Review Article

Evidence-Based Diagnostic Performance of Novel Biomarkers for the Diagnosis of Malignant Mesothelioma in Effusion Cytology

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Cytology effusions are often the only material available for diagnosing malignant pleural mesothelioma (MPM). However, the cytomorphological features alone are not always diagnostic, and cytology samples preclude an assessment for pleural tissue invasion. Accordingly, immunohistochemical, soluble, and molecular biomarkers have been developed. The aim of this study is to provide quantitative evidence regarding the diagnostic performance of novel biomarkers. To that end, a systematic literature review was performed of articles dealing with a loss of BRCA1-associated protein 1 (BAP1), methylthioadenosine (MTAP), 5-hydroxymethylcitosine (5-hmC), glucose transporter 1 (GLUT1), insulin like-growth factor II messenger RNA–binding protein 3 (IMP3), enhanced zeste homologue 2 (EZH2) staining, cyclin-dependent kinase inhibitor 2A (*CDKN2A*) homozygous deletion (HD) testing, soluble mesothelin, and microRNA quantification in cytological samples for the diagnosis of MPM versus reactive atypical mesothelial cells. Sensitivity and specificity were extracted, and a meta-analysis was performed. The quality of the studies was assessed with Quality Assessment of Diagnostic Accuracy Studies 2, and the quality of the evidence was evaluated with the Grading of Recommendations Assessment, Development, and Evaluation approach. Seventy-one studies were included. BAP1 loss showed a sensitivity of 0.65 (confidence interval [CI], 0.59-0.71) and a specificity of 0.99 (CI, 0.93-1.00). MTAP loss and p16 HD showed 100% specificity with sensitivities of 0.47 (CI, 0.38-0.57) and 0.62 (CI, 0.53-0.71), respectively. BAP1 loss and *CDKN2A* HD combined showed maximal specificity and a sensitivity of 0.83 (CI, 0.78-0.89). GLUT1 and IMP3 showed sensitivities of 0.82 (CI, 0.70-0.90) and 0.65 (CI, 0.41-0.90), respectively, with comparable specificity. Mesothelin showed a sensitivity of 0.73 (CI, 0.68-0.77) and a specificity of 0.90 (CI, 0.84-0.93). In conclusion, some of the recently emerging biomarkers are close to 1.00 specificity. Their moderate sensitivity on their own, however, can be significantly improved by the use of 2 biomarkers, such as a combination of BAP1 and *CDKN2A* with fluorescence in situ hybridization or a combination of BAP1 and MTAP immunohistochemistry. ***Cancer Cytopathol* 2022;130:96-109**.

KEY WORDS: biomarker; cytology; diagnostic specificity and sensitivity; effusion; immunohistochemistry; mesothelioma; meta-analysis; pleura; systematic review.

INTRODUCTION

Malignant mesothelioma arises from the serosal surfaces lining the pleural, peritoneal, and pericardial cavities.[1] Exposure to asbestos fibers is regarded as the major etiological factor, but the role of genetic predisposition is increasingly recognized.[2] Malignant pleural mesothelioma (MPM) carries a poor prognosis with an overall

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survival of less than 18 months.[1,3] Afflicted patients are usually elderly and are not always fit enough to tolerate thoracoscopic surgery to obtain a pleural biopsy. A cytological examination of the pleural effusion, on the other hand, can be performed with minimal invasion and with less morbidity. Because tissue invasion is a major diagnostic criterion, mesothelioma guidelines recommend that a cell block preparation be performed whenever possible.[3] Prior published sensitivities for rendering a diagnosis of mesothelioma based on a cytologic evaluation alone range from 0.30 to 0.75.[4] Although reliable immunohistochemistry (IHC) markers have been well established to assist in the differential diagnosis between MPM and metastatic adenocarcinoma in pleural effusions,[5,6] a panel of biomarkers has not been established to reliably differentiate between MPM and reactive atypical mesothelial cells.

According to recent International Mesothelioma Interest Group guidelines,[7] the most valuable biomarkers for discriminating MPM from benign mesothelial proliferations include a loss of IHC expression of BRCA1-associated protein 1 (BAP1) and the homozygous deletion (HD) of the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene (also known as *p16*) as evaluated by fluorescence in situ hybridization (FISH). A meta-analysis to evaluate the diagnostic performance of BAP1 loss has shown that the sensitivity by IHC is 0.74 in epithelioid mesothelioma, 0.50 in biphasic mesothelioma, and 0.07 in sarcomatoid,[8,9] with confirmation provided by subsequent reports.[10,11] *CDKN2A* has the same unsatisfactory sensitivity (0.48-0.88), which is slightly higher (up to 0.80-1.00) for sarcomatoid mesothelioma,[10,12] and high specificity according to recent reviews[13,14] also in cytological material.[11]

Recently, methylthioadenosine (MTAP) IHC loss has emerged as another potentially useful biomarker. MTAP has shown high specificity for diagnosing MPM with a sensitivity comparable to that of BAP1 and *CDKN2A* testing.[15] Other IHC biomarkers, such as insulin like-growth factor II messenger RNA–binding protein 3 (IMP3) and glucose transporter 1 (GLUT1), also have been tested. Despite some studies suggesting high specificity and variable sensitivity (0.30-0.75),[16,17] this is not truly established, with reporting of a large quota of benign lesions incorrectly staining.[18,19] Overexpression of enhanced zeste homologue 2 (EZH2) appears to also play a role in mesothelioma development and progression, and its IHC staining is nuclear; this allows it to be easily used in combination with other membranous or cytoplasmic markers. Early reports regarding EZH2 involved tissue specimens and showed a specificity of 1.00 when a 50% or high-staining/expression pattern was used as the cutoff, with moderate sensitivity (0.45-0.66) when it was used alone; this increased in combination with BAP1/MTAP.[20,21] 5-Hydroxymethylcitosine (5-hmC) is a modified nucleotide, and its diagnostic use for pleural mesothelioma has been reported only in histological specimens.[22]

Soluble biomarkers include mesothelin, soluble mesothelin-related peptides (SMRPs), and fibulin-3 detected by enzyme-linked immunosorbent assays or chemiluminescent enzyme immunoassays. They are relatively simple to detect and would permit a rapid diagnosis, but none of them have been proven to be highly sensitive for discriminating between benign lesions and MPM.[18] Soluble mesothelin is the only Food and Drug Administration–approved biomarker for MPM.[23] Elevated levels of mesothelin in pleural effusions have high specificity but low sensitivity,[23] and no unequivocal conclusions on its value have been reached.[24,25] Results on fibulin-3 are even more conflicting, with this biomarker working better in plasma and with only 1 study dealing with pleural effusions.[26,27] MicroRNAs (miRNAs) are the newest potential diagnostic and prognostic markers. miRNAs are short RNA molecules involved in posttranslational gene regulation, and they have a well-established role in carcinogenesis, cancer progression, and metastasis.[28] miRNAs may be extracted from biological samples, including liquid specimens such as plasma, saliva, and serous effusions.[29] However, the usefulness of miRNA profiling in effusions as a biomarker of MPM remains unknown.[30]

The aim of this study, therefore, was to conduct a systematic review to assess published evidence for the diagnostic accuracy of the aforementioned diagnostic biomarkers for the diagnosis of MPM in pleural effusion cytological materials.

MATERIALS AND METHODS

A systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for diagnostic test accuracy.[31] The review was registered with the PROSPERO database (registration CRD42020198334).[32]

Search Strategy

Electronic searches were performed in the PubMed (MEDLINE), Embase, and Cochrane Library databases until September 15, 2020, with separate searches performed for each biomarker. No study type filters were used, nor were language restrictions applied. Where possible, filters to exclude animal and in vitro studies were applied. A search for gray literature was performed with the Open Grey (http://opengrey.eu/) and OAIster (https://oaister.worldcat.org/) public resources. The references listed in all included studies and previous reviews on biomarkers, even if not applicable to cytology, were hand-searched to retrieve potential additional studies. The complete search strategies are available in Supporting Table 1.

Article Screening

The initial screening of articles by title/abstract was performed with the aid of the online systematic review web application QRCI.[33] The eligibility of published studies was determined independently by 2 reviewers, with disagreements resolved through consensus. After full-text screening, a list of excluded studies with reasons for exclusion was provided in a standardized PRISMA flow chart.

Eligibility Criteria and Data Extraction

The criteria for including relevant studies were built according to the Population/Participants, Target Condition, Index Test, Reference Test, Outcome, and Study Design Type model. We considered studies dealing with patients who had a pleural effusion and for whom MPM was in the differential diagnosis with atypical mesothelial reactive cells. We considered only studies in which the mesothelial origin of the atypical cellular component was already determined and an epithelial nature was excluded; we thus excluded all studies dealing with the differential diagnosis of metastatic carcinoma. The index test was represented by several biomarkers of interest. The reference test was represented by a final diagnosis of MPM reached by means of definitive histology or by means of follow-up long enough to demonstrate clinical evidence of MPM.

Data were extracted by a single reviewer, with all outcome data verified by a second reviewer, according to the review question as for the protocol.[32]

The complete eligibility criteria for full-text inclusion and a list of the extracted data are reported in detail in Supporting Table 2.

Quality Assessment of Studies

The quality of the included studies was assessed with the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.[34] The risk of bias assessments was determined by 1 reviewer, with another reviewer providing subsequent verification. Briefly, the QUADAS-2 tool comprises 4 domains, for which basic yes/no questions guide the evaluation. The domains are the patient selection, which deals mainly with the avoidance of a case-control design (which is a source of bias) and the adoption of random/consecutive inclusion of patients; the index and reference test domains, for which a clear and exhaustive description with stating of the cutoff is required; and the flow and timing domain, with attention paid to the inclusion of all cases in the analysis and the timing of performing both the index test and the reference test. Additionally, an evaluation of the applicability of the study to the review question is required. The risk of bias for all the domains and then overall judgment for a single study are rated qualitatively as low, high, or unclear. On the basis of the QUADAS-2 guidance, we tailored the tool according to our review question. The adapted QUADAS-2 tool that we used is shown in Supporting Table 3.

Statistical Analysis

Data from 2 × 2 tables for each biomarker (true positive, false negative, false positive, and true negative) were used to summarize accuracy estimates. A graphical representation of the studies was provided via the plotting of sensitivity and specificity estimates with their 95% confidence intervals (CIs) as forest plots. Because there was no explicit threshold reported for the biomarkers evaluated, a bivariate model was fitted with the METANDI function in Stata to calculate pooled estimates for sensitivity and specificity with their 95% CIs. For markers with very little variation in specificity, we fitted a univariate random model with the METAPROP function in Stata to calculate pooled estimates for sensitivity with its 95% CI. A subgroup analysis was performed according to the geographical area of study (Eastern countries vs Western countries), cell blocks versus smears, and the risk of bias according to QUADAS-2 (high risk vs low risk).

All of the graphical depictions were performed with RevMan 5.3 (Nordic Cochrane Centre, Cochrane Collaboration, 2014) and Stata 15 (StataCorp, College Station, Texas).

Quality of Evidence Assessment

For the evaluation of the certainty of the evidence of the pooled estimates of the diagnostic performance, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used.[35] Briefly, GRADE is a rating system that applies to the outcome explored in a review; it takes into account factors derived both from singles studies and from the final estimate from the meta-analysis. The rating uses 4 grades (very low, low, moderate, and high) to describe the overall quality of the final evidence obtained. The factors are the risk of bias of included studies, which concerns mainly the study design; the inconsistency of estimates derived from visual inspections of forest plots not showing overlapping CIs among single studies; the indirectness for the applicability of the final estimate when cases not entirely matching the population of interest are present (eg, the presence of data from biopsies is not eliminable); the imprecision of the pooled estimate, which is mainly due to the limited pooled sample size; and the publication bias. Because all the studies were likely to be observational and retrospective, the certainty of evidence was always downgraded by at least 1 point for a risk of bias due to the study design. According to GRADE, judgment remains subjective, but an explanation of decisions is required. Indeed, we were conservative and decided to always treat any issue of imprecision and inconsistency as serious by downgrading further the quality of evidence.

RESULTS

Search Algorithm

After the removal of duplicates, 2929 studies underwent title and abstract screening. Among these studies, 222 were checked in full-text form. There were 71 studies included in the qualitative synthesis, and 65 provided data for the quantitative meta-analysis. The flowchart for the screening of articles is portrayed in Figure 1.

Study Characteristics

The studies included 57 full articles and 14 abstracts. They incorporated a total of 5354 patients from Europe (n = 25; 35%), North America (n = 19; 27%), Far East Asia (n = 16; 23%), Oceania (n = 6; 8%), and the Middle East (n = 5; 7%). As for study design, 49 (69%) were retrospective, 19 (27%) collected cases prospectively, and 3 (4%) declared a case-control design. Information on asbestos exposure was available in 7 studies (10%). Detailed histological subtyping was available in 26 studies (37%), and in all of them, the majority of cases in their population were epithelioid MPM. Three studies dealt with epithelioid MPM only, 5 dealt with both epithelioid and biphasic types, and none considered the sarcomatoid type only. The cytological specimens were cell blocks only (n = 27; 38%), pleural effusion fluids for soluble markers (n = 26; 37%), cytological smears (n = 17; 24%), and a mixed population of smears and cell blocks (n = 1; 1%). Among soluble biomarkers, mesothelin/SMRP alone was investigated in 22 studies (31%), fibulin-3 was investigated in 3 studies (4%), and both biomarkers together were investigated in 1 study (1%). Among the IHC markers, BAP1 was investigated in 21 studies (30%), MTAP was investigated in 8 studies (11%), GLUT1 was investigated in 11 studies (16%), and IMP3 was investigated in 5 studies (7%). The newest IHC biomarkers EZH2 and 5-hmC were each investigated in 1 study. *CDKN2A* HD was investigated in 26 studies (37%). Finally, miRNA signatures were investigated in 2 studies (3%). The essential data of the included studies are reported in Table 1, whereas the full list of included studies with all references can be found in Supporting Table 4.

Quality Assessment

The overall quality of the studies was considered moderate. The parameter with the highest risk of bias was patient selection in 14 studies (20%), with some studies clearly declaring a case-control design and almost all studies being observational and retrospective. Critical points regarding the index test domain concerned cutoffs in 12 studies (17%) with an unclear or high risk of bias, whereas the reference test was the issue with less risk of bias. Applicability concerns were generally limited, with only a few cases for which the presence of data from biopsy materials or peritoneal specimens could not be excluded. The quality appraisal of the studies is depicted in Figure 2 and Supporting Figure 1.

Diagnostic Outcome: Quantitative Analysis

A bivariate model was used for the following biomarkers: BAP1 loss, p16 HD, GLUT1, IMP3, MTAP loss, mesothelin, and the combination of BAP1 loss and p16 HD. In the case of p16, IMP3, and the combination of BAP1 loss and p16 HD, a univariate random model analysis was

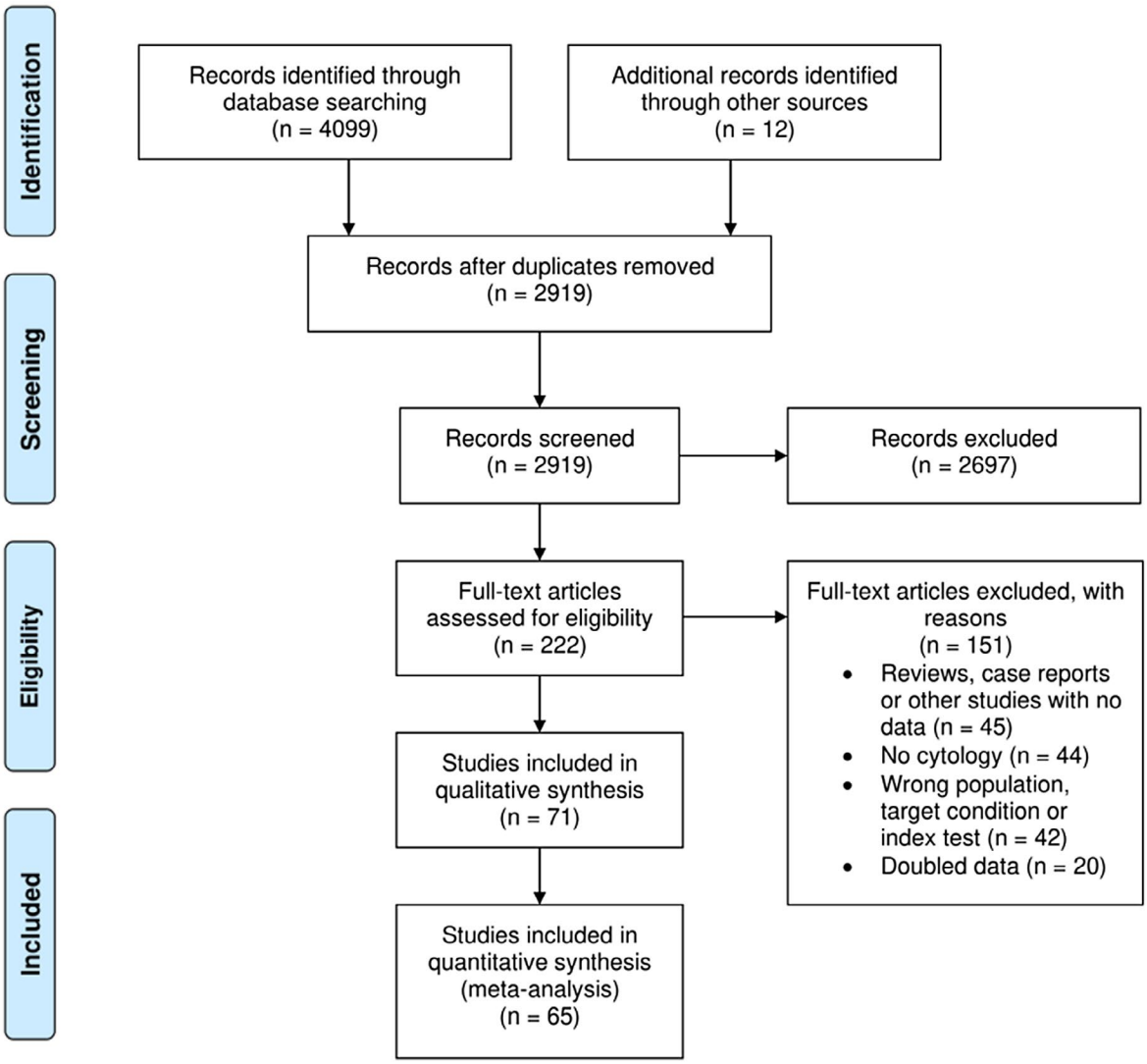

Figure 1. Search flow according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram.

performed because the specificity was always maximal at 1.00 or hampered by low variability around the maximal value. We also attempted a comparison of the sensitivities of the biomarkers by using the best performing one, the combination of BAP1 loss and p16 HD, as the reference, and we found that almost all the markers significantly differed from the reference with lower sensitivity. Only GLUT1 did not show statistical significance for sensitivity, but its pooled specificity remained lower than that of the best performing combination.

As for the quality of the evidence, at least 1 point of downgrading was always present because of the retrospective and possibly case-control study design: moderate was the highest grade and was achieved by BAP1 loss, GLUT1, p16 HD, and mesothelin/SMRP. Evidence judged to be of low quality (MTAP loss and the combination of BAP1 loss and p16 HD) or very low quality (IMP3) was downgraded mainly because of imprecision, the limited pooled sample size, and/or inconsistency from the inspection of forest plots.

The forest plots for the diagnostic performance of the single biomarkers with the relative ranges of sensitivity and specificity are shown in Figures 3 to 9, and a summary of the findings, highlighting the pooled estimates, comparisons, and quality of evidence, is presented in Table 2.

TABLE 1. Summary of the Included Studies

Source (Country)[a]	Sample Size	Specimen	MPM Subtype	Diagnostic Test	Cutoff for Positivity
Agha 2014[1] (Egypt)	34	PE	10 E, 6 S, 9 B	Fibulin-3	127.5 ng/mL
Agrawal 2019[2] (United States)[b]	33	PE	NS	BAP1	NS
Aleman 2009[3] (Spain)	39	PE	NS	SMRP/mesothelin	6 nmol/L
Amany 2013[4] (Egypt)	40	PE	NS	SMRP/mesothelin	3.5 nmol/L
Andrici 2015[5] (United States)	168	CB	NS	BAP1	Retention of staining up to 5% of presumed target cells
Battolla 2017[6] (Italy)[c]	97	PE	22 E, 4 S, 3 B	Fibulin-3	0.183 ng/mL
				SMRP/mesothelin	0.3 nM/L
Battolla 2012[7] (Italy)	181	PE	35 E, 9 S, 4 B	SMRP/mesothelin	9.3 nmol/L
Berg 2020[8] (United States)[c]	39	CB	NS	BAP1	100% loss; positive control present
				MTAP	Less than 25% cells with staining
Birnie 2019[9] (United States)[c]	36	PE	17 E, 2 S, 1 B	miRNAs	Variable
Blanquart 2012[10] (France)	76	PE	49 E, 4 S, 4 B	SMRP/mesothelin	14.6 nM/L
Bradley 2013[11] (United Kingdom)[b]	18	PE	NS	SMRP/mesothelin	NS
Bruno 2019[12] (Italy)	27	CB	NS	BAP1	100% loss in atypical mesothelial cells in presence of positive control
				p16	More than 11% HD in atypical mesothelial cells
				BAP1 + p16	—
Canessa 2013[13] (Italy)[b]	86	PE	NS	SMRP/mesothelin	20 nM/L
Canessa 2013[14] (Italy)	104	PE	25 E, 9 S 0 B	SMRP/mesothelin	19.6 nM/L
Canessa 2012[15] (Italy)	181	PE	35 E, 9 S, 4 B	SMRP/mesothelin	9.30 nM/L
Cappellesso 2016[16] (Italy)	53	PE	NS	miRNAs	0.49 folds
Chen 2020[17] (China)[b,c]	110	CB	NS	BAP1	NS
				p16	NS
Cigognetti 2015[18] (Italy)	70	CB	NS	BAP1	NS
Cozzi 2017[19] (Italy)	114	CB	NS	BAP1	NS; all or nothing staining with internal control present
Creaney 2014[20] (Australia)	829	PE, CB	59 E, 19 S, 23 B	SMRP/mesothelin	20 nM/L
Creaney 2013[21] (Australia)	98	PE	NS	SMRP/mesothelin	20 nM/L
Creaney 2007[22] (Australia)	136	PE		SMRP/mesothelin	20 nM/L
Dagli 2011[23] (Turkey)[b]	20	CB	NS	GLUT1	NS
				IMP3	NS
Davies 2009[24] (United Kingdom)	99	PE	11 E, 5 S, 4 B	SMRP/mesothelin	20 nM/L
Deng 2013[25] (United States)[b]	32	CB	NS	GLUT1	More than 5% of target cells
	32			IMP3	More than 5% of target cells
Ferro 2013[26] (Italy)	79	PE	NS	SMRP/mesothelin	12.7 nM/L
Flores-Staino 2010[27] (Sweden)	39	PE	NS	p16	12 nuclei with deletion
Fujimoto 2010[28] (Japan)	49	PE	15 E, 4 S, 2 B	SMRP/mesothelin	8 nM/L
Galateau-Salle 2008[29] (France)[b]	37	CB		p16	NS
Hanley 2007[30] (United States)	26	CB	NS	IMP3	NS
Hasteh 2010[31] (United States)	58	CB	NS	GLUT1	>20% mesothelial cells with membrane staining
Hamasaki 2012[32] (Japan)[b]	13	PE	NS	p16	More than 10% HD
Hamasaki 2019[33] (Japan)[b,c]	74	CB	NS	Combination: p16, MTAP, BAP1, and NF2	NS
Hatem 2018[34] (United States)	30	CB	13 E, 2 S, 3 B	BAP1	More than 50% loss of staining
Hida 2015[35] (Japan)	45	PE	NS	p16	More than 10% HD
Hiroshima 2016[36] (Japan)	39	CB	15 E, 1 S, 6 B	p16	More than 15% HD
Hiroshima 2020[37] (Japan)	67	CB	24 E, 9 B	HEG1	Score > 2
				BAP1	NS
				MTAP	NS
				BAP1 + MTAP	—
Hooper 2012[38] (United Kingdom)	54	PE	23 E, 3 S, 2 B	SMRP/mesothelin	20 nM/L
Hwang 2016[39] (Canada)	16	PE	NS	BAP1	NS
				p16	More than 12% HD
				BAP1 + p16	
Ikeda 2010[40] (Japan)	50	PE	NS	IMP3	NS
Ikeda 2011[41] (Japan)	61	PE	11 E	IMP3	NS
				GLUT1	NS
				GLUT1	NS
Illei 2003[42] (United States)	32	PE	NS	p16	More than 15% HD
Javadi 2020[43] (Sweden)[c]	82	PE	NS	SMRP/mesothelin	NS

TABLE 1. Continued

Source (Country)[a]	Sample Size	Specimen	MPM Subtype	Diagnostic Test	Cutoff for Positivity
Kee 2010[44] (New Zealand)	34	CB	15 E, 1 S, 1 B	GLUT1	NS
Kinoshita 2018[45] (Japan)	66	CB	NS	BAP1	More than 50% loss
				MTAP	More than 50% loss
				p16	More than 10% HD
				BAP1 + MTAP	—
				BAP1 + p16	—
Kinoshita 2020[46] (Japan)[b]	42	CB	NS	p16	NS
				MTAP	NS
				BAP1	NS
Kirschner 2015[47] (Australia)[c]	90	PE	27 E, 1 S, 2 B	Fibulin-3	NS
Kuperman 2013[48] (United States)	88	CB	NS	GLUT1	Any positivity
Leong 2015[49] (Australia)	37	PE	NS	SMRP/mesothelin	20 nM/L
Matsumoto 2013[50] (Japan)	35	PE	NS	p16	More than 10% HD
Matsumoto 2019[51] (Japan)	88	PE	NS	p16	More than 10% HD
				BAP1	More than 50% loss
				BAP1 + p16	—
McCroskey 2017[52] (United States)	32	CB	19 E	BAP1	Retention of staining up to 5% of presumed target cells
Mutlu 2012[53] (Turkey)	40	CB	19 E, 1 B	GLUT1	More than 10%
Nabeshima 2012[54] (Japan)	20	PE	NS	p16	More than 10% HD
Önder 2019[55] (Turkey)	46	PE, CB	NS	BAP1	100% loss
				GLUT1	More than 1%
Onofre 2008[56] (Germany)	72	PE	NS	p16	More than 5 nuclei
Pass 2008[57] (United States)	72	PE	NS	SMRP/mesothelin	12.6 nM/L
Pass 2012[58] (United States)		PE		SMRP/mesothelin	378.33 ng/mL
Pinheiro 2012[59] (Portugal)	20	PE	NS	GLUT1	Combined score > 2
Raza 2020[60] (United States)[b]	33	PE	11 E, 2 B	BAP1	NS
				MTAP	NS
Savic 2010[61] (Switzerland)	80	PE	44 E, 1 S, 7 B	p16	More than 15% HD
Scherpereel 2006[62] (France)	64	PE	NS	SMRP/mesothelin	10.4 nM/L
Schürch 2018[63] (Switzerland)	148	CB	NS	BAP1	100% loss
				GLUT1	Any positivity
Shahi 2020[64] (United States)[b]	108	CB	62 E, 20 B	BAP1	NS
				MTAP	NS
				5-hmC	NS
				BAP1 + 5-hmC	—
				BAP1 + 5-hmC + MTAP	—
Shen 2009[65] (United States)	73	CB	28 E	GLUT1	Any positivity
					Any positivity
Stockhammer 2020[66] (Germany)	72	PE	35 E, 6 S, 7 B	SMRP/mesothelin	13.1 nM/L
Vigani 2011[67] (Italy)[b]	140	PE	NS	SMRP/mesothelin	10.8 nM/L
Walts 2016[68] (United States)	63	CB	25 E, 6 B	BAP1	More than 50% loss with internal control present
				p16	More than 15% HD
				BAP1 + p16	—
Yamada 2011[69] (Japan)	69	PE	37 E, 5 S, 3 B	SMRP/mesothelin	10 nmol/L
Yoshimura 2020[70] (Japan)	60	CB	NS	BAP1	More than 50% loss
				MTAP	More than 50% loss
				EZH2	More than 50% expression
				p16	10% HD
				BAP1 + EZH2	—
				MTAP + EZH2	—
				p16 + EZH2	—
				BAP1 + MTAP	—
				BAP1 + p16	—
				BAP1 + MTAP/p16 + EZH2	—
Zhu 2020[71] (United States)[b]	59	CB	NS	MTAP	NS

Abbreviations: 5-hmC, 5-hydroxymethylcitosine; B, biphasic type; BAP1, BRCA1-associated protein 1; CB, cell block; E, epithelioid type; EZH2, enhanced zeste homologue 2; GLUT1, glucose transporter 1; HD, homozygous deletion; IMP3, insulin like-growth factor II messenger RNA–binding protein 3; miRNA, microRNA; MPM, malignant pleural mesothelioma; MTAP, methylthioadenosine; NS, not stated; PE, pleural effusion (fluid or smear); S, sarcomatoid type; SMRP, soluble mesothelin-related peptide.

[a]The reference citations for the studies refer to the references listed in Supporting Table 4.

[b]The study was represented by an abstract only.

[c]The study did not provide data for quantitative analysis.

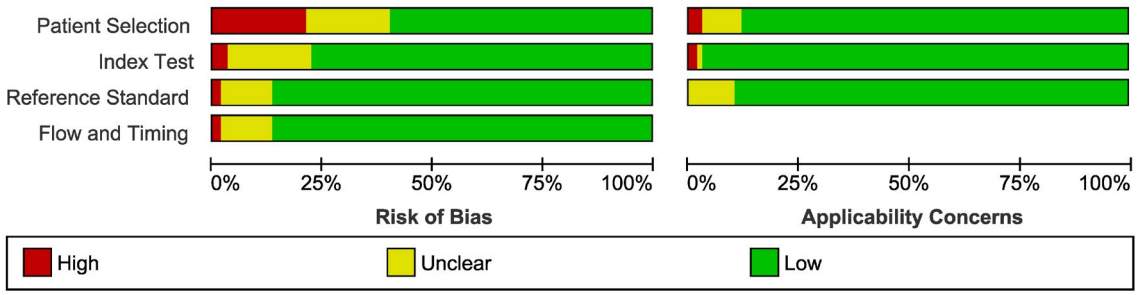

Figure 2. Quality assessment of the included studies according to Quality Assessment of Diagnostic Accuracy Studies 2.

Study	TP	FP	FN	TN	Sample type	Country	Sensitivity (95% CI)	Specificity (95% CI)
Hatem 2018	7	0	10	13	Cell block	Western	0.41 [0.18, 0.67]	1.00 [0.75, 1.00]
Matsumoto 2019	21	0	22	17	Smear	Far Eastern	0.49 [0.33, 0.65]	1.00 [0.80, 1.00]
Walts 2016	14	5	14	30	Cell block	Western	0.50 [0.31, 0.69]	0.86 [0.70, 0.95]
Raza 2020	7	0	6	20	Cell block	Western	0.54 [0.25, 0.81]	1.00 [0.83, 1.00]
Yoshimura 2020	22	0	18	20	Cell block	Far Eastern	0.55 [0.38, 0.71]	1.00 [0.83, 1.00]
Schuerch 2018	21	0	16	103	Cell block	Western	0.57 [0.39, 0.73]	1.00 [0.96, 1.00]
Bruno 2019	10	0	7	8	Cell block	Western	0.59 [0.33, 0.82]	1.00 [0.63, 1.00]
Andrici 2015	49	6	34	143	Cell block	Western	0.59 [0.48, 0.70]	0.96 [0.91, 0.99]
Kinoshita 2018	27	0	18	21	Cell block	Far Eastern	0.60 [0.44, 0.74]	1.00 [0.84, 1.00]
Hwang 2016	8	0	5	3	Smear	Western	0.62 [0.32, 0.86]	1.00 [0.29, 1.00]
Cigognetti 2015	35	0	17	19	Cell block	Western	0.67 [0.53, 0.80]	1.00 [0.82, 1.00]
Hiroshima 2020	32	0	13	9	Cell block	Far Eastern	0.71 [0.56, 0.84]	1.00 [0.66, 1.00]
Oender 2019	12	0	4	30	Mixed	Middle Eastern	0.75 [0.48, 0.93]	1.00 [0.88, 1.00]
Cozzi 2017	52	4	16	30	Cell block	Western	0.76 [0.65, 0.86]	0.88 [0.73, 0.97]
Shahi 2020	21	2	6	19	Cell block	Western	0.78 [0.58, 0.91]	0.90 [0.70, 0.99]
Kinoshita 2020	18	0	4	20	Cell block	Far Eastern	0.82 [0.60, 0.95]	1.00 [0.83, 1.00]
Agrawal 2019	24	0	4	5	Smear	Western	0.86 [0.67, 0.96]	1.00 [0.48, 1.00]
McCroskey 2017	19	0	3	11	Cell block	Western	0.86 [0.65, 0.97]	1.00 [0.72, 1.00]

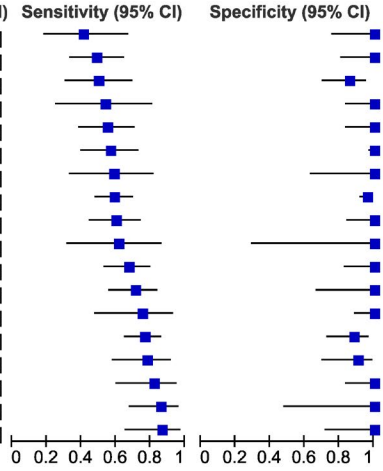

Figure 3. Forest plot of BAP loss. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. BAP indicates BRCA1-associated protein; CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Exploration of Heterogeneity

A subgroup analysis according to the specimen type was possible only for p16 HD. For the other biomarkers, a minimum number of studies per subgroup was not reached.

The complete results of the subgroup analyses are reported in Supporting Table 5. As for p16 HD, the pooled sensitivity was slightly higher with cytology smears than cell blocks, but there was no statistically significant difference with the overall estimate. The pooled sensitivity and specificity of GLUT1 staining and BAP1 loss in cell block–only cases showed substantial overlapping with the overall estimates, and the same applied to the combination of BAP1 loss and p16 HD. Geographical area was not a moderator of heterogeneity. The extractable data did not allow for subgroup analysis according to histological subtyping or asbestos exposure, but we noticed that in all studies, the absolute majority of MPM cases were of the epithelioid subtype. Formal metrics such as Q and I^2 were not produced because they are not appropriate for a meta-analysis of diagnostic tests: they are univariate, and sensitivity and specificity are correlated.[36]

Diagnostic Outcome: Qualitative Synthesis

For the biomarkers fibulin-3, EZH2, and 5-hmC and the miRNA signatures, it was not possible to perform a meta-analysis. The sensitivity and specificity of fibulin-3 ranged from 0.79 to 0.88 and from 0.78 to 0.95, respectively, in the 2 studies providing data.[37,38] The role of miRNAs was investigated in 2 studies,[39,40] but only 1 provided extractable data.[40] Other combinations that were investigated were EZH2 overexpression combined with i) BAP1 loss or ii) p16 HD or iii) MTAP loss or iv) both,[41] BAP1 loss with MTAP loss,[42,43] and BAP1 loss with 5-mhC overexpression with or without MTAP loss.[44] The combination

Study	TP	FP	FN	TN	Sample type	Country	Sensitivity (95% CI)	Specificity (95% CI)
Hasteh 2010	7	5	8	38	Cell block	Western	0.47 [0.21, 0.73]	0.88 [0.75, 0.96]
Schuerch 2018	23	7	16	80	Cell block	Western	0.59 [0.42, 0.74]	0.92 [0.84, 0.97]
Shen 2009	22	7	13	31	Cell block	Western	0.63 [0.45, 0.79]	0.82 [0.66, 0.92]
Kee 2010	14	0	3	17	Cell block	Western	0.82 [0.57, 0.96]	1.00 [0.80, 1.00]
Oender 2019	12	6	2	24	Mixed	Middle Eastern	0.86 [0.57, 0.98]	0.80 [0.61, 0.92]
Deng 2013	7	1	1	23	Cell block	Western	0.88 [0.47, 1.00]	0.96 [0.79, 1.00]
Pinheiro 2012	8	1	1	10	Smear	Western	0.89 [0.52, 1.00]	0.91 [0.59, 1.00]
Dagli 2011	9	0	1	10	Cell block	Middle Eastern	0.90 [0.55, 1.00]	1.00 [0.69, 1.00]
Mutlu 2012	18	1	2	19	Cell block	Middle Eastern	0.90 [0.68, 0.99]	0.95 [0.75, 1.00]
Kuperman 2013	39	7	4	14	Cell block	Western	0.91 [0.78, 0.97]	0.67 [0.43, 0.85]
Ikeda 2011	11	10	0	40	Smear	Far Eastern	1.00 [0.72, 1.00]	0.80 [0.66, 0.90]

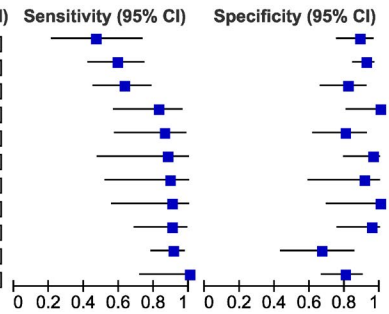

Figure 4. Forest plot of GLUT1 staining. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. CI indicates confidence interval; FN, false negative; FP, false positive; GLUT1, glucose transporter 1; TN, true negative; TP, true positive.

Study	TP	FP	FN	TN	Sample type	Country	Sensitivity (95% CI)	Specificity (95% CI)
Raza 2020	3	0	10	20	Cell block	Western	0.23 [0.05, 0.54]	1.00 [0.83, 1.00]
Shahi 2020	9	0	18	19	Cell block	Western	0.33 [0.17, 0.54]	1.00 [0.82, 1.00]
Berg 2020	7	0	14	15	Cell block	Western	0.33 [0.15, 0.57]	1.00 [0.78, 1.00]
Kinoshita 2018	19	0	26	21	Cell block	Far Eastern	0.42 [0.28, 0.58]	1.00 [0.84, 1.00]
Kinoshita 2020	12	0	10	20	Cell block	Far Eastern	0.55 [0.32, 0.76]	1.00 [0.83, 1.00]
Yoshimura 2020	22	0	18	20	Cell block	Far Eastern	0.55 [0.38, 0.71]	1.00 [0.83, 1.00]
Zhu 2020	25	1	20	13	Cell block	Western	0.56 [0.40, 0.70]	0.93 [0.66, 1.00]
Hiroshima 2020	16	0	5	5	Cell block	Far Eastern	0.76 [0.53, 0.92]	1.00 [0.48, 1.00]

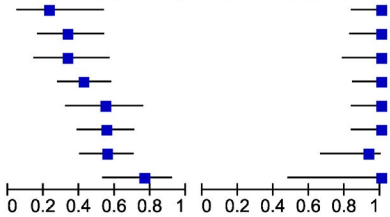

Figure 5. Forest plot of MTAP loss. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. CI indicates confidence interval; FN, false negative; FP, false positive; MTAP, methylthioadenosine; TN, true negative; TP, true positive.

Study	TP	FP	FN	TN	Sample type	Country	Sensitivity (95% CI)	Specificity (95% CI)
Ikeda 2010	4	2	7	37	Smear	Far Eastern	0.36 [0.11, 0.69]	0.95 [0.83, 0.99]
Ikeda 2011	4	3	7	47	Smear	Far Eastern	0.36 [0.11, 0.69]	0.94 [0.83, 0.99]
Deng 2013	5	0	3	24	Cell block	Western	0.63 [0.24, 0.91]	1.00 [0.86, 1.00]
Dagli 2011	9	1	1	9	Cell block	Middle Eastern	0.90 [0.55, 1.00]	0.90 [0.55, 1.00]
Hanley 2007	11	1	1	13	Cell block	Western	0.92 [0.62, 1.00]	0.93 [0.66, 1.00]

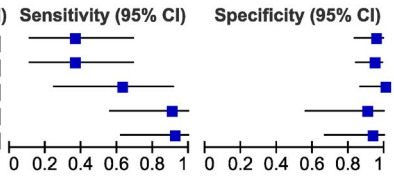

Figure 6. Forest plot of IMP3 staining. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. CI indicates confidence interval; FN, false negative; FP, false positive; IMP3, insulin like-growth factor II messenger RNA–binding protein 3; TN, true negative; TP, true positive.

of BAP1 and MTAP loss with or without p16 HD and the combination of EZH2 overexpression with BAP1 or MTAP loss and p16 HD all showed a specificity of 1.00, with the sensitivity ranging from 0.72 to 0.91. A loss of 5-hmC alone or in combination with BAP1 loss showed a sensitivity ranging from 0.67 to 0.89 and a specificity ranging from 0.89 to 1.00.

DISCUSSION

The cytological diagnosis of MPM typically cannot rely solely on cytomorphological features because of the significant overlapping cytologic features between reactive and malignant mesothelial proliferations. Previous systematic reviews attempting to summarize the diagnostic utility of biomarkers for MPM have failed to draw a strong conclusion. Our analysis indicates that BAP1 loss with IHC alone carries a high pooled specificity (0.99; CI, 0.93-1.00) but still lower sensitivity (0.65; CI, 0.59-0.71). This is in line with the only previous meta-analysis focused on cytological materials.[8] *CDKN2A* HD showed comparable diagnostic performance with a pooled sensitivity of 0.62 (CI, 0.53-0.71) and a specificity of 1.00. These 2 biomarkers are diagnostically powerful in that a positive result from either of them is diagnostic of MPM. However, a negative result does not exclude a diagnosis of MPM because of their only moderate sensitivity when

Study	TP	FP	FN	TN	Sample type	Country	Sensitivity (95% CI)	Specificity (95% CI)
Walts 2016	6	0	11	21	Cell block	Western	0.35 [0.14, 0.62]	1.00 [0.84, 1.00]
Galateau-Salle 2008	7	0	10	20	Cell block	Western	0.41 [0.18, 0.67]	1.00 [0.83, 1.00]
Matsumoto 2019	21	0	22	28	Smear	Far Eastern	0.49 [0.33, 0.65]	1.00 [0.88, 1.00]
Bruno 2019	9	0	8	8	Cell block	Western	0.53 [0.28, 0.77]	1.00 [0.63, 1.00]
Onofre 2008	18	0	15	39	Smear	Western	0.55 [0.36, 0.72]	1.00 [0.91, 1.00]
Flores-Staino 2010	12	0	9	18	Smear	Western	0.57 [0.34, 0.78]	1.00 [0.81, 1.00]
Yoshimura 2020	23	0	17	20	Cell block	Far Eastern	0.57 [0.41, 0.73]	1.00 [0.83, 1.00]
Kinoshita 2020	13	0	9	20	Cell block	Far Eastern	0.59 [0.36, 0.79]	1.00 [0.83, 1.00]
Kinoshita 2018	28	0	17	21	Cell block	Far Eastern	0.62 [0.47, 0.76]	1.00 [0.84, 1.00]
Hida 2015	14	0	6	25	Smear	Far Eastern	0.70 [0.46, 0.88]	1.00 [0.86, 1.00]
Hiroshima 2016	17	0	5	17	Cell block	Far Eastern	0.77 [0.55, 0.92]	1.00 [0.80, 1.00]
Savic 2010	41	0	11	28	Smear	Western	0.79 [0.65, 0.89]	1.00 [0.88, 1.00]
Illei 2003	12	0	1	19	Smear	Western	0.92 [0.64, 1.00]	1.00 [0.82, 1.00]
Nabeshima 2012	10	0	0	10	Smear	Far Eastern	1.00 [0.69, 1.00]	1.00 [0.69, 1.00]
Hamasaki 2012	7	0	0	6	Smear	Far Eastern	1.00 [0.59, 1.00]	1.00 [0.54, 1.00]
Matsumoto 2013	15	0	0	20	Smear	Far Eastern	1.00 [0.78, 1.00]	1.00 [0.83, 1.00]
Hwang 2016	9	0	0	3	Smear	Western	1.00 [0.66, 1.00]	1.00 [0.29, 1.00]

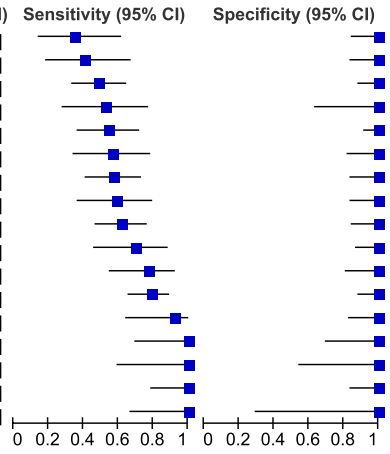

Figure 7. Forest plot of p16 homozygous deletion with fluorescence in situ hybridization. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. CI indicates confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Study	TP	FP	FN	TN	Sample type	Country	Sensitivity (95% CI)	Specificity (95% CI)
Walts 2016	10	0	6	21	Cell block	Western	0.63 [0.35, 0.85]	1.00 [0.84, 1.00]
Bruno 2019	13	0	4	8	Cell block	Western	0.76 [0.50, 0.93]	1.00 [0.63, 1.00]
Matsumoto 2019	35	0	8	17	Smear	Far Eastern	0.81 [0.67, 0.92]	1.00 [0.80, 1.00]
Kinoshita 2018	38	0	7	21	Cell block	Far Eastern	0.84 [0.71, 0.94]	1.00 [0.84, 1.00]
Yoshimura 2020	34	0	6	20	Cell block	Far Eastern	0.85 [0.70, 0.94]	1.00 [0.83, 1.00]
Hwang 2016	12	0	1	3	Smear	Western	0.92 [0.64, 1.00]	1.00 [0.29, 1.00]

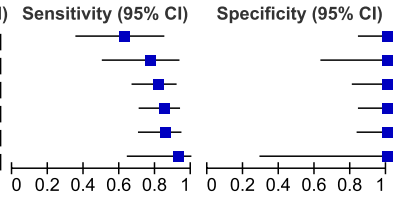

Figure 8. Forest plot of BAP loss combined with p16 homozygous deletion. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. BAP indicates BRCA1-associated protein; CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Study	TP	FP	FN	TN	Country	Sensitivity (95% CI)	Specificity (95% CI)
Leong 2015	13	0	11	9	Western	0.54 [0.33, 0.74]	1.00 [0.66, 1.00]
Canessa 2013	4	2	3	75	Western	0.57 [0.18, 0.90]	0.97 [0.91, 1.00]
Canessa 2013 (2)	20	1	14	34	Western	0.59 [0.41, 0.75]	0.97 [0.85, 1.00]
Vigani 2011	25	6	13	96	Western	0.66 [0.49, 0.80]	0.94 [0.88, 0.98]
Creaney 2014	122	54	61	592	Western	0.67 [0.59, 0.73]	0.92 [0.89, 0.94]
Creaney 2007	35	2	17	82	Western	0.67 [0.53, 0.80]	0.98 [0.92, 1.00]
Fujimoto 2010	16	8	7	18	Far Eastern	0.70 [0.47, 0.87]	0.69 [0.48, 0.86]
Ferro 2013	30	3	13	33	Western	0.70 [0.54, 0.83]	0.92 [0.78, 0.98]
Creaney 2013	30	3	13	52	Western	0.70 [0.54, 0.83]	0.95 [0.85, 0.99]
Davies 2009	17	2	7	73	Western	0.71 [0.49, 0.87]	0.97 [0.91, 1.00]
Hooper 2012	18	2	7	24	Western	0.72 [0.51, 0.88]	0.92 [0.75, 0.99]
Aleman 2009	13	11	5	10	Western	0.72 [0.47, 0.90]	0.48 [0.26, 0.70]
Battolla 2012	38	9	14	120	Western	0.73 [0.59, 0.84]	0.93 [0.87, 0.97]
Canessa 2012	38	9	14	120	Western	0.73 [0.59, 0.84]	0.93 [0.87, 0.97]
Pass 2008	34	5	11	25	Western	0.76 [0.60, 0.87]	0.83 [0.65, 0.94]
Scherpereel 2006	33	5	10	16	Western	0.77 [0.61, 0.88]	0.76 [0.53, 0.92]
Stockhammer 2020	38	8	10	16	Western	0.79 [0.65, 0.90]	0.67 [0.45, 0.84]
Yamada 2011	36	4	9	20	Far Eastern	0.80 [0.65, 0.90]	0.83 [0.63, 0.95]
Bradley 2013	5	3	1	9	Western	0.83 [0.36, 1.00]	0.75 [0.43, 0.95]
Blanquart 2012	57	4	4	11	Western	0.93 [0.84, 0.98]	0.73 [0.45, 0.92]
Amany 2013	19	2	1	18	Middle Eastern	0.95 [0.75, 1.00]	0.90 [0.68, 0.99]

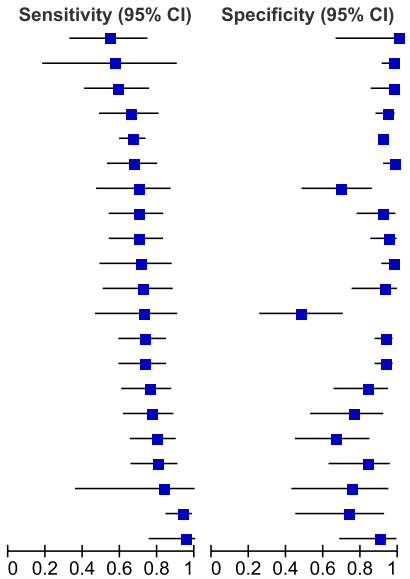

Figure 9. Forest plot of soluble mesothelin/soluble mesothelin-related peptides. References' citations for all studies are reported in Table 1 and refer to the references listed in Supporting Table 4. CI indicates confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

TABLE 2. Summary of the Sensitivity and Specificity Ranges and Pooled Estimates With Comparisons and Certainty of Evidence

Marker	Sensitivity Range	Specificity Range	Specificity: Pooled Estimate (95% CI)	Sensitivity: Pooled Estimate (95% CI)	Univariate Comparison of Sensitivities: P	Certainty of Evidence: Summary
BAP1 loss + p16 HD	0.63-0.92	1.00	1.00[a]	0.83 (0.78-0.89)[a]	Reference	⊕⊕◯◯: low
BAP1	0.41-0.86	0.96-1.00	0.99 (0.93-1.00)	0.65 (0.59-0.71)	.00	⊕⊕⊕◯: moderate
GLUT1	0.47-1.00	0.67-1.00	0.88 (0.81-0.92)	0.82 (0.80-0.90)	.29	⊕⊕⊕◯: moderate
MTAP	0.23-0.76	0.93-1.00	0.99 (0.88-1.00)	0.47 (0.38-0.57)	.00	⊕⊕◯◯: low
IMP3	0.36-0.92	0.90-1.00	0.90-1.00[a]	0.65 (0.41-0.90)[a]	.04	⊕◯◯◯: very low
p16 HD	0.35-1.00	1.00	1.00[a]	0.62 (0.53-0.71)[a]	.00	⊕⊕⊕◯: moderate
Mesothelin/SMRP	0.54-0.95	0.48-1.00	0.90 (0.84-0.93)	0.73 (0.68-0.77)	.00	⊕⊕⊕◯: moderate

Abbreviations: BAP1, BRCA1-associated protein 1; CI, confidence interval; GLUT1, glucose transporter 1; HD, homozygous deletion; IMP3, insulin like-growth factor II messenger RNA–binding protein 3; MTAP, methylthioadenosine; SMRP, soluble mesothelin-related peptide.
Certainty of Evidence: 1 point, very low certainty; 2 points, low certainty; 3 points, moderate certainty; 4 points, high certainty.
[a]Univariate model.

they are applied alone. Another important finding is that the combination of BAP1 loss and *CDKN2A* HD yields a pooled sensitivity of 0.83 (CI, 0.78-0.89) with a specificity of 1.00. The sensitivity is significantly increased with the combination (as shown by the CIs not overlapping), and this implies that, even though a negative result cannot exclude malignancy, a significantly greater quota of mesotheliomas can be detected with their combined use. Our subgroup analysis showed that the diagnostic performance of these 2 biomarkers was not significantly different from the overall estimate when we considered cell block–only cases; this implies that even though cell block processing is always recommended, the diagnostic value is maintained, regardless of the specimen.

This study revealed interesting findings concerning the role of MTAP loss by IHC, which has shown high concordance with *CDKN2A*/p16 HD by FISH[45] and could potentially replace FISH analysis in low-resource settings. Unfortunately, the minimum number of studies to perform a pooled analysis after the combination of BAP1 and MTAP loss was not reached, but in all 3 studies[41-43] that reported this combination, there was a tendency toward improved sensitivity, which ranged from 0.7 to 0.85. This observation means that the addition of MTAP testing to BAP1 likely portends an improvement of sensitivity. It appears that the sensitivity of a single marker remains suboptimal or, in other words, too different from the specificity, and it could be more advantageous to always combine the markers in order to not miss MPM cases without a loss of specificity and with optimization of the diagnostic yield; this is in line with proposed diagnostic algorithms for tissue biopsy material.[10] Because these markers are intended to aid and guide the establishment of a diagnosis and not to be screening or triage tests, the search for higher sensitivity has to be balanced with the preservation of higher specificity so that a positive result confirms the diagnosis but without the risk of false positives and overdiagnosis. Moreover, what is not always addressed in primary studies is the necessary presence of a positive internal control for markers such as BAP1 and MTAP, which are deemed positive when they show a loss of staining. All these markers maintain high specificity, and this also could be important in the case of a discordant result from 2 markers because in the case of a strongly suspicious case, the discordant marker could be reperformed, or the addition of a third marker could counterbalance this situation (eg, p16 after BAP1 and MTAP).

This study also found a pooled sensitivity of 0.82 (CI, 0.70-0.90) and a pooled specificity of 0.88 (CI, 0.81-0.92) for GLUT1 IHC staining. Our results are in agreement with previous systematic research.[19] Overall, these values indicate an unfavorable diagnostic performance for GLUT1 and suggest that, even if an important quota of mesotheliomas are correctly detected, there is likely to still be a proportion of negative cases that stain unreliably. Similar considerations apply to IMP3, which showed an unsatisfactory pooled sensitivity of 0.65 (CI, 0.41-0.90). Therefore, IMP3 is not recommended for diagnosing MPM.

We also attempted to compare the performance of markers according to sensitivity; we took the best performing one, the combination of BAP1 loss and p16 HD, as the reference. The pooled sensitivity of other markers was significantly lower, and this implied a potential advantage of the use of this combination in detecting MPM. The only exception was GLUT1, which did not reach significance, but its pooled specificity was, however,

lower than the reference. Moreover, these comparisons are statistically indirect because no primary studies compared a marker against another, so the comparative results should be regarded only as an indirect evaluation of their diagnostic performance.

Newer biomarkers such as EZH2 and 5-hmC showed a diagnostic profile similar to that of MTAP or BAP1 loss alone and an increase in sensitivity when they were used in combination.[41] Because of the limited study, these 2 markers are not recommended for general use in diagnosing MPM in effusion cytology. Interestingly, these newer markers are deemed positive when overexpressed oppositely to MTAP and BAP1 without the need for an internal positive control, and this could be useful (eg, as a third marker of a combination or in case of discordance in a pair), so we may expect that with future studies their use as adjunctive markers will increase. However, it is to be kept in mind that the marker itself or the combination does not make the diagnosis alone, but it always has to be evaluated together with the morphology and the clinical context.

No quantitative analysis was possible for miRNA signatures with the 2 included studies.[39,40] Most of the studies about miRNA signatures retrieved during the search process dealt with tissue specimens.[46] Finally, mesothelin showed pooled estimates for sensitivity and specificity of 0.73 (CI, 0.68-0.77) and 0.90 (CI, 0.84-0.93), respectively. Although these results should be interpreted with caution because there was evidence of a threshold effect (the correlation between logit sensitivity and logit specificity was –0.98), they are in keeping with results from a previous systematic review.[25] This finding confirms the unsatisfactory profile of mesothelin/SMRPs in pleural effusion. Indeed, high specificity indicates that mesothelin could be helpful in confirming MPM, but a result below the cutoff for positivity does not exclude malignancy. Moreover, it must be kept in mind that elevated levels of mesothelin/SMRP are present also in other malignancies. It could be interesting to evaluate whether the addition of mesothelin/SMRP measurement to a combination of 2 IHC markers or to the combination of BAP1 loss and p16 HD could further improve the diagnostic yield because when pleural fluid is collected, it can be easy and advantageous to perform different investigations on the same material. Unfortunately, no primary studies addressed this issue, and this marker, even if used together with others, has to be evaluated as a standalone test.

This study has both strengths and some limitations. The strengths reside in the methodology of performing a formal systematic review and the evaluation of the quality of evidence. The limitations of this study are related mainly to the primary studies with limited information included. Some risk of bias cannot be eliminated when studies declare a case-control design, are unclear about the selection of cases, use different cutoffs for positivity, or do not state clearly the distinction of targeted mesothelial cells for the evaluation of staining or its loss. We chose to be conservative in judging the quality of evidence, with all findings downgraded at least 1 point for this reason. Moreover, for some markers, the quality of evidence was downgraded for imprecision due to the limited pooled sample size and/or inconsistency from the inspection of forest plots. We had to balance the pros and cons and keep all the studies with a minimum of available quantitative information to maximize the number of cases; we balanced this with the consideration that the quality of evidence would be critically evaluated in light of this choice. This leads also to future directions for research emerging from this systematic review: because the case-control design and the selection of cases are the main sources of bias, prospective studies or even retrospective studies with uncontrolled selection could allow evidence of a higher quality to be drawn.

In conclusion, our systematic review highlights how IHC showing BAP1 loss and MTAP loss and p16 HD by FISH have high specificity but suboptimal sensitivity when used alone. A combination of BAP1 loss and p16 HD yields a significant increase in MPM detection capability, and this makes these dual biomarkers suitable for rendering a definitive diagnosis. Historical biomarkers such as GLUT1 and IMP3 as well as mesothelin/SMRPs, when used alone, have an unsatisfactory diagnostic performance, and we should not rely on them for diagnosing MPM in pleural effusions.

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