Diagnosis and prognosis—review of biomarkers for mesothelioma

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Abstract: Malignant pleural mesothelioma (MPM) is an aggressive disease arising in pleural cell lining and is associated with asbestos exposure. Today, there is a rising incidence of MPM reaching 3,000 annual cases nationally, primarily from the large population occupationally exposed to asbestos between 1940 and 1980. With a prolonged latency period, presenting clinically 10 to 40 years after exposure, MPM is often diagnosed in late stages and presents median survival time of less than 12 months. There is a serious need for improvement in prognostic and diagnostic tools for MPM. Recent investigation and discovery of various biomarkers has shown promise, including Osteopontin, Fibulin-3, Soluble Mesothelin-Related Proteins (SMRP), High Mobility Group Box 1 (HMGB1), micro-RNA's, peripheral blood-based markers, and Slow Off-rate Modified Aptamer (SOMAmer) proteomic assays. In this review, we explore these current major biomarkers and their prognostic and diagnostic potential, highlighting the most recent large studies and developments for each. While progress has been made in mesothelioma research, many questions remain unanswered. Increased international cooperation is necessary for improving validity of results for current biomarkers through repeated investigation and increasing cohort sizes, as well as for the continued search for new and better markers.

Keywords: Mesothelioma; biomarker; prognosis; diagnosis; asbestos

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Malignant pleural mesothelioma (MPM) is a highly aggressive tumor arising from pleural cell lining and is associated with asbestos exposure. Between 1940 and 1979, approximately 27.5 million people in the United States were occupationally exposed to asbestos. Over the years, there has been a rising incidence of MPM, reaching approximately 3,000 cases annually. In addition, MPM has a prolonged latency period of presenting clinically 10 to 40 years after initial exposure. Most patients are diagnosed with advanced stage disease and have median survival time of less than 12 months (1). Given the increasing incidence of MPM and its lengthy latency period, there is an urgent need for earlier diagnosis and better prognostication.

Currently, the best known clinical prognostic scoring systems for MPM patients are from European Organization for Research and Treatment of Cancer (EORTC) and Cancer and Leukemia Group B (CALGB) (2,3). Specifically, they have found that poor performance status, non-epithelioid histology, male gender, anemia, thrombocytosis, leukocytosis, and elevated LDH were poor prognostic indicators in patients with MPM. Despite the utility of these scoring systems, overall survival remains dismal and there is still a need for better prognostic biomarkers. Over the past decade, advances in molecular biology have led to the identification of several biomarkers in MPM patients with potential to serve as screening tools and for early diagnosis in high-risk populations. Here, we explore the most recent and promising markers that can play a role in improving the

treatment and outlook for future patients.

Soluble mesothelin-related proteins (SMRPs)

SMRPs are found in normal mesothelin cells and are over-expressed in various cancers. They are membrane-bound peptides that can be processed to yield megakaryocyte-potentiating factor (MPF) and mesothelin, which remains attached to the cell membrane via glycophosphatidylinositol linkage (4). Further studies have shown that mesothelin promotes tumor cell survival and proliferation via activation of NF-kB pathway, resulting in increase of interleukin-6 level (5). Hollevoet and colleagues have shown that as a diagnostic marker, mesothelin has high specificity of 96% but low sensitivity of only 47% (6). With regard to prognosis, the results are inconclusive. Several studies have shown no correlation between serum mesothelin level and progression-free or overall survival (7-9). On the other hand, some have shown that at cut off values of 1 and 3.5 nmol/L, SMRP levels are inversely associated with overall survival (10-13). However, in multivariate analysis limited to epithelial MPM, the prognostic impact of SMRP on overall survival was lost. This suggests that histology remains a critical determinant of prognosis. Possible explanations for the mixed results on mesothelin as a prognostic marker include small sample sizes and heterogeneous treatment among the different studies. Therefore, studies with more standardized treatments and larger numbers of patients are needed to better understand the role of SMRP as a prognostic marker.

Osteopontin

Osteopontin is an extracellular cell adhesion protein that mediates cell-matrix interaction and cell-signaling via interaction with integrin and CD44 receptors (14). Studies have shown that osteopontin is up-regulated in cells exposed to asbestos in-vitro, as well as in rat models of asbestos-induced carcinogenesis (15). A landmark study by Pass and colleagues compared 69 patients with asbestos-related non-malignant pulmonary disease with 45 patients without exposure to asbestos and 76 patients with surgically staged pleural mesothelioma (16). They found that serum osteopontin levels were significantly higher in patients with pleural mesothelioma than in those with exposure to asbestos ($P<0.001$). Specifically, with a cutoff value of 48.3 ng/mL, the ROC curve in the group exposed to asbestos compared with the group with mesothelioma had sensitivity of 77.6% and a specificity of 85.5%. Further subgroup analysis showed that at cutoff value of 62.4 ng/mL, ROC curve comparing patients with stage I mesothelioma and patients with exposure to asbestos showed sensitivity of 84.6% and specificity of 88.4%. Collectively, these results initially established osteopontin as a potential diagnostic marker for MPM patients. Unfortunately, results from this study led to a whirlwind of controversy as it was able to be validated in certain studies (17-19) but not in others (10,20). Some potential explanations include the different ELISA assays used for osteopontin and different control populations used, which may not be reflective of high-risk screening populations. Nevertheless, lack of validation in separate cohorts has left the value of osteopontin as a diagnostic marker in question.

Despite controversy over diagnostic value, several studies have investigated osteopontin's potential in prognosis, demonstrating encouraging results. Cappia and colleagues studied immunohistochemical (IHC) expression of osteopontin in short-term and long-term survivors of MPM (21). At a cutoff value of 145 histologic scoring (HScore), they found osteopontin to be an independent prognostic predictor. Similarly, others showed that low baseline plasma osteopontin levels were independently associated with favorable progression-free and overall survival (7). Most recently, Pass and colleagues combined MPM plasma biomarkers with EORTC prognostic index (PI) to determine whether it will improve the risk stratification for MPM patients (22). The authors found that higher levels of osteopontin and mesothelin were individually associated with worse prognosis after adjusting for PI. Using Harrell's C-index to formally assess the predictive ability of the biomarker, they also showed that incorporating either plasma osteopontin or mesothelin into the predictive PI model led to a statistically significant improvement in Harrell's C-statistic. In the final prognostic model, log-osteopontin level, EORTC clinical PI and hemoglobin level remained as independently significant predictors. This further validates the role of osteopontin as a potential prognostic marker in MPM patients.

Fibulin-3

Fibullin-3 is a conserved member of the extracellular glycoprotein fibulin family encoded by the gene epidermal growth factor, containing fibulin-like extracellular matrix protein 1 (EFEMP1) (23). Fibullin-3 has been implicated in involvement with cell morphology, growth, adhesion,

and motility, especially with regard to tumorigenesis (24). Previous studies have investigated fibulin-3 levels in plasma and pleural effusion, as well as fibulin-3 IHC expression in tumor tissues (25). They found that plasma fibulin-3 levels were significantly higher in patients with MPM, compared to those with only asbestos exposure. Similarly, effusion fibulin-3 levels were significantly higher in patients with MPM compared to those with pleural effusion unrelated to MPM. The authors also showed that at a cutoff value of 52.8 ng/mL, the ROC curve for plasma fibulin-3 level in patients with and without MPM had sensitivity of 96.7% and specificity of 95.5%. Collectively, these results have established fibulin-3 as a potential biomarker for patients with MPM, but it still needs to be prospectively validated. While no prospective validation studies have been done for fibulin-3, recent retrospective analysis of two cohorts of patients with MPM showed that plasma fibulin-3 level had low diagnostic accuracy as it was significantly elevated in one (Sydney cohort) but not the other (Vienna cohort) (26). Even though pleural effusion fibulin-3 level was not significantly different between cases and control groups, low levels were significantly associated with prolonged survival and therefore, independently associated with prognosis with a hazard ratio of 9.92. While fibulin-3 still holds promise as a biomarker for MPM patients, further prospective validation is needed.

High-mobility group box 1 (HMGB1)

HMGB1is a typical damage associated molecular pattern (DAMP) and a key mediator of inflammation. Recent studies have shown that asbestos exposure leads to necrosis of primary human mesothelial cells, resulting in release of HMGB-1, which binds to its main receptor and causes Nalp3 inflammasome activation and IL-1b secretion (27-29). This cascade has been linked to asbestos-related carcinogenesis. Studies have shown that higher serum HMGB1 level is found in patients with MPM compared to control group with only asbestos-exposure (no MPM) (30). Furthermore, at a cutoff value of 9 ng/mL, there is significant negative correlation between serum HMGB1 level and survival, suggesting a potential role for HMGB1 as a prognostic marker. Napolitano and colleagues have also shown that total HMGB1 level in blood was significantly higher in MPM patients and asbestos-exposed patients, when compared to healthy controls (31). Specifically, hyperacetylated HMGB1 level was significantly higher in MPM patients, compared with asbestos-exposed patients and healthy controls. At a cutoff value of 2.0 ng/mL, they found that serum hyperacetylated HMGB1 had sensitivity and specificity of 100% in differentiating MPM patients from asbestos-exposed individuals and healthy controls. These results thus suggest a role for hyperacetylated HMGB1 as a potential diagnostic marker to differentiate MPM patients.

Micro-RNA (miRNA)

miRNAs are a family of small non-coding RNAs, approximately 21–25 nt long, responsible for regulating gene expression by inhibiting translation of target messenger RNAs by pairing with messenger RNA recognition elements (32). In recent years, miRNAs from MRM cells or sera have been proposed as new biomarkers. Specifically, Bononi and colleagues analyzed circulating miRNAs from serum samples of MPM patients, asbestos-exposed workers, and healthy subjects (33). Using microarray and RT-qPCR technologies, they identified three circulating miRNAs that were upregulated in MPM patients compared to the control groups—miRNA 197-3p, miRNA-1281 and miRNA 32-3p. They further elucidated that miR-197 down-regulates the FOXO3 gene, while miR-32-3p down-regulates the tumor suppressor gene PTEN and the anti-proliferative factor BTG2, which suggest that these events may participate in MPM carcinogenesis.

Other studies have suggested miRNAs as potential prognostic markers. Pass and colleagues performed microarray analyses on 9 MPM cell lines and 129 fresh-frozen samples from resected MPM patients (34). They found that miRNA-29 expression levels were higher in patients with epithelioid histology. Furthermore, in the epithelial cohort of patients, miRNA-29 was found to be an independent prognostic factor since its higher expression was able to predict a more favorable prognosis (OS 21.6 months) as compared to low expression level (OS 9.1 months). Analysis of the entire cohort of patients, irrespective of histology, showed that miRNA-29 remained an independent predictor of survival, together with stage and lymph node involvement. Collectively, these results suggest that miRNA-29 is an independent prognostic marker for predicting time to progression and time of survival after surgery in MPM patients. More recently, Kirschner and colleagues constructed an miRNA signature from microarray profiling and differential expression of six miRNAs from patients who underwent extra-pleural

pneumonectomy (35). ROC analysis of the six-miRNA signature showed a good prognostic role with AUC of 0.867 and accuracy in survival prediction of approximately 90%. When validated in separate cohort of patients who underwent palliative surgery, the ROC curve reported an accuracy of 71.9%, in which positive patients showed a differential survival benefit of 8.9 months between good and poor prognosis groups (15.4 *vs.* 6.5 months, respectively). These results suggest that the clinical utility of miRNAs should be further explored.

Proteomics

Slow Off-Rate Modified Aptamers (SOMAmers) are short, single stranded deoxynucleotides with ability to bind discrete molecular targets and they have been used in recent studies to develop proteomic assays (36). SOMAmers, as capture reagents, have several advantages over traditional antibody-based immunoassays, including high sensitivity and specificity, dynamic range, accurate quantification and reproducibility, and the ability of measure thousands of human proteins in small volumes of biological samples with low limits of detection (36,37). Utilizing the SOMAmer technology, Ostroff and colleagues discovered a candidate 13 biomarker panel for the detection of MPM in asbestos-exposed individuals with an AUC of 0.95 and an overall accuracy of 92% (38). The candidate biomarker panel consisted of both inflammatory and proliferative proteins, none of which had been previously associated with MPM, but both processes have been implicated in asbestos-induced carcinogenesis. Furthermore, sensitivity of the biomarker panel correlated with pathologic stages, such that 77% of stage I, 93% of stage II, 96% of stage III and 96% of stage IV cases were detected. These results provide the foundation for surveillance and early diagnosis of MPM in high-risk population.

Peripheral blood based markers

Chronic inflammation is critically involved in the pathogenesis of MPM and inflammation-based prognostic scores, such as lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), have been studied as potential prognostic markers. One retrospective review study included 150 patients with biopsy-proven MPM and found that elevated LMR was significantly associated with prolonged overall survival (39). Specifically, patients with LMR greater than 2.74 had median overall survival of 14 months compared to 5 months in patients with LMR less than 2.74. This association between LMR and overall survival was confirmed in the same study using multivariate analysis and led the authors to conclude that LMR is an independent prognostic marker for overall survival in MPM patients (39). With regard to other inflammation-based prognostic scores, such as NLR, the results are somewhat conflicting. In a cohort of consecutive, previously untreated patients diagnosed with MPM, Meniawy and colleagues found that baseline NLR greater than 5 did not predict worse overall survival (40). On multivariate analysis, age, histology, performance status, weight loss, chest pain and platelet count remained significant such that they concluded the EORTC and CALGB prognostic groups were validated as predictive of overall survival, but not NLR (40). On the other hand, there have been several studies that found baseline NLR to be an independent predictor of better survival and this has been subsequently validated in other independent studies (41-46). More recently, one study found that in a cohort of 52 patients diagnosed with MPM, patients with epithelial histology, performance status, NLR less than 5 and positive Wilms' tumor gene (WT1) expression were significant prognostic factors for overall survival on univariate analysis (46). However, on multivariate analysis, only epithelial histology and WT1 expression remained as significant prognostic factors for overall survival (46). Possible explanations to account for the varying results from different studies on NLR include different cut-off values for NLR, heterogeneous patient populations with different treatment modalities and non-randomized allocation with its inherent biases. Therefore, further randomized, prospective validation studies are needed to better elucidate the role of NLR as a prognostic marker for MPM patients.

Conclusions

Recent years have seen a number of developments in biomarker research for malignant mesothelioma prognosis and diagnosis *(Table S1)*. Multiple studies have shown promising results for both new and previously explored markers, with the most potential coming from those most widely investigated, such as osteopontin, SMPR, fibulin-3, HMGB1, and miRNAs. Others are beginning

to be explored as well, including Neutrophil-Lymphocyte Ration (LMR), integrin, BAP-1, calretrin, caveolin-1, and P16-CDKN2A, but these will require much more work and validation in the future. However, common to many studies are limitations such as lack of standardized treatments and assays that may affect results and analysis. Furthermore, low patient numbers in certain studies limit the conclusiveness of results and suggest the need for increased cooperation among research centers in combining cohorts and increasing study sizes.

Malignant mesothelioma is an aggressive disease with diffuse nature, low median survival, and prolonged latency presenting difficulty in prognosis, diagnosis, and treatment. Incidence is increasing annually, as millions exposed to asbestos during the second half of the 20th Century are being diagnosed decades later. Thus, while progress is being made in mesothelioma biomarker discovery and investigation, much work remains to be done for improved prognosis and diagnosis.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Supplementary

Table S1 Recent studies and results by biomarker

Biomarker	Year	Author	N	Prognostic/ diagnostic	Controls	Methods	Results	Conclusions
Osteopontin	2017	Bonotti (47)	56	Clinical response	None	Serum; commercial ELISA kit	Osteopontin change varies w/disease status	Particularly effective for partial response, disease progression
	2016	Pass (22)	194	Prognostic	None	Plasma, adjusted for EORTC PI	Higher osteopontin—worse prognosis; w/EORTC PI improves Harrell's C Index	Independently significant predictor
	2014	Hu (48)	–	Diagnostic	–	Serum, plasma	Diagnostic sensitivity: 0.65; specificity: 0.81	Effective for MPM diagnosis
	2014	Felten (49)	2,262	Diagnostic	Non-asbestos exposed	Blood; Commercial ELISA	Osteopontin rise assoc. w/age	Must account for age specific effects on the biomarker
	2014	Bayram (50)	546	Diagnostic	Pleural plaques, healthy asbestos exposed, unexposed	Serum; Kruskal-Wallis Test	Osteopontin independently assoc. w/MPM, age, smoking pack years; sensitivity: 75; specificity: 86	Combination w/mesothelin distinguishes MPM
Mesothelin	2017	Bonotti (47)	56	Clinical response	None	Serum; commercial ELISA kit	Mesothelin change varies w/disease status	Particularly effective for partial response, disease progression
	2016	Pass (22)	194	Prognostic	None	Plasma, adjusted for EORTC PI	Higher osteopontin—worse prognosis; w/EORTC PI improves Harrell's C Index	Independently significant predictor
	2014	Creaney (9)	153	Diagnostic + prognostic	Non-MM malignant, benign	Plasma, Pleural Fluid; ELISA	Plasma and pleural fluid mesothelin, NLR not independent prognostic for survival	Mesothelin, NLR better as diagnostic markers
	2014	Linch (13)	53	Prognostic	None	Serum, Kruskal-Wallis Test	Low/high mesothelin—PFS: 8.0/5.1 months, OS: 17.2/11.3 months	Serum mesothelin provides prognosis, not correlated w/treatment response
	2014	Felten (49)	2,262	Diagnostic	Non-asbestos exposed	Blood; commercial ELISA	Mesothelin rise assoc. w/age; steep rise w/MPM	Consistent w/Mesothelin rise 6-18 months before clinical symptoms
	2014	Bayram (50)	546	Diagnostic	Pleural plaques, healthy asbestos exposed, unexposed	Serum; Kruskal-Wallis Test	Mesothelin independently assoc. w/MPM, age, smoking pack years, BMI; sensitivity: 58, specificity: 83	Combination w/osteopontin distinguishes MPM
	2013	Creaney (51)	213	Diagnostic	Other malignant, benign, asbestos exposed healthy, kidney disease	Pleural Fluid, serum	Pleural and serum soluble mesothelin elevated in MM relative to all controls	Mesothelin conveys equivalent diagnostic accuracy in pleural fluid and serum
SMRP	2016	Demir (52)	131	Diagnostic	Asbestos exposed, healthy	Serum	SMRP graded increase: control-asbestos-MPM; sensitivity: 0.976, specificity: 0.689	SMRP and w/TRX provide better diagnosis than EGRF, mesothelin, SDC-1, fibulin-3
	2015	Santarelli (53)	188	Diagnostic	Asbestos exposed, healthy	Serum	SMRP w/miR-126 + Met-TM outperforms SMRP in diagnosis	SMRP w/two epigenetic factors overcomes sensitivity limits of SMRP alone
	2014	Filiberti (54)	1,715	Prognostic	None	Blood; ELISA	1- and 2-year samples pre-MPM diagnosis showed no SMPR variation	SMRP is not an early marker for detection at 1 year interval
	2013	Ferro (55)	102	Diagnostic	Non-MPM pleural metastasis, benign	Pleural effusion, serum; MesoMark ELISAs	MPM *vs.* controls—Pleural SMRP: sensitivity, 0.698, specificity, 0.881. Serum SMRP: sensitivity, 0.465; specificity, 0.847	Pleural SMRP has better diagnostic accuracy than serum SMRP
	2013	Filiberti (56)	1,704	Prognostic	Asbestos-related pleural lesions, benign, healthy	Serum; ELISA	Increased SMRP predictors—age >57, current smoking, BMI <25, positive anamnesis for cancer, pleural lesions	SMRP is a candidate marker predictive of mesothelioma
HMGB1	2016	Napolitano (31)	100	Diagnostic	Asbestos exposed, benign effusion, other malignant effusion, healthy	Serum	At 2.0 ng/mL cutoff value, hyperacetylated HMGB1 specificity, sensitivity both 100%; Total HMGB1 (w/non-acetylated) higher	Hyperacetylated HMGB1 is effective for differentiating MPM
	2013	Tabata (57)	58	Diagnostic	Benign Asbestos Diseases	Serum	DMPM patients displayed significantly higher serum HMGB1	Serum HMGB1 useful marker for Diffuse Malignant Peritoneal Mesothelioma
	2013	Tabata (30)	106	Prognostic	Benign asbestos diseases	Serum, ELISA	Higher serum HMGB1 associated with MPM diagnosis, lower OS	Further study of prognostic importance of HMGB1 is warranted
Fibulin-3	2016	Napolitano (31)	100	Diagnostic	Asbestos exposed, benign effusion, other malignant effusion, healthy	Serum	Combined w/HMGB1, increased sensitivity, specificity for differentiating MPM	With HMGB1, is effective differentiating marker for MPM
	2015	Kirschner (26)	–	Diagnostic + prognostic	Benign mesothelial cell lines, non-MPM patients	Plasma, Pleural Fluid; ELISA	Plasma higher in MPM, but diagnostic accuracy low Pleural no significant diff. from controls; but prognostic value	Pleural fibulin-3 independently associated w/prognosis; plasma, pleural not effective for diagnosis
	2015	Kaya (58)	83	Diagnostic + prognostic	Healthy	Serum	Higher in MPM, cutoff 36.6 ng/mL, sensitivity 0.93 specificity 0.90; Prognostic factors—advanced stage, high WBC, platelet, C-Reactive Protein counts	Serum fibulin-3 more useful for diagnosis than prognosis
	2015	Rapisarda (59)		Prognosis	Non fluoro-edenite exposed	Pleural plaques, plasma	Higher plasma fibulin-3 in exposed subjects	Plasma levels show high predictive value for pleural plaques
	2014	Creaney (9)	153	Diagnostic + prognostic	Non-MM malignant, benign	Plasma, pleural fluid; ELISA	Higher pleural fibulin-3 associated w/lower survival	Pleural fibulin-3 independent prognostic factor for survival; not as effective for diagnosis
Integrin ITGA7	2015	Laszlo (60)	200	Prognostic	Non-malignant mesothelial cells	MPM cell lines; genome-wide expression array analysis	Deceased ITGA7 expression in MPM cells at transcription and protein levels; high tumor cell ITGA7 expression—higher OS	ITGA7 is independent prognostic factor; no correlation w/histological type
Neutrophil-lymphocyte ratio (NLR)	2014	Cedres (46)	52	Prognostic	None	Peripheral blood	NLR <5 associated with significant increase in OS	NLR is significant prognostic factor for MPM survival
	2013	Meniawy (40)	274	Prognostic	None	Peripheral blood	Baseline NLR >5 does not predict worse OS Multivariate prognostic—age, histology, performance status, weight loss, chest pain, platelet count	NLR at diagnosis not a significant prognostic factor
Lymphocyte-monocyte ratio (LMR)	2015	Yamagishi (39)	150	Prognostic	None	Peripheral blood	LMR <2.74: OS lower; LMR is independently associated with OS	LMR is independent prognostic marker for MPM OS
Micro RNA	2016	Bononi (33)	30	Diagnostic	Asbestos exposed workers; healthy	Serum; microarray, RT-qPCR	miR-197-3p, miR 1281, miR-32-3p upregulated in MPM	Three mRNAs proposed as potential new biomarkers
	2015	Santarelli (53)	188	Diagnostic	Asbestos exposed; healthy	Serum	miR-126 combined with SMRP, methylated thrombomodulin (Met-TM) differentiates MPM	Combination of the 3 markers serves as early diagnostic; overcomes SMRP low sensitivity limit
	2014	Matsumoto (61)		Diagnostic + prognostic	Reactive mesothelial proliferations	RT-qPCR, formaldehyde-fixed paraffin embedded samples	miR-31 expression reduced in MPM, inverse relationship with OS, particularly sarcomatoid type	miR-31 level may serve as good diagnostic of histological typing and prognostic
	2014	Gayosso-Gómez (62)	92	Diagnostic	Adenocarcinoma; healthy	Serum; Deep Sequencing	7 miRNAs upregulated in MPM, 12 upregulated in AD; 3 unique to MPM—associated w/p38 pathway	Differing upregulation in MPM and AD can be useful for diagnosis; Possible p38 pathway MPM association
	2014	Andersen (63)	45	Diagnostic	Reactive mesothelial Proliferations	RT-qPCR, formaldehyde-fixed paraffin embedded Pre Op biopsy samples	4 miRNA group—miR-126, miR-143, miR145, miR-652 differentiated MPM, sensitivity 0.95, specificity 0.93	All four miRNA's may be suitable for differentiating MPM from RMP's
	2013	Wright (64)	85	Diagnostic	Benign pleura	RT-qPCR, microarrays	2 candidate long ncRNA's separated benign pleura from MPM; high sensitivity, specificity	LncRNA's have potential as MPM biomarkers
BAP1	2016	Hwang (65)	33	Diagnostic	Sarcomatoid carcinoma	Tumor tissue; FISH testing, immunohistochemistry	BAP1 loss by immunohistochemistry insensitive to sarcomatous, desmoplastic mesotheliomas; loss in 15% of tumors	BAP1 loss insensitive, but favors mesothelioma
	2015	Cigognetti (66)	266	Prognostic	Reactive mesothelial proliferations, benign tumors	Tissue; immunohistochemistry, FISH, cytology	BAP1 loss predictive of mesothelial malignancy and most common in epithelioid/biphasic type MM; Differentiation from benign specificity 1.00	BAP1 immunostain is an effective marker for benign and malignant differentiation
	2015	Farzin (67)	229	Prognostic	None	Tissue microarray, immunohistochemistry	BAP1 loss strongly assoc. w/younger onset age, epithelioid differentiation; BAP1 loss predicted improved median survival by 16.11 mo	BAP1 loss IHC may be predictive of prolonged survival
P16-CDKN2A	2016	Hwang (65)	33	Diagnostic	Sarcomatoid carcinoma	Tumor tissue; FISH testing	P16 deletion by FISH in 80% of mesothelioma tumors, 15% sarcomatoid carcinoma; good sensitivity	Potential diagnostic w/BAP1 loss; alone can't differentiate sarcomatous mesothelioma and sarcomatoid carcinoma
	2014	Hwang (68)	18	Diagnostic	None	Pleural, Peritoneal; FISH	Homozygous P16 FISH deletion in mesothelial surface proliferations assoc. w/deletion in mesotheliomas; absence of p16 deletion did not rule out diagnosis	P16 homozygous FISH deletion could provide a diagnostic marker for mesothelioma
Calretrin	2016	Thapa (69)	329	Prognostic	None	Tissue Microarray	Higher expression assoc. with epithelioid histology, better survival	Calretrin expression is an independent predictor of survival; can predict histology
	2014	Cedres (46)	52	Prognostic	None	Immunohistochemistry analysis staining	Higher expression assoc. w/epithelioid histology. No significant increase in OS	Calretrin is not a useful prognostic factor for MPM OS
Caveolin-1	2016	Thapa (69)	329	Prognostic	None	Tissue microarray	Higher expression in mesothelioma; not assoc. with histology or better survival	Caveolin-1 expression is a sensitive MM biomarker; does not predict histology, survival
	2014	Righi (70)	131	Prognostic	–	Immunohistochemistry	CAV1 detected in neoplastic cells of 77% of epithelial, 100% of biphasic, sarcomatoid MPM. Stromal cell CAV1 in 67% of epithelial MPM	CAV1 expression differs by low- to high-grade histotypes Stromal CAV1 expression assoc. w/worse prognosis, particularly MPM epithelial

MPM, malignant pleural mesothelioma.

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