). (D) A case of RMH showing negative staining for EMA (x400).

To the best of our knowledge, there are no published reports regarding immunohisto-chemical expression of MTAP in peritoneal mesothelioma. However, Chapel et al., (2020b) presume that the number of lesions exhibiting MTAP loss would be low since CDKN2A homozygous deletion in peritoneal mesothelioma is a relatively rare phenomenon. Thus, the current work focused on analyzing the expression status of MTAP in mesothelioma of pleural origin only.

Hamasaki et al. (2019) concluded that the loss of cytoplasmic MTAP expression correlates best with CDKN2A homozygous deletion, unlike the loss of nuclear staining. Thus, the cytoplasmic MTAP expression is a more reliable and consistent parameter for the assessment of MTAP immunoreactivity of examined specimens and is easier as well to evaluate for diagnostic purposes (Berg et al., 2018). The calculated cut-off point in the current study for distinguishing EPM from RMH cases was 52.5%. This cut-off point was very close to the one adopted by Hida et al. (2017), Kinoshita et al. (2018a) and Yoshimura et al. (2019), which was 50%. The previously mentioned studies reported that the percentage of MTAP-positive cells exhibited a bimodal distribution. Accordingly, a simplified cut-off point like 50% would be of a practical advantage.

In the current study, MTAP sensitivity was equal to 63.3% (64.7% for tissue specimens and 61.5% for cell blocks). This result was higher than those obtained by Yoshimura et al., (2019) who reported a sensitivity of 47.4%. Hida et al., (2017) recorded a sensitivity of 45.1% but argued that the employment of a ROC-based cut-off value in their work may be the

underlying cause of decreased sensitivity, compared with the result obtained by Zimling et al., (2012) who recorded a sensitivity as high as 71% when applying a semi-quantitative H score. Meanwhile, the current study applied the ROC curve-based method for the calculation of the optimal cut-off point. The higher sensitivity recorded by Zimling et al. (2012) compared with the present work could be explained by the difference in the methods used for the determination of the optimal cut-off point. Moreover, Berg et al. (2020) obtained much lower sensitivity equal to 33%, which could be attributed to the application of a higher cut-off point equal to 75%. In the work of Kinoshita et al. (2018b) and Terra et al. (2022), who conducted their studies on sarcomatoid mesothelioma cases only, the sensitivity was 80% and 61%, respectively.

Regarding the specificity of MTAP, the current study revealed that all of the 30 cases diagnosed with reactive mesothelial hyperplasia retained the cytoplasmic MTAP staining, rendering that lost immunohistochemical expression for MTAP represents a 100% specific marker for diagnosis of mesothelioma. This result is identical to those obtained by Hida et al. (2017), Kinoshita et al. (2018a), Yoshimura et al. (2019) and Berg et al. (2020). 100% specificity was reported as well in the study of Kinoshita$_{(b)}$ et al. (2018). Only the work of Zimling et al. (2012) recorded lower specificity equal to 90%. To summarize, the reported sensitivity of MTAP for the detection of pleural mesothelioma showed variability among different publications, ranging from 33% to 80%. Inversely, most recent reports stated that MTAP has 100% specificity.

One of the well-known early-generation markers is EMA, which has been reported to be one of the most reliable markers in the differential diagnosis of mesothelioma and reactive mesothelial proliferation with considerably high sensitivity. Nevertheless, it has been recommended to be a member of the panel to achieve significant diagnostic advantage (Sato et al., 2010).

In the current study, EMA sensitivity was 93.3% (88.2% for tissue specimens and 100% for cell blocks). This result is near to what was obtained by Sato et al. (2010), who recorded sensitivity of 95%. Many studies stated that EMA is an excellent negative marker for the diagnosis of mesothelioma with a 100 % sensitivity for the detection of malignant cells (Hasteh et al., 2010; Ikeda et al., 2011). Meanwhile, other publications recorded lower values as Shen et al. (2009) and Minato et al. (2014) who reported sensitivity of 86% and 79%, respectively.

However, EMA showed a specificity equal to 66.7% (58.3% for tissue specimens and 72.2% for cell blocks). This result came much lower than those obtained by King et al. (2006) and Hasteh et al. (2010), who reported a specificity of 89% and 91%, respectively, as well as Sato et al. (2010) and Minato et al. (2014) who both reported specificity of 88%. This notable difference between the specificity recorded by the present study and the other publications may be explained by the variation in methods applied for the interpretation of EMA expression. Studies that support or argue the specificity of EMA immunoreactivity for mesothelioma continue to be published.

However, many studies define EMA as a reliable sensitive marker (Churg et al., 2016). Within each of the two study groups (EPM and RMH), no statistically significant difference was found in the expression of both MTAP and EMA between tissue biopsies and cell blocks, suggesting that cell block preparations could be a fairly reliable surrogate for tissue biopsies to distinguish EPM from RMH.

CONCLUSION

MTAP could be considered a reliable and highly specific marker for distinguishing EPM from RMH. However, its sensitivity is considered low compared with that of EMA. The diagnostic value of MTAP is similar in both cytological and histological preparations, and the same is true regarding EMA, signifying that cell block preparations are a reliable surrogate for tissue biopsies to distinguish EPM from RMH.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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No fund was received for this work.

AUTHOR CONTRIBUTION

All authors contributed equally and approved the manuscript.

REFERENCES

Berg KB, Churg AM, Cheung S, Dacic S (2020). Usefulness of methylthioadenosine phosphorylase and BRCA-associated protein 1 immunohistochemistry in the diagnosis of mesothelioma in effusion cytology specimens. Cancer Cytopathology, 128(2):126–132.

Berg KB, Dacic S, Miller C, Cheung S, Churg A (2018). Utility of Methylthioadenosine Phosphorylase Compared with BAP1 Immunohistochemistry, and CDKN2A and NF2 Fluorescence In Situ Hybridization in Separating Reactive Mesothelial Proliferations From Epithelioid Malignant Mesotheliomas. Archives of Pathology & Laboratory Medicine, 142(12):1549–1553.

Bruno R, Alì G and Fontanini G (2018). Molecular markers and new diagnostic methods to differentiate malignant from benign mesothelial pleural proliferations: a literature review. Journal of Thoracic Disease, 10(2):S342–S352.

Chapel DB, Schulte JJ, Berg K, Churg A, Dacic S, Fitzpatrick C, Galateau-Salle F, Hiroshima K, Krausz T, Le Stang N, McGregor S, Nabeshima K, Husain AN. (2020a). MTAP immunohistochemistry is an accurate and reproducible surrogate for CDKN2A fluorescence in situ hybridization in diagnosis of malignant pleural mesothelioma. Modern Pathology, 33(2):245–254.

Chapel DB, Schulte JJ, Husain AN, Krausz T (2020b). Application of immunohistochemistry in diagnosis and management of malignant mesothelioma. Translational Lung Cancer Research, 9(1):S3–S27.

Cho HY, Lee M, Takei H, Dancer J, Ro JY, Zhai QJ (2009). Immunohistochemical Comparison of Chordoma With Chondrosarcoma, Myxopapillary Ependymoma, and Chordoid Meningioma. Applied Immunohistochemistry & Molecular Morphology, 17(2):131–138.

Churg A, Sheffield BS, Galateau-Salle F (2016). New Markers for Separating Benign From Malignant Mesothelial Proliferations: Are We There Yet?. Archives of Pathology & Laboratory Medicine, 140(4):318–321.

Davidson B, Tötsch M, Wohlschlaeger J, Hager T, Pinamonti M (2018). The diagnostic role of BAP1 in serous effusions. Human Pathology, 79:122–126.

Hamasaki M, Kinoshita Y, Yoshimura M, Matsumoto S, Kamei T, Hiroshima K, Sato A, Tsujimura T, Kawahara K, Nabeshima K (2019). Cytoplasmic MTAP expression loss detected by immunohistochemistry correlates with 9p21 homozygous deletion detected by FISH in pleural effusion cytology of mesothelioma. Histopathology, 75(1):153–155.

Hasteh F, Lin GY, Weidner N, Michael CW (2010). The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. Cancer Cytopathology, 118(2): 90–96.

Hida T, Hamasaki M, Matsumoto S, Sato A, Tsujimura T, Kawahara K, Iwasaki A, Okamoto T, Oda Y, Honda H, Nabeshima K (2017). Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: Comparison with 9p21 FISH and BAP1 immunohistochemistry. Lung Cancer, 104:98–105.

Hiroshima K, Wu D, Hamakawa S, Tsuruoka S, Ozaki D, Orikasa H, Hasegawa M, Koh E, Sekine Y, Yonemori Y, Nabeshima K, Tsuji S, Miyagi Y, Imai K (2021). HEG1, BAP1, and MTAP are useful in cytologic diagnosis of malignant mesothelioma with effusion. Diagnostic Cytopathology, 49(5):622–632.

Husain AN, Colby T, Ordonez N, Krausz T, Attanoos R, Beasley MB, Borczuk AC, Butnor K, Cagle PT, Chirieac LR, Churg A, Dacic S, Fraire A, Galateau-Salle F, Gibbs A, Gown A, Hammar S, Litzky L, Marchevsky AM, Nicholson AG, Roggli V, Travis WD, Wick M (2013). International Mesothelioma Interest Group. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma: 2012 Update of the Consensus Statement from the International Mesothelioma Interest Group. Archives of Pathology & Laboratory Medicine, 137(5):650–651.

Husain AN, Colby TV, Ordóñez NG, Allen TC, Attanoos RL, Beasley MB, Butnor KJ, Chirieac LR, Churg AM, Dacic S, Galateau-Sallé F, Gibbs A, Gown AM, Krausz T, Litzky LA, Marchevsky A, Nicholson AG, Roggli VL, Sharma AK, Travis WD, Walts AE, Wick MR (2018). Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. Archives of Pathology & Laboratory Medicine, 142(1):89–108.

Ikeda K, Tate G, Suzuki T, Kitamura T, Mitsuya T (2011). Diagnostic Usefulness of EMA, IMP3, and GLUT-1 for the Immunocytochemical Distinction of Malignant Cells From Reactive

Mesothelial Cells in Effusion Cytology Using Cytospin Preparations. Diagnostic Cytopathology, 39(6):395–401.

King J, Thatcher N, Pickering C, Hasleton P (2006). Sensitivity and specificity of immunohistochemical antibodies used to distinguish between benign and malignant pleural disease: a systematic review of published reports. Histopathology, 49(6):561–568.

Kinoshita Y, Hida T, Hamasaki M, Matsumoto S, Sato A, Tsujimura T, Kawahara K, Hiroshima K, Oda Y, Nabeshima K (2018a). A Combination of MTAP and BAP1 Immunohistochemistry in Pleural Effusion Cytology for the Diagnosis of Mesothelioma. Cancer Cytopathology, 126(1): 54–63.

Kinoshita Y, Hamasaki M, Yoshimura M, Matsumoto S, Sato A, Tsujimura T, Ueda H, Makihata S, Kato F, Iwasaki A, Nabeshima K (2018b). A combination of MTAP and BAP1 immunohistochemistry is effective for distinguishing sarcomatoid mesothelioma from fibrous pleuritis. Lung Cancer, 125:198–204.

Lin W, Liu X, Cen Y (2016). Diagnostic accuracy of epithelial membrane antigen for malignant effusions: a meta-analysis. International Journal of Biological Markers, 31(1): e11–e16.

Mavrakis KJ, McDonald ER 3rd, Schlabach MR, Billy E, Hoffman GR, deWeck A, Ruddy DA, Venkatesan K, Yu J, McAllister G, Stump M, deBeaumont R, Ho S, Yue Y, Liu Y, Yan-Neale Y, Yang G, Lin F, Yin H, Gao H, Kipp DR, Zhao S, McNamara JT, Sprague ER, Zheng B, Lin Y, Cho YS, Gu J, Crawford K, Ciccone D, Vitari AC, Lai A, Capka V, Hurov K, Porter JA, Tallarico J, Mickanin C, Lees E, Pagliarini R, Keen N, Schmelzle T, Hofmann F, Stegmeier F, Sellers WR (2016). Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. Science, 351(6278):1208–1213.

Minato H, Kurose N, Fukushima M, Nojima T, Usuda K, Sagawa M, Sakuma T, Ooi A, Matsumoto I, Oda M, Arano Y, Shimizu J (2014). Comparative Immunohistochemical Analysis of IMP3, GLUT1, EMA, CD146, and Desmin for Distinguishing Malignant Mesothelioma from Reactive Mesothelial Cells. American Journal of Clinical Pathology, 141(1):85–93.

Nautiyal N, Bhardwaj A, Acharya S, Kishore S, Kudesia S (2017). Diagnostic utility of epithelial membrane antigen (EMA) and calretinin (CAL) in effusion cytology. Journal of Clinical and Diagnostic Research, 11(5):EC36–EC39.

Sato A, Torii I, Okamura Y, Yamamoto T, Nishigami T, Kataoka TR, Song M, Hasegawa S, Nakano T, Kamei T, Tsujimura T (2010). Immunocytochemistry of CD146 is useful to discriminate between malignant pleural mesothelioma and reactive mesothelium. Modern Pathology, 23(11):1458–1466.

Schürch CM, Forster S, Brühl F, Yang SH, Felley-Bosco E, Hewer E (2017). The "don't eat me" signal CD47 is a novel diagnostic biomarker and potential therapeutic target for diffuse malignant mesothelioma. Oncoimmunology, 7(1): e1373235

Shen J, Pinkus GS, Deshpande V, Cibas ES (2009). Usefulness of EMA, GLUT-1, and XIAP for the cytologic diagnosis of malignant mesothelioma in body cavity fluids. American Journal of Clinical Pathology, 131(4):516–523.

Terra S, Roden AC, Yi ES, Aubry MC, Boland JM (2022). Loss of Methylthioadenosine Phosphorylase by Immunohistochemistry Is Common in Pulmonary Sarcomatoid Carcinoma and Sarcomatoid Mesothelioma. American Journal of Clinical Pathology, 157(1):33–9.

Thomas A, Chen Y, Yu T, Gill A, Prasad V (2015). Distinctive clinical characteristics of malignant mesothelioma in young patients. Oncotarget, 6(18):16766–73.

Yoshimura M, Kinoshita Y, Hamasaki M, Matsumoto S, Hida T, Oda Y, Iwasaki A, Nabeshima K (2019). Highly expressed EZH2 in combination with BAP1 and MTAP loss, as detected by immunohistochemistry, is useful for differentiating malignant pleural mesothelioma from reactive mesothelial hyperplasia. Lung Cancer, 130:187–93.

Zimling ZG, Jørgensen A, Santoni-Rugiu E (2012). The diagnostic value of immunohistochemically detected methylthioadenosine phosphorylase deficiency in malignant pleural mesotheliomas. Histopathology, 60(6B): E96–E105.