Mesothelioma Biomarkers
Discovery in Search of Validation

Harvey I. Pass, MD[a,*], Marjan Alimi, MD[b], Michele Carbone, MD, PhD[c], Haining Yang, PhD[c], Chandra M. Goparaju, PhD[b]

KEYWORDS

• Mesothelioma • Biomarkers • Asbestos • Prognosis • Diagnosis

KEY POINTS

- Malignant pleural mesothelioma (MPM) is an asbestos-related neoplasm that can only be treated successfully when correctly diagnosed and treated in early stages.
- The asbestos-exposed population serves as a high-risk group that could benefit from sensitive and specific blood- or tissue-based biomarkers.
- The literature of the last 20 years is reviewed to comment on the most promising of the blood- and tissue-based biomarkers.
- SMRP remains as the only validated blood-based biomarker with diagnostic, monitoring, and prognostic value. Other biomarkers, such as calretinin, fibulin 3, and HMGB1, remain under study and need international validation trials with large cohorts of cases and controls to demonstrate any utility.
- To strengthen the development and testing of MPM biomarkers, cohorts for validation must be established by enlisting collaborations from all over the world.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an aggressive type of cancer originating from the serosal surface of the lungs, and is thought to be primarily caused by asbestos exposure and less commonly due to exposure to high-dose radiation and certain mineral fibers. The role of SV40 infection continues to be controversial.[1–8] Although the incidence is low and estimated around 3200 cases per year in the US, the mortality is particularly high due to its aggressiveness and diagnosis at late stages.[9–11] There is approximately 20 to 40 years latency between the time of exposure and clinical diagnosis, during which chronic asbestos exposure creates a persistent inflammatory response (the inflammasome). This response has a myriad of actions on cytokines and reactive oxygen species (ROS) that potentially lead to MPM carcinogenesis pathway.[12–16] Data from various studies strongly support that MPM has a low mutation burden and the most frequently mutated genes involved in MPM pathogenesis are tumor suppressor genes, including BAP1, NF2, and CDKN2A.[17–21]

Histologically, MPM is classified into 3 main different subtypes. The epithelioid subtype characterizes the most common and least aggressive type, consisting approximately of 70% of MPMs. The sarcomatoid subtype is the most aggressive type and is highly resistant to chemotherapy and associated with the worst prognosis. The biphasic subtype denotes an intermediate type corresponding to a transition between the other 2 histologic subtypes.[1,9] Kadota and colleagues[22] reported the median survival length for epithelioid, biphasic and sarcomatoid subtypes to be 16.2, 7.0, and 3.8 months, respectively.

[a] Research, Department of Cardiothoracic Surgery, General Thoracic Surgery, NYU Langone Medical Center, 530 First Avenue, 9V, New York, NY 10016, USA; [b] Department of Cardiothoracic Surgery, NYU Langone Medical Center, 530 First Avenue, 9V, New York, NY 10016, USA; [c] Department of Thoracic Oncology, John A. Burns School of Medicine, University of Hawaii Cancer Center, 701 Ilalo Street, Room 437, Honolulu, HI 96813, USA
* Corresponding author.
E-mail address: harvey.pass@nyumc.org

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Early-stage MPM is associated with significantly better outcome and overall survival (OS) compared with late-stage disease that is minimally responsive to aggressive multimodality therapy with surgery, chemotherapy, and radiation therapy.[1,23–28] Unfortunately, the former group constitutes only 5% of the patients with MPM and, therefore, the average survival is about 13 months in all patients.[9] Given that early diagnosis is of critical importance in improving the outcome and chance for cure, extensive research has focused on identifying optimal screening strategies for populations at risk.[29,30] This is particularly important since, contrary to common assumption, asbestos continues to be used worldwide and thus MPM rate is not expected to decrease. An optimal screening test is noninvasive, easily accessible, concomitantly highly sensitive and specific (highly accurate), and ideally cost-effective. Although imaging methods are mostly noninvasive, current modalities, including computed tomography scans, are found to be ineffective and nonspecific in early diagnosis of MPM.[31] Pleural biopsy remains the diagnostic method of choice for MPM, but introduces potential morbidity and cost.[32] As a result, there is a critical need for effective noninvasive screening methods for high-risk, asbestos-exposed populations to assist in diagnosis and treatment of patients with MPM at earlier stages. Accordingly, over the past 2 decades, biomarkers have gained a high level of attention and have been extensively studied (**Table 1**).

Mesothelin, Soluble Mesothelin-Related Proteins, and Megakaryocyte Potentiating Factor

Mesothelin (MSLN), a protein believed to have a role in cellular adhesion, was the first biomarker studied for MPM and was originally discovered and described by Chang and Pastan.[33] After initial translation into a precursor protein, MSLN and megakaryocyte potentiating factor (MPF) are formed by cleavage of the preprotein into 41K and 30K molecules, respectively.[34–36] MSLN was found to activate the nuclear factor κB pathway, thereby promoting cell proliferation and survival.[37] Although low levels of MSLN are produced by normal mesothelial cells, overexpression has been seen in certain cancers, including MPM, pancreatic adenocarcinoma, and ovarian and lung cancers.[38,39] Soluble mesothelin-related peptide (SMRP) is a soluble form of MSLN released by tumor cells into the circulation and is the only Food and Drug Administration-approved biomarker for diagnosis of MPM.[40,41] Following the original description of SMRP by Hellstrom and colleagues,[34] it was commercialized by Fujirebio.[35,42] The diagnostic value of SMRP in MPM was first studied and described by Robinson and colleagues.[35] After technical and clinical validation of the MESOMARK assay in serum and pleural effusion (PE) of North American population,[42,43] the serum level of SMRP was found to be capable of differentiating patients with MPM from asbestos-exposed and asbestos-unexposed individuals, as well as those with benign pleural diseases.[44–47] These early studies were followed by an international blinded trial of MESOMARK levels in 165 MPM cases and 652 asbestos-exposed controls, where serum samples from Australia and North America were independently measured by Fujirebio and the UCLA Early Detection Research Laboratory Biomarker Reference Laboratory. Discrimination between cases and controls was validated at both sites with very similar sensitivity and specificity, as seen in **Fig. 1**, and later studies confirmed these findings.[42] In a systematic review and meta-analysis of SMRP for MPM discrimination, Luo and colleagues[48] pooled data from 12 studies with 717 patients with MPM and 2851 controls (including healthy individuals and patients with non-MPM diseases) and found 64% sensitivity and 89% specificity for serum SMRPs in diagnosis of MPM. On the other hand, Hollevoet and colleagues[49] performed a meta-analysis of 16 studies with 1026 MPM cases and 4491 controls that revealed 32% sensitivity and 95% specificity for MSLN in diagnosis of MPM. Other studies suggested the potential capability of MSLN in discriminating between MPM and pleural metastases.[42,45,47]

Utility of SMRP in screening for MPM in high-risk asbestos-exposed population was further explored in a subset of patients from Beta-Carotene and Retinol Efficacy Trial (CARET), a large study in which the potential role of vitamin supplementation in chemoprevention of lung cancer was investigated. A total of 4060 heavily asbestos-exposed US men were followed for 9 to 17 years, among whom 49 developed MPM while on the CARET. Forty-nine MPM cases and 96 matched controls were studied to elucidate whether SMRP was able to predict development of MPM years before the clinical presentation. Accordingly, serum markers were measured blindly at 2 separate sites, and unsurprisingly showed an overall ROC (receiver operating curve) AUC (area under the curve) of 0.604 (95% CI, 0.489–0.699), likely given that MPM markers may not be increased several years before the diagnosis. Conversely, serum SMRP levels measured less than a year before diagnosis had an AUC of

Table 1
Summary of major biomarkers for diagnosis of MPM

Biomarker	Author, Year	N (MPM/Total)	Compared Groups	Method	Results	Conclusion
Mesothelin	Creaney et al,[64] 2014	82/153	Non-MPM malignant, benign	Plasma, pleural fluid; ELISA (mesothelin and fibulin-3)	Mesothelin showed high diagnostic accuracy for MPM Plasma AUC, 0.822 Pleural AUC, 0.815	Mesothelin is a superior diagnostic biomarker for MPM compared with fibulin-3
	Bayram et al,[239] 2014	24/546	Pleural plaques, healthy asbestos exposed, healthy unexposed	Serum; ELISA (mesothelin and osteopontin)	Mesothelin level was independently associated with MPM, age, smoking pack years, and BMI. It differentiated MPM from other groups Sensitivity, 58% Specificity, 83%	Combination of mesothelin with osteopontin provides higher diagnostic accuracy
	Creaney et al,[40] 2013	66/213	Other malignant, benign, asbestos exposed, healthy, kidney disease	Pleural fluid, serum ELISA	Serum and pleural mesothelin was increased in MPM compared with all controls Serum AUC, 0.829 Pleural AUC, 0.928	Mesothelin conveys diagnostic accuracy in both serum and pleural fluid (equivalent to MPF
	Hollevoet et al,[49] 2012	1026/5517	Non-MPM (various controls)	Review and meta-analysis	At 95% specificity, sensitivity was 32% AUC, 0.77 (95% CI, 0.73–0.81)	Mesothelin is highly specific for diagnosis of MPM, but lacks adequate sensitivity for screening
SMRP	Burt et al,[54] 2017	102	–	Serum, ELISA	Percentage of change in serial postop SMRP values at cutoff of 48%, was highly predictive of disease recurrence AUC, 0.96	Serial SMRP level measurements can aid in detection of recurrence after resection of MPM
	Demir et al,[98] 2016	42/131	Asbestos exposed, healthy	Serum (various markers, including SMRP, thioredoxin-1 (TRX), EGFR, mesothelin, syndecan-1, fibulin-3)	SMRP showed graded increase: control-asbestos-MPM, and was able to distinguish MPM from other groups AUC, 0.86	SMRP and TRX provide better diagnostic accuracy than EGFR, mesothelin, syndecan-1, fibulin-3

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Biomarker	Author, Year	N (MPM/Total)	Compared Groups	Method	Results	Conclusion
	Santarelli et al,[144] 2015	45/188	Asbestos exposed, healthy	Serum (various markers, including SMRP, miR-126 and methylated thrombomodulin [Met-TM])	Combination of SMRP, miR-126 and Met-TM has higher diagnostic accuracy compared with isolated SMRP AUC, 0.857 vs 0.818	Combined panel of SMRP with other biomarkers improves diagnostic value of SMRP for MPM
	Filiberti et al,[50] 2013	–/1704	Asbestos-related pleural lesions, benign, healthy	Blood, ELISA	Predictors of increased SMRP were age >57, current smoking, BMI <25, positive anamnesis for cancer and for asbestos-related pleural lesions	SMRP is a candidate marker predictive of MPM
	Hollevoet et al,[55] 2011	215(->179->137)	–	Serum, ELISA	SMRP and MPF showed a high intraclass correlation Single biomarker measurement and fixed threshold are suboptimal in screening	Biomarker-based screening approach can be improved by incorporation of serial measurements and adjustment for age and GFR
	Hollevoet et al,[69] 2010	85/507	Healthy, healthy asbestos-exposed, benign asbestos-related disease, benign respiratory disease, lung cancer	Serum; MesoMark ELISAs (MPF and SMRP)	SMRP (and MPF) levels were significantly higher in MPM compared with all other groups AUC for SMRP, 0.871 (AUC for MPF, 0.849)	SMRP has shown to be a highly performant MPM biomarker
	Luo et al,[48] 2010	717/3568	Non-MPM (various controls)	Review and meta-analysis	SMRPs had a pooled sensitivity of 0.64 (95% CI, 0.61–0.68), specificity of 0.89 (95% CI, 0.88–0.90), positive likelihood ratio of 7.10 (95% CI, 4.44–11.35), negative likelihood ratio of 0.39 (95% CI, 0.31–0.48), and diagnostic odds ratio of 19.35 (95% CI, 10.95–34.17)	Serum SMRP level is a candidate biomarker for diagnosis of MPM
	Rodriguez Portal et al,[46] 2009	36/362	Healthy, asbestos exposed without pleural disease, asbestos exposed with benign pleural disease	Serum; ELISA	Serum SMRP levels were higher in MPM compared with other groups AUC, 0.75	Serum SMRP level is a potential biomarker for diagnosis of MPM

	Pass et al,[43] 2008	90/326	Lung cancer, asbestos exposed	Serum, pleural effusion; MesoMark ELISAs	Serum SMRP levels were higher in MPM compared with asbestos exposed AUC, 0.81 SMRP levels were higher in stages 2–4 MPM compared with stage 1 MPM	Serum and pleural SMRP levels can be used in screening asbestos-exposed individuals
	Pass et al,[53] 2008	30/85	–	MesoMark ELISAs (response to therapy with a copper reducing agent tetrathiomolybdate, assessed by target ceruloplasmin levels and VGEF levels)	SMRP levels decreased immediately postsurgery and increased over time during progression of disease	SMRPs can have a role in monitoring posttreatment patients with MPM
	Park et al,[51] 2008	–/538	–	Serum; ELISA	Mean SMRP in healthy subjects was significantly lower than in subjects with pleural plaques	SMRP has a high false positive rate and seems unlikely to prove useful in screening for MPM
	Scherpereel et al,[45] 2006	74/137	Pleural metastasis of carcinomas, benign pleural lesions associated with asbestos exposure	Serum, pleural effusion; ELISA	SMRP level was higher in MPM than in metastasis or benign lesions Serum AUC for differentiating MPM and benign, 0.872 Serum AUC for differentiating MPM and metastasis, 0.693 Pleural AUC for differentiating MPM and benign, 0.831 Pleural AUC for differentiating MPM and metastasis, 0.793	Serum and pleural SMRP levels can be used as biomarkers in diagnosis of MPM
	Robinson et al,[35] 2003	44/272	Healthy, asbestos exposed, other inflammatory or malignant lung and pleural diseases	Serum; ELISA	SMRP levels were increased in the vast majority of patients with MPM. Increased SMRP levels in asbestos-exposed individuals may predict development of MPM SMRP concentrations correlated with tumor size and progression	SMRP level can be helpful in diagnosis of MPM and screening of asbestos-exposed high-risk individuals
MPF	Creaney et al,[40] 2013	66/213	Other malignant, benign, asbestos exposed, healthy, kidney disease	Pleural fluid, serum; ELISA	Serum and pleural MPF were increased in MPM compared with all controls Serum AUC, 0.813 Pleural AUC, 0.945	MPF conveys diagnostic accuracy in both serum and pleural fluid (equivalent to mesothelin)
	Hollevoet et al,[69] 2010	85/507	Healthy, healthy asbestos-exposed, benign asbestos-related disease, benign respiratory disease, lung cancer	Serum; MesoMark ELISAs (MPF and SMRP)	MPF (and SMRP) levels were significantly higher in MPM compared with all other groups AUC for MPF, 0.849 (AUC for SMRP, 0.871)	MPF is validated as a highly performant MPM biomarker (equivalent to SMRP)

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Biomarker	Author, Year	N (MPM/Total)	Compared Groups	Method	Results	Conclusion
	Onda et al,[68] 2006	56/126	Healthy	Serum, ELISA (MPF and SMRP)	MPF level was increased in 91% of patients with MPM compared with healthy controls	MPF can aid in diagnosis of MPM
Osteopontin	Hu et al,[89] 2014	360/906	Non-MPM (various controls)	Review and meta-analysis	Osteopontin pooled diagnostic sensitivity and specificity for MPM was 0.65 and 0.81, respectively AUC, 0.83	Osteopontin is an effective biomarker for MPM diagnosis
	Bayram et al,[239] 2014	24/546	Pleural plaques, healthy asbestos exposed, healthy unexposed	Serum; ELISA (osteopontin and mesothelin)	Osteopontin level was independently associated with MPM, age, smoking pack years, and BMI. It was able to differentiate MPM from other groups Sensitivity, 75% Specificity, 86%	Combination of osteopontin with mesothelin provides higher diagnostic accuracy
	Felten et al,[82] 2014	–/2262	Formerly asbestos exposed, unknown history of asbestos exposure, nonasbestos exposed	Blood; commercial ELISA (osteopontin and mesothelin)	Osteopontin rise was associated with age	Age effects on biomarkers need to be taken into account
	Creaney et al,[88] 2011	66/176	Nonmalignant asbestos-related lung or pleural disease, other benign pleural and lung diseases, lung cancer	Serum and plasma (osteopontin and mesothelin)	Serum and plasma osteopontin levels were significantly higher in patients with MPM compared with benign lung and pleural disease AUC for serum, 0.639 AUC for plasma, 0.763 Combining the serum mesothelin and plasma osteopontin did not increase AUC	Plasma osteopontin has a superior diagnostic accuracy to serum osteopontin
	Cristaudo et al,[84] 2011	31/235	Healthy, benign respiratory disease	Plasma; ELISA (plasma osteopontin and serum SMRP)	Plasma osteopontin level was significantly higher in patients with MPM compared with other groups AUC, 0.795	Combined osteopontin and SMRP panel provides a high accuracy for diagnosis of MPM AUC, 0.873
	Rai et al,[86] 2010	205/286	Healthy, nonmesothelioma other patients with cancer	Plasma; ELISA (plasma osteopontin and serum SMRP)	Osteopontin level was significantly higher in patients with MPM compared with other groups AUC for osteopontin, 0.68 AUC for SMRP, 0.89	Both osteopontin and SMRP can be used as biomarkers in diagnosis of MPM

	Grigoriu et al,[61] 2007	96/284	Pleural metastases of various carcinomas, benign pleural lesions associated with asbestos exposure, asbestos-exposed healthy	Serum, pleural fluid; ELISA	Osteopontin was able to distinguish MPM from healthy asbestos exposed (AUC, 0.724); however, could not distinguish between MPM and pleural metastatic carcinoma or benign pleural lesions associated with asbestos exposure	Insufficient specificity limits osteopontin utility as a diagnostic marker
	Pass et al,[80] 2005	76/190	Asbestos-related nonmalignant pulmonary disease, healthy nonasbestos exposed	Serum; ELISA	Serum osteopontin level was able to distinguish patients with MPM from asbestos-exposed patients with high sensitivity and specificity AUC, 0.888	Serum osteopontin can be used a biomarker for diagnosis of MPM in asbestos-exposed individuals
Fibulin-3	Pei et al,[103] 2017	468/1132	Non-MPM (various controls)	Review and meta-analysis	Serum fibulin-3 level had a pooled sensitivity of 62% (95% CI, 45%–77%) and specificity of 82% (95% CI, 73%–89%) for in diagnosis of MPM AUC, 0.81	Fibulin-3 has a relatively high diagnostic efficacy for identification of MPM
	Napolitano et al,[183] 2016	22/100	Asbestos exposed, benign effusion, other malignant effusion, healthy	Serum; ELISA	Fibulin-3 was able to distinguish MPM with high accuracy AUC, 0.959 Combining fibulin-3 with HMGB1 resulted in higher sensitivity, and specificity for differentiating MPM	Combined panel of fibulin-3 and HMGB1, is effective in diagnosis of MPM
	Kaya et al,[100] 2015	43/83	Healthy	Serum; ELISA	Serum fibulin-3 level was significantly higher in patients with MPM compared with controls AUC, 0.976	Serum fibulin-3 is a useful biomarker for diagnosis of MPM
	Creaney et al,[64] 2014	82/153	Non-MM malignant, benign	Plasma, pleural fluid; ELISA (fibulin-3 and mesothelin)	Fibulin-3 showed lower diagnostic accuracy for MPM compared with mesothelin Plasma AUC, 0.671 Pleural AUC, 0.588	Pleural fibulin-3 is an independent prognostic factor for survival; not as effective for diagnosis

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Biomarker	Author, Year	N (MPM/Total)	Compared Groups	Method	Results	Conclusion
	Pass et al,[96] 2012	92/364	Asbestos exposed without cancer, patients with effusions not due to mesothelioma, healthy controls	Plasma, pleural fluid; ELISA	Plasma fibulin-3 was significantly higher in MPM compared with asbestos-exposed persons without mesothelioma AUC, 0.99 Effusion fibulin-3 was significantly higher in MPM compared with effusions not due to mesothelioma AUC, 0.93	Plasma fibulin-3 can aid in diagnosis of MPM in asbestos-exposed individuals Pleural fibulin-3 can better aid in differentiation of MPM from other pathologies
Proteomic	Ostroff et al,[107] 2012	117/259	Asbestos exposed	Serum; SOMAmer proteomic technology	The identified 13-marker SOMAmer panel was able to accurately distinguish patients with MPM AUC, 0.99 It showed better performance than mesothelin Sensitivity correlated with pathologic stage	SOMAmer biomarker panel provides a strong surveillance method for diagnosis of MPM in population at risk
Glycomic	Cerciello et al,[111] 2013	23/75	Healthy, non-small cell lung cancer (NSCLC)	Serum; selected reaction monitoring (SRM) assay technology (glycopeptides and mesothelin)	The identified 7-glycopeptide signature discriminated patients with MPM from healthy donors (AUC, 0.94), but not from patients with NSCLC	Glycomic technology can provide a helpful diagnostic tool for MPM in adjunction with other biomarkers
miRNAs	Sun et al,[150] 2018	93/146	Asbestos exposed	Serum; HTG EdgeSeq miRNA whole transcriptome assay	An identified 7-miRNA signature was able to differentiate MPMs from asbestos exposed AUC, 0.953	The 7-miRNA signature can aid in early diagnosis of MPM
	Bononi et al,[151] 2016	10/30	Asbestos-exposed workers; healthy	Serum; microarray, real-time qPCR	miR-197-3p, miR 1281, miR-32-3p upregulated in MPM	Distinct mRNAs are potential new biomarkers for diagnosis of MPM
	Santarelli et al,143 2015	45/188	Asbestos exposed, healthy	Serum (various markers, including miR-126, SMRP, and Met-TM)	Combination of miR-126, SMRP, and Met-TM has higher diagnostic accuracy compared to isolated SMRP AUC, 0.857 vs 0.818	Combined 3 biomarker panel including miR-126, provides high diagnostic accuracy for MPM
	Andersen et al,[148] 2014	45/76	Reactive mesothelial proliferations (RMP)	Tissue Real-time qPCR, formaldehyde-fixed paraffin embedded preoperative biopsy samples	A 4 miRNA group including miR-126, miR-143, miR145, miR-652 was able to accurately differentiate MPM AUC, 0.96	The identified 4 miRNA group can aid in differentiation of MPM from RMPs

	Tomasetti et al,[143] 2012	45/121	NSCLC, healthy	Serum real-time qPCR	miR-126-3p was able to distinguish patients with MPM from healthy controls (AUC, 0.894) and NSCLC (AUC, 0.751)	miR-126-3p can serve as a diagnostic (and prognostic) biomarker for MPM
	Busacca et al,[139] 2010	24/24	–	Tissue; miRNA microarray analysis; real-time qPCR	Analysis of MPM specimen revealed overexpression of miR-17-5p, miR-21, miR-29a, miR-30c, miR-30e-5p, miR-106a, and miR-143. Certain miRNA correlate with specific pathologic subtypes	The identified miRNA points can be helpful diagnostic and prognostic biomarkers
	Guled et al,[140] 2009	17/17	–	Tissue; miRNA microarray analysis	miRNA microarray analysis of MPM revealed overexpression of miR-30b, miR-32, miR-483-3p, miR-584, and miR-885-3p and downregulation of miR-9, miR-7-1, and miR-203	Certain combination of miRNA points can serve as diagnostic biomarkers for MPM
DNA methylation	Guarrera et al,[170] 2019	163/300	Cancer-free asbestos exposed	Blood; genome-wide methylation array technique	The identified set of methylation markers was able to distinguish MPM AUC, 0.81–0.89	Blood DNA methylation array can serve as a complementary tool in screening high-risk group for MPM
HMGB1	Ying et al,[182] 2017	15/497	Healthy, asbestos exposed <10 y, asbestos exposed >10 y, pleural plaques, diagnosed with asbestosis	Serum, ELISA	HMBG1 was able to differentiate MPM from all other groups (except for asbestosis) with high sensitivity and specificity AUC for differentiating MPM from healthy, 0.94	HMBG1 is a potential biomarker for diagnosis of MPM in asbestos-exposed population
	Napolitano et al,[183] 2016	22/100	Asbestos exposed, benign effusion, other malignant effusion, healthy	Serum	Fibulin-3 was able to distinguish MPM with high accuracy AUC, 0.959 Combining fibulin-3 with HMGB1 resulted in higher sensitivity, and specificity for differentiating MPM	Combined panel of fibulin-3 and HMGB1, is effective in diagnosing MPM
Calretinin		34/170	Healthy	Plasma, ELISA		

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Biomarker	Author, Year	N (MPM/Total)	Compared Groups	Method	Results	Conclusion
	Johnen et al,[199] 2018				Calretinin was able to distinguish MPM from controls AUC, 0.74 Combining calretinin and mesothelin resulted in higher performance AUC, 0.83	Calretinin is highly specific but not very sensitive for MPM. Calretinin-mesothelin combined panel can provide a test with high performance in diagnosis of MPM
	Johnen et al,[196] 2017	199/434	Healthy	Serum/plasma, ELISA	Calretinin was able to differentiate MPM from controls with high sensitivity and specificity AUC, 0.87–0.95 depending on the county of origin	Calretinin can serve as a biomarker for diagnosis of MPM along with other markers
	Raiko et al,[195] 2010	42/174	Asbestos exposed, healthy	Plasma, ELISA	Calretinin was significantly higher in MPM compared with asbestos exposed and healthy (no AUC provided)	Calretinin has high sensitivity in diagnosis of MPM and can be used a biomarker for diagnosis of MPM
TRX	Demir et al,[98] 2016	42/131	Asbestos exposed, healthy	Serum (various markers, including TRX, SMRP, EGFR, mesothelin, syndecan-1, fibulin-3)	TRX (and SMRP) showed graded increase: control-asbestos-MPM, and was able to distinguish MPM from other groups AUC, 0.72	TRX and SMRP provide better diagnostic accuracy than EGFR, mesothelin, syndecan-1, fibulin-3

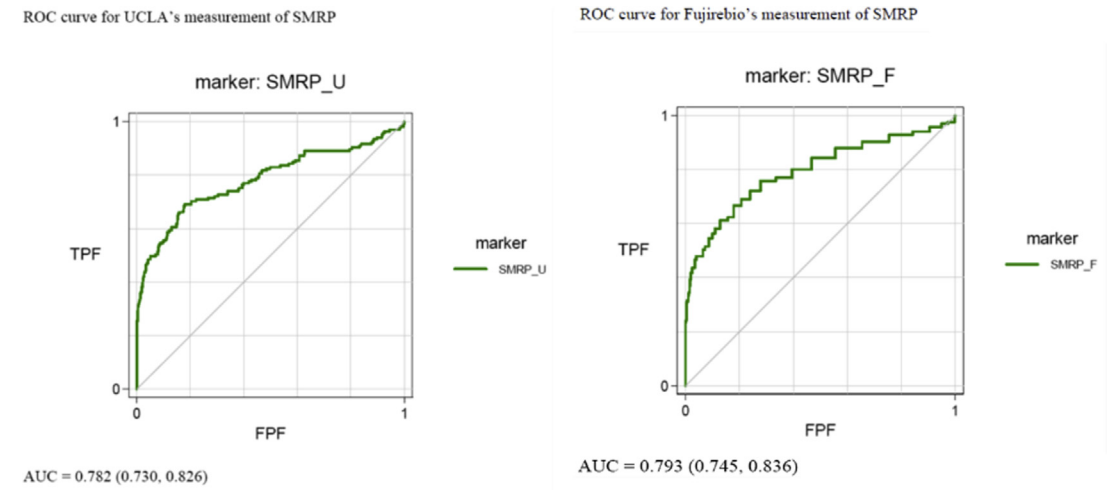

Fig. 1. EDRN validation trial results for SMRP as an MPM biomarker.

0.720 (95% CI, 0.562–0.853) with statistical significance, revealing evidence that SMRP can be increased within a year before diagnosis (Fig. 2). Analysis of data at 1- to 2-year intervals, however, did not show a statistically significant AUC, most likely due to small case number. Other studies similarly show an increase in SMRP levels in the year before diagnosis, but SMRP levels lack adequate sensitivity to serve as a reliable standalone screening test, and current evidence collectively suggests that it lacks sufficient sensitivity to be used for screening in high-risk population.[50–52]

SMRP has been found to be valuable in monitoring therapy. In a study of 28 patients with MPM undergoing therapy with copper reducing agent tetrathiomolybdate,[53] MESOMARK assays were used to measure serum SMRP levels during the therapeutic period and over the progression time. SMRP levels significantly decreased immediately after surgery. Interestingly, a gradual increase over time was seen in 82% of patients with progressive disease, whereas such increases were not observed in 45.5% of patients with stable disease, the difference of which was statistically significant. Utility of SMRP in monitoring therapy was subsequently validated by other studies.[54–58] Schneider and colleagues[59] found that, at a cutoff value of 1.35 nmol/L, SMRP levels inversely correlated with OS. Nonetheless, prognostic value of SMRP on OS was lost in multivariate analysis limited to epithelial MPM. Conversely, Burt and colleagues[54] showed that, in patients with epithelial histology, serum SMRP level significantly decreased immediately after macroscopic complete resection, and preoperative SMRP levels were independently associated with poor disease-free survival. Specifically, the study showed that percentage change in serial postoperative SMRP values at cutoff at 48% was able to predict disease recurrence with 90% sensitivity and 93% specificity. Three prospective studies with a total of 304 patients with MPM, also showed that high baseline SMRP levels were significantly associated with shorter survival.[59–61] In addition, Creaney and colleagues[57] showed that postchemotherapy decrease in SMRP strongly correlated with longer survival. A meta-analysis of 8 studies with 579 patients with MPM showed that serum

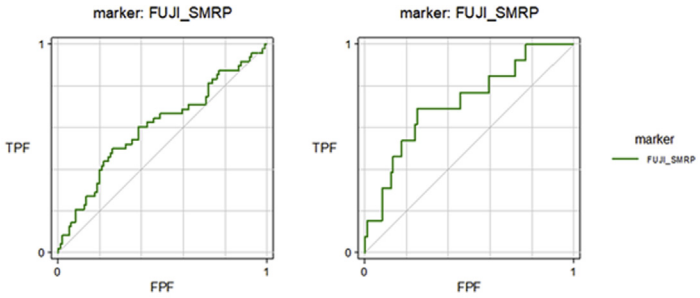

Fig. 2. ROC curve for SMRP, all cases, AUC = 0.604 (95% CI, 0.489, 0.699); ROC curve for SMRP, cases less than 1 year before diagnosis, AUC = 0.720 (95% CI, 0.562, 0.853).

SMRP level significantly correlated with survival.[62] Overall, current evidence suggest good prognostic value for SMRP.

Currently, the prognostic role of MSLN in MPM is inconclusive. Several studies showed no association between serum MSLN level and progression-free survival or OS.[52,63,64] On the other hand a prospective study of 41 patients with MPM showed that a 10% increase in serum MSLN was able to predict radiographic progression with 96% sensitivity. In addition, a rising MSLN level at 6 months was associated with significantly worse OS compared with stable or falling levels (175 versus 448 days, respectively).[65] A systematic review of 8 studies showed that serum MSLN levels correlated with radiographic progression and survival.[66]

MPF has not been studied in as much detail as the other mesothelin-related markers. Previous studies showed that serum MPF levels were significantly higher in patients with MPM compared with patients with benign asbestos-related diseases, lung cancer, or healthy individuals.[67,68] Current evidence suggests that MPF and SMRPs have comparable diagnostic performance in differentiating MPM from other diseases.[40,69] Nonetheless, a key limitation in using SMRPs and MPF for diagnosis of MPM is that their levels can significantly be affected by many physiologic and/or pathologic factors, including age, renal function, and body mass index (BMI).[55,63,70,71]

Osteopontin

Osteopontin (OPN) is a glycoprotein secreted into the extracellular matrix that has major roles in several physiologic processes, including cell-matrix interaction, cell migration via integrin and CD44 receptors, immunologic regulation, as well as tumor development.[72–76] Increased serum OPN levels are seen in various cancers, such as colon, breast, and lung cancer, and in MPM.[77–82]

Both in vitro asbestos-exposed cells and in vivo rat models for asbestos-induced carcinogenesis, exhibited upregulation of OPN.[83] Pass and colleagues[80] were the first to discover and describe the potential role of OPN in MPM pathogenesis, using HG1 Affymetrix array to detect a 9-fold increase in OPN RNA expression in 48 MPMs compared with matched normal peritoneum as a normal mesothelial control. The early study used a commercially available OPN ELISA and found serum OPN levels to be significantly higher in patients with MPM compared with asbestos-exposed and asbestos-unexposed individuals, although the last 2 were not significantly different. Furthermore, OPN showed high accuracy in differentiating stage I patients with MPM from asbestos-exposed individuals, but it was not able to distinguish MPM from other asbestos exposure-related pathologies.[61] Despite initial promising results, due to low specificity and conflicting results of later studies, utility of OPN in early diagnosis of MPM became contentious.[61,84–87] Two studies showed that OPN had higher diagnostic performance when measured in serum compared with plasma, likely due to higher stability in the serum.[84,88] A meta-analysis of 6 studies with 360 patients with MPM and 546 non-MPM controls found a pooled sensitivity of 65% and specificity of 81% for OPN, with an AUC of ROC of 0.83.[89]

Although collective data support the utility of OPN as an adjunctive biomarker in diagnosis of MPM, its prognostic role might be more prominent. Cappia and colleagues[90] investigated the expression of OPN in short-term and long-term survivors of MPM and found that, at a cutoff value of 145 histologic scoring (HScore), OPN was an independent prognostic predictor for MPM. Furthermore, other studies showed independent association of low baseline plasma OPN levels with favorable progression-free survival and OS in patients with MPM.[52,61] Grigoriu and colleagues[61] found serum OPN and serum MSLN to be independent prognostic factors in MPM. In addition, Hollevoet and colleagues[52] conducted a study on 45 patients with MPM during and after chemotherapy, and compared OPN levels in patients with stable disease, partial response, and progressive disease. Multivariate analysis confirmed an association of unfavorable prognosis with increased OPN. The landmark study by Pass and colleagues[91] was an international blinded study with a discovery set of 83 MPMs from the US and a validation set of 111 MPMs from Canada that expanded prognostic accuracy of MPM by using OPN and MSLN as biomarkers. In both sets, there were individual associations between higher levels of OPN and MSLN and worse prognosis. Consequently, plasma OPN or MSLN were incorporated into a baseline predictive prognostic index model, resulting in substantially and statistically significant improvement in Harrell's C-statistic. The final combined model consisting of log-OPN, the EORTC clinical prognostic index, and hemoglobin was an independently significant predictor of survival. Furthermore, the combined biomarker and clinical model significantly improved Harrell's C-index from the clinical model, from 0.718 (0.67–0.77) to 0.801 (0.77–0.84). Of note, recent studies showed a prognostic value for OPN in malignant peritoneal mesothelioma.[92] Bonotti and colleagues[93] performed consecutive

measurements of OPN, SMRP, and vimentin during follow-up of 56 patients with MPM and assessed their response to therapy. They found that percentage differences between 2 consecutive measurements of each of those biomarkers, significantly correlated with the clinical course, specifically disease category: stable disease, partial response, and disease progression.

Fibulin-3

Fibulin-3 (FBLN3) is a glycoprotein encoded by the epidermal growth factor (EGF)-containing fibulin-like extracellular matrix protein-1 (EFEMP-1) gene and belongs to a family of extracellular proteins expressed in the basement membranes of blood vessels. It is involved in cell growth, adhesion, motility, and particularly tumorigenesis.[94,95] An early study using an HG1 Affymetrix array showed a 7-fold increase in EFEMP1 RNA expression ($P = 10^{-9}$) in 48 MPMs compared with matched normal peritoneum.[96] Tert-transformed mesothelial cell transfection with EFEMP1 exhibits increased proliferation, colony formation, and migration, whereas siRNA FBLN3 transfection into 2 MPM cell lines reveals the opposite functional characteristics (Pass H,unpublished data, 2017). Using the only available ELISA in 2012 (Cloud Clone, China), Pass and colleagues[96] found FBLN3 levels capable of differentiating patients with MPM from healthy asbestos-exposed controls and those affected by other types of cancers. In the study set, a plasma FBLN3 level cutoff of 52.8 ng/mL exhibited 96.7% sensitivity and 95.5% specificity in differentiating patients with and without MPM. Although the blinded validation cohort from the Princess Margaret Cancer Center corroborated earlier results, the level of accuracy was slightly lower.[96] In addition, they found that PE FBLN3 level could discriminate patients with MPM from those with PEs unrelated to MPM. Moreover, the pleural effusion FBLN3 level had prognostic value, with patients having high levels surviving shorter times than those with low levels.[96] Agha and colleagues[97] compared serum and PE FBLN3 levels in 25 patients with MPM, 11 patients with metastatic pleural carcinoma (Mets) and 9 patients with benign PEs and observed similar results to those of Pass and colleagues[96]: serum and PE FBLN3 levels were significantly higher in patients with MPM compared with those with metastatic effusion of carcinoma or benign pleural effusion. At cutoff points of 150 and 66.5 ng/mL, PE and serum FBLN3 levels successfully discriminated between patients with MPM and patients with Mets, respectively (PE AUC, 0.878; serum AUC, 0.776). Moreover, PE and serum FBLN3 levels were capable of distinguishing patients with MPM from those with benign PEs at cutoff points of 127.5 and 18 ng/mL, respectively (PE AUC, 0.909; serum AUC, 0.931).[97] In a later study, including 42 patients with MPM, serum FBLN3 levels were significantly higher in patients with MPM compared with asbestos-exposed individuals and healthy controls.[98] Jiang and colleagues[99] also explored the diagnostic role of FBLN3 in MPM, and reported that serum FBLN3 was able to distinguish patients with MPM from healthy controls, asbestos-exposed individuals, patients with pleural plaques, and patients with asbestosis with AUCs of 0.92, 0.88, 0.90, and 0.81, respectively.

Nonetheless, the role of FBLN3 as a biomarker has been controversial since later studies had conflicting results. The comparative study by Kaya and colleagues[100] of 43 patients with MPM and 40 healthy controls revealed a significantly higher serum FBLN3 in patients with MPM and, at the best cutoff point of 36.6 ng/mL, FBLN3 had 93.0% sensitivity and 90.0% specificity for diagnosis of MPM (AUC, 0.976). It is of interest that studies that did not find diagnostic power of FBLN3 in the plasma indeed validated the prognostic value for PE FBLN3 in MPM. Kirschner and colleagues[101] investigated serum and PE FBLN3 levels in 2 different series and reported plasma FBLN3 to be significantly higher in patients with MPM in only 1 series. Diagnostic accuracy was found to be low for plasma FBLN3. On the other hand, although PE FBLN3 levels in patients with MPM and controls were not significantly different, lower levels of PE FBLN3 correlated with significantly improved survival in patients with MPM, and were independently associated with prognosis with a hazard ratio of 9.92. Creaney and colleagues[64] performed a study of 82 patients with MPM and similarly found PE FBLN3 level to be a significant prognostic predictor in patients with MPM. However, their study indicated low sensitivity for both serum and PE FBLN3 levels, inferior to that of MSLN. Furthermore, in a later study, including 33 patients with MPM, PE FBLN3 level could not distinguish patients with MPM from those with other pathologies.[102]

A meta-analysis of studies using serum FBLN3 for diagnosis of MPM included 7 studies with a total of 468 MPM and revealed a pooled sensitivity of 62% (95% CI, 45%–77%) and a specificity of 82% (95% CI, 73%–89%) (AUC of ROC, 0.81).[103]

Due to inconsistencies observed with the sole use of FBLN3 USCN ELISA, further investigations were initiated by the National Cancer Institute's

Early Detection Research Network Mesothelioma Biomarker Discovery Laboratory (EDRN MPM BDL) for the development of alternative assays. Consequently, a custom MRM mass spectroscopy assay for FBLN3 was reported to distinguish 15 MPMs from 15 asbestos-exposed controls, revealing markedly better AUC of 0.82 compared with earlier results observed by Australian investigators (AUC, 0.69) using the USCN ELISA. This was further supported by later studies using newer FBLN3 ELISAs, including the LS-Biosciences Chemoluminscent human EFEMP1 ELISA, to compare MPM and asbestos-exposed serum FBLN3 levels that reported an AUC of 0.93 (Pass H, personal communication, IASLC Targeted Meeting, 2020). Another significant contribution by MPM BDL was development of a unique Slow Off-Rate Modified Aptamer (SOMAmer) Luminex assay using a FBLN3 SOMAmer that revealed an AUC of 0.98. In addition, the SOMAmer assay was able to differentiate plasma obtained from patients with MPM PE, from those with non-MPM PE, with a remarkable AUC of 0.93. More recently, using a novel FBLN 3 monoclonal antibody, mAB382, a novel sandwich ELISA has been constructed in the MPM BDL, in collaboration with researchers who first described EFEMP1 expression in glioblastoma,[104] and has yielded promising results. Results from currently ongoing blinded validations of FBLN3 with other MPM and asbestos-exposed cohorts are pending.

Proteomics (Multiplex Protein Signature) and Glycomics

The proteome is defined as the complete set of proteins produced in an organism, system, or biological context, at a certain time, under specific circumstances. Proteomics has resulted in identification of various useful protein signatures that can assist in diagnosis and management of different malignancies, including MPM.[105–107] Somalogic, Boulder, Colorado, developed a remarkable proteomic platform for MPM, using over 1100 SOMAmers. SOMAmers are short, single-stranded deoxynucleotides designed to attach to specific protein targets. They are modified to be selectively eluted from the protein during steps to concentrate and quantitate the proteins that they bind.[108,109] One unique feature of SOMAmers is that multiplexing them allows for quantification of many proteins with very small amounts of sample. In a multicenter case-control study of 117 patients with MPM and 142 asbestos-exposed controls, SOMAmers were used to screen more than 1000 proteins, identified 64 candidate biomarkers, and created a 13-marker random forest classifier that was able to differentiate patients with MPM from asbestos-exposed controls with both sensitivity and specificity of greater than 90% (AUC, 0.99), revealing superior performance to MSLN.[107] Importantly, the 13 SOMAmer panel was later validated in 2 other cohorts.

More recently, White and colleagues[110] performed a study using quantitative mass spectrometry to explore MPM proteomic profile. They identified an explicit group of upregulated proteins in MPM effusions, capable of distinguishing MPM PE from benign reactive and adenocarcinoma-associated PEs.

Glycomics is a technique that allows for quantification of serum glycosylated moieties. Although earlier research had promising results, later studies were unable to validate the previously identified signatures for diagnosis and prognosis of MPM.[111] Therefore, further research is required to elucidate the role of glycomics in MPM.[110,112]

Genomics and Epigenomics

Some of the first attempts for genomic modeling of mesothelioma involved transcriptomic prognostication. Using a study and validation set, Pass and colleagues[113] identified a 27-gene classifier for patients with MPM that was able to predict time to progression and survival after cytoreduction and postoperative adjuvant therapy.[114] Brigham and Women's Hospital has focused on detection and validation of microRNA (miRNA) ratios for MPM prognostication.[114–117] In a recent study, Zhou and colleagues[118] developed and validated a 3-gene prognostic signature for MPM that was able to classify patients with MPM into low- and high-risk patients with significantly different OS. In an early study, representative oligonucleotide microarray analysis was used to quantify copy-number abnormalities (CNA) in patients with MPM who presented with recurrence at variable intervals after surgery.[119] Patients with early recurrence were found to have significantly greater increase in CNA and frequent deletions in chromosomes 22q12.2, 19q13.32, and 17p13.1 (55%–74%). These data suggested a prognostic role for CNA in MPM. More complex techniques, such as next-generation sequencing (NGS) moved the biomarker discovery in MPM to new levels. Whole-exome sequencing of pleural MPM in collaboration with the Broad Institute, resulted in identification of 517 somatic mutations across 490 mutated genes.[19] The most frequent genetic alterations included BAP1, NF2, CDKN2A, and CUL1. These findings were subsequently

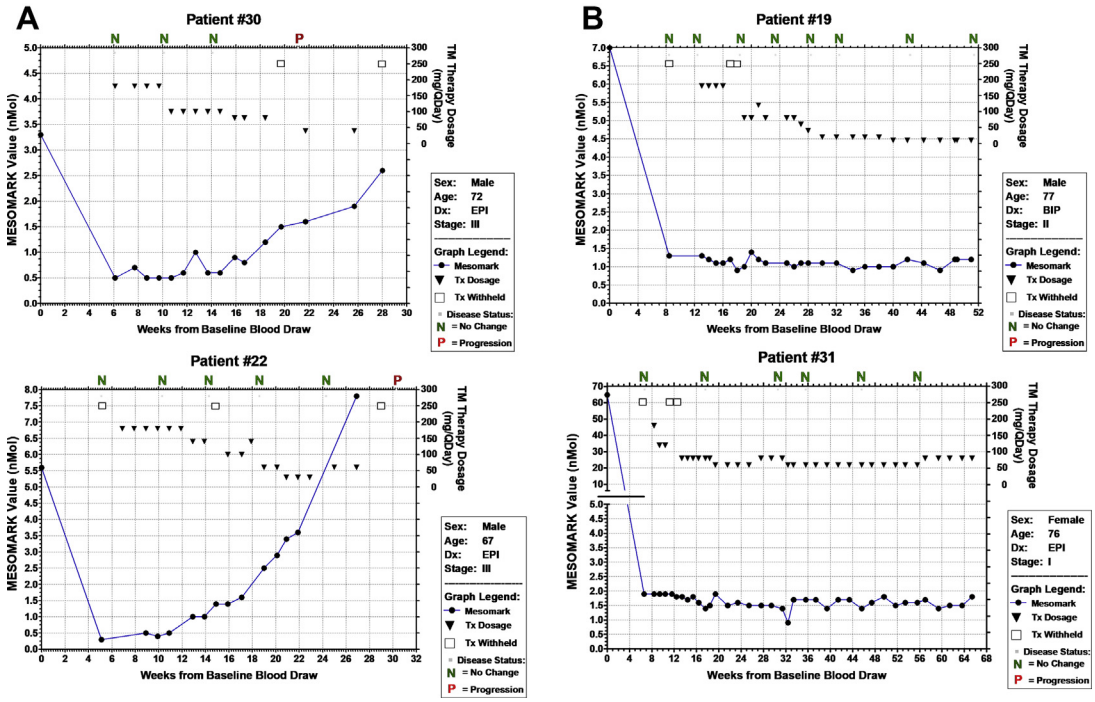

Fig. 3. Examples of patients with recurring disease (*A*) and with stable disease (*B*); patients diagnosed with mesothelioma were monitored using MESOMARK during the course of chemotherapy. Serum concentrations of mesothelin were measured before surgery and during the course of treatment postsurgery.

validated and expanded by larger studies from Brigham and Women's[120] and The Cancer Genome Atlas (TCGA).[121] BAP1 has emerged as the gene with the highest number of derangements in MPM, with diagnostic and prognostic implications. Studies from Bott and colleagues[17] were seminal in the discovery of mutated BAP1 in MPM, and Testa and colleagues[122] and Carbone and colleagues[123] were the first to describe germ line alterations of the gene and the association of these germ line mutations with familial mesothelioma. Shinozaki and colleagues[21] found that BAP1 loss and high EZH2 expression were highly specific in differentiating MPM from benign mesothelial proliferations, and that combination of both biomarkers improved diagnostic accuracy to a sensitivity of 90% and specificity of 100%. Moreover, Carbone and colleagues reported that germline mutations of BAP1 were associated with longer than expected survival,[18,124] and this has been validated in at least 1 other study.[125]

Few other studies found homozygous deletion of CDKN2A to be a prognostic factor in MPM.[121,126,127] These results corroborated with earlier findings of Chou and colleagues[128] that BAP1-negative and p16-positive (CDKN2A-positive) phenotype was associated with significantly longer survival.

Tissue and Circulating MicroRNAs

miRNAs are small noncoding RNAs involved in RNA silencing and posttranscriptional regulation of gene expression, and they have a key role in regulating various cellular processes, such as proliferation, differentiation, apoptosis, angiogenesis, and invasion.[129–133] Specific signatures of miRNAs are exhibited by tumor cells, either secreted actively or released passively after cell death.[134,135] MPM miRNA expression profiles have been extensively explored over the past few years.[136–148] An early tissue-based study found that mir-29c* was an independent predictor of survival, along with stage and lymph node involvement, regardless of histology. Data suggested mir-29c* to be an independent prognostic marker for predicting time to progression and OS after surgery, and increased miR-29c* expression was associated with significantly higher survival.[141] Furthermore, the mechanism of mir-29c* was found to be through epigenetic regulation of the tumor via downregulation of DNA methyltransferases, along with upregulation of demethylating genes. Subsequently, the prognostic role of mir-29c* in MPM was validated by TCGA (**Figs. 3** and **4**) (Gordon Robertson, personal communication, 2015).

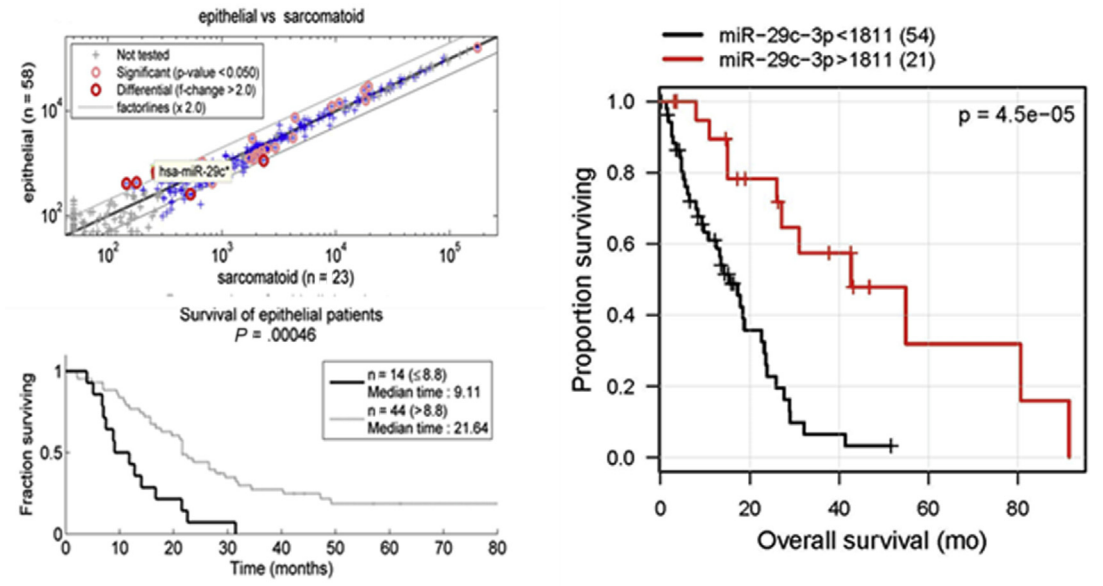

Fig. 4. mir 29c* and MPM. Left panel: significant increase of the mir in epithelial mesothelioma and loss of the mir associated with poor prognosis. Right panel: validation in 75 TCGA patients with MPM.

Although various studies have attempted to elucidate the diagnostic and prognostic role of miRNAs in MPM, there has been minimal overlap in findings of different studies and most models lack validation. The observed discrepancies may partially be explained by differences in normalizers/housekeepers. Two previous studies reported overexpression of miR-30b, miR-32, miR-483-3p, miR-584, miR-885-3p, miR-17-5p, miR-21, miR-29a, miR-30c, miR-30e-5p, miR-106a, and miR-143, and downregulation of miR-9, miR-7-1, and miR-203 in MPM.[139,140] Interestingly, correlation between certain miRNAs and specific histologic subtypes was observed in both studies. Loss of miR-31 (associated with homozygous loss of 9p21.3 chromosome in MPM) has correlated with tumor suppressor activity.[142,143] Although an early study found miR-126-3p capable of discriminating patients with MPM from healthy controls,[143] a later study provided conflicting results and found it unable to distinguish between patients with MPM and asbestos-exposed controls.[147] In the study by Matboli and colleagues[149] of 60 patients with MPM, miR-548a-3p and miR-20a sera levels could individually distinguish patients with MPM from 20 asbestos-exposed and 20 healthy controls with high sensitivity. A combined panel of both biomarkers resulted in sensitivity of 100%.

Most recently, the novel platform HTG EdgeSeq miRNA whole transcriptome assay was used to measure expression of 2083 human miRNA transcripts. When combined with NGS, this platform uses only 15 μL of serum or plasma. Preliminary results were promising, showing that a 7-serum miRNA signature was able to differentiate 93 MPMs from 53 asbestos-exposed pipe fitters with an AUC of 0.953.[150]

Further upregulated miRNAs reported in MPM are miRNA 197-3p, miRNA-1281, miRNA 32-3p,[151,152] miR-625-3p,[138] miR-103a-3p,[137,153] miR-30e-3p,[152] and miR-2053.[154]

One study found that expression of miR-17-5p and miR-30c correlated with survival in sarcomatoid MPM.[139] In addition, upregulation of miR-31 was associated with the presence of a sarcomatoid component and worse prognosis in patients with that histologic subtype.[155] Fassina and colleagues[156] found that tissue levels of miR-205 were lower in biphasic and sarcomatoid MPM histologic subtypes and higher in epithelioid type.

Kirschner and colleagues[157] performed a study on patients undergoing extrapleural pneumonectomy (EPP) or palliative surgery (P/D) to investigate the association between miRNA expression and OS. They developed a miR-Score consisting of a 6-miRNA signature (miR-21-5p, miR-23a-3p, miR-30e-5p, miR-221-3p, miR-222-3p, and miR-31-5p), which was able to predict long survival in patients undergoing EPP and P/D with 92.3% and 71.9% accuracy, respectively. In addition, Lamberti and colleagues[136] found 2 distinct serum miRNA signatures with diagnostic and prognostic significance in patients with MPM. Consequent to earlier findings, miR-29c*, miR-92a, and miR-625-

3p were validated as encouraging diagnostic markers for MPM.[138,141] In a strong validation test, De Santi and colleagues[158] reported significant differential expression of miR-185, miR-197, and miR-299 in patients with MPM and identified a 2-miRNA prognostic signature, consisting of Let-7c-5p and miR-151a-5p. Andersen and colleagues[159] performed a comparative analysis of MPM tumor tissues and nonneoplastic control specimen and found that DNA-hypermethylation downregulated miR-126 and its host gene EGFL7 and resulted in lower survival in patients with MPM.

Other studies found high tissue levels of miR-137,[160] miR-1, miR-335-5p, and miR-566 associated with a poor prognosis, whereas high tissue expression of miR-16, miR-486, miR-146a-5p, miR-378a-3p, miR-451a, and miR-1246 correlated with better outcome.[161] A study of 60 patients with MPM revealed hsa-miR-2053 to be an independent prognostic factor for MPM.[154]

Circulating Tumor DNA

Circulating tumor DNA (ctDNA) is defined as the portion of circulating cell-free DNA (cfDNA) that is released into the blood either through active secretion, or via tumor apoptosis and necrosis.[162,163] cfDNAs containing tumor mutations are detected at higher concentrations in patients with cancer and contain mutations present in the tumor.[164] Contrary to other malignancies, studies on ctDNA in MPM are limited. Although data support the feasibility of the technique, sensitivity is reported to be low. Whole-exome sequencing with validation of the tumor-specific variants by digital droplet PCR was performed in 10 patients with MPM and detection of patient-specific, selected variants was observed only in 3 treatment-naive patients with MPM, either in one or both independent droplet digital PCR runs.[165] Based on earlier findings that miR-34b/c is downregulated in 90% of MPMs, a Japanese group specifically investigated the degree of miR-34b/c methylation in serum-circulating DNA using a digital methylation-specific PCR (MSP) assay.[166] They used a technique utilizing digital droplet PCR methods in combination with MSP, originally evolved from MSP.[167] The reported sensitivity in the discovery and validation sets were 76.9% and 59.1%, and specificities were 90% and 100%, respectively. Further improvement of accuracy was observed with advancing stages of disease.

DNA Methylation

DNA methylation is an epigenetic modification occurring at specific regulatory regions of genes. Methylation patterns can be affected by several factors, including environmental exposure, aging, and various types of disease and therapy.[168] DNA methylation profile has been shown to successfully distinguish MPM cells from normal pleural cells.[169] Furthermore, the DNA methylation profile in peripheral blood, in isolation or combined with other biomarkers, might be helpful in the diagnosis of MPM.[144,170] In one study with a discovery test and a test set of MPMs and asbestos-exposed controls, a genome-wide methylation array was used to identify novel DNA methylation markers from whole blood. Aiming the top differentially methylated signals, 7 single cytosine-guanine dinucleotides and 5 genomic regions of coordinated methylation were identified in both cohorts. Subsequently, a model was created using cytosine-guanine dinucleotides (CpG) methylation levels, along with clinical characteristics of age, sex, and asbestos exposure levels, and revealed an AUC of 0.89.[170] Change in the methylation profiles over time in at-risk asbestos-exposed populations is currently unknown and yet to be explored.

Circulating Tumor Cells

Circulating tumor cells (CTCs) are cells that have detached from a primary tumor or a metastatic site and are circulating in the bloodstream. They become more abundant as cancer develops toward more advanced stages. In contrast to other cancers where methods of counting CTCs have been found helpful for diagnosis, in MPM these methods have limited utility due to low sensitivity.[171–173] Recently, however, CTC capture with microfluidics has improved the efficacy of CTC quantitation in MPM. The CTC-Chip is a novel microfluidic device developed by Chikaishi and colleagues,[174] in which the capture antibody was one against podoplanin, which is abundantly expressed on MPM. The CTC-Chip was found to have higher diagnostic value for MPM. Earlier stages of disease showed that lower numbers of cells and CTCs were positive in only 68% of clinical samples. Highest accuracy was seen in comparison of CTCs in earlier stages to those with stages IIIB and IV (AUC, 0.851). In addition, CTC count $\geq$2 cells/mL was associated with significantly worse prognosis ($P = .030$).

INFLAMMATORY AND ANGIOGENIC FACTORS

High-Mobility Group Box 1

High-mobility group box 1 (HMGB1) is among the most important chromatin proteins that interacts with nucleosomes and transcription factors and facilitates binding of DNA to other proteins. Accordingly, it regulates transcription, DNA

repair, proliferation, and inflammation.[175,176] Recognized as a damage-associated molecular pattern, HMGB1 is released by cells undergoing necrosis in physiologic states. Conversely, in pathologic states a hyperacetylated form of HMGB1 can be secreted by inflammatory and cancer cells.[177–179] Exposure of human mesothelial cells to asbestos provokes programmed necrosis and results in the release of HMGB1, along with activation of Nalp3 inflammasome, and eventually cell alteration.[12,15,16,180,181] In a study of 15 patients with MPM, serum HMGB1 level was significantly higher in patients with MPM compared with non-MPM asbestos-exposed individuals.[182] In another study, total blood HMGB1 levels were found to be higher in patients with MPM and asbestos-exposed individuals compared with healthy controls. More importantly, hyperacetylated HMGB1 was significantly higher in patients with MPM compared with asbestos-exposed and healthy controls.[183] Validation of these findings is forthcoming. Combined use of HMGB1 and FBLN3 resulted in improved accuracy. One study showed that, at a cutoff value of 9 ng/mL, there was a significant negative correlation between serum HMGB1 level and survival, suggesting a potential prognostic role for HMGB1.[86] Furthermore, in a systematic review and meta-analysis by Wu and colleagues[184] of 18 studies on HMGB1 overexpression in various types of cancer, HMGB1 was found to be a prognostic marker for MPM.

Peripheral Blood-Based Markers

Given the well-established role of chronic inflammation in pathophysiology of various cancers, including MPM, many studies have investigated the role of inflammation-based scores, such as lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio in diagnosis and prognosis of MPM. A retrospective study of 150 patients with MPM found increased LMR significantly associated with prolonged OS. Specifically, median OS was 14 months in patients with LMR $\geq$2.74 compared with 5 months in patients with LMR less than 2.74. Multivariate analysis confirmed LMR to be an independent prognostic marker for OS in MPM.[185] Conversely, the prognostic role of NLR is somewhat controversial. Although many studies consistently found baseline NLR to be an independent predictor of improved survival,[186–189] other studies failed to show similar results.[190,191]

A study of 55 patients with MPM showed that late-stage patients with MPM had significantly higher plasma circulating complement component 4d (C4d) levels compared with early-stage patients, and that high circulating C4d levels correlated with higher tumor volume. Furthermore, after induction chemotherapy, plasma C4d levels were significantly higher in patients with stable and progressive disease compared with those with partial or major response. In multivariate analysis, patients with low C4d levels at diagnosis were found to have significantly better OS.[192]

Calretinin

Calretinin is a vitamin D-dependent calcium-binding protein similar to S-100 and is a member of EF-hand protein family. Encoded by the CALB2 gene, calretinin was first detected in neurons and later on mesothelial cell surfaces and has been found to be overexpressed in MPM.[193,194] A calretinin assay developed by Raiko and colleagues[195] could differentiate patients with MPM from asbestos-exposed and healthy controls. In addition, the assay was able to distinguish asbestos-exposed individuals from healthy controls. Later studies showed corroborative results. In comparing MPM and controls, calretinin had sensitivity of 71% for a predefined specificity of 95%, with AUC ranging between 0.77 and 0.95 depending on the gender and the country of origin of the specimen.[196–198] Furthermore, blood calretinin levels could prediagnose mesothelioma 1 to 15 months before definitive diagnosis in an asbestos-exposed population with an AUC of 0.77.[199] Additional use of serum MSLN enhanced the accuracy to AUC of 0.85. In another study, calretinin expression was found to be an independent predictor of survival in patients with MPM.[200] Following earlier promising results, validation studies for calretinin and FBLN3 are currently underway.

ENOX2

ENOX2 (Ecto-NOX disulfide-thiol exchanger 2) is a cell surface protein, a member of the NOX family of NADPH oxidases that is involved in oxidization of reduced pyridine nucleotides and is essential for cell growth.[201] Specific cancer cells exhibit tissue-specific patterns of ENOX2 transcript variants.[202] ENOX2 proteins are released in circulation and can be detected at an early stage in particular cancers, including breast, lung, colon, prostate, and ovarian cancer.[202] Specific ENOX2 protein transcript variants characteristic of MPM were identified by Morré and colleagues[203] that could be detected in sera of patients 4 to 10 years before developing clinical symptoms.

Thioredoxin-1

Thioredoxin-1 (TRX) is a conserved antioxidant protein, well known for its regulatory disulfide reductase activity, and has a critical role in decreasing ROS levels.[204] Overexpression of TRX has been detected in patients with MPM.[15] In a study by Demir and colleagues,[98] TRX and SMRP exhibited a graded increase among controls, asbestos-exposed individuals, and patients with MPM, respectively. TRX showed a sensitivity of 92.9% and specificity of 77.6% in diagnosis of MPM.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a signal protein and a key stimulator of neoangiogenesis and has been found to be overexpressed in many malignant tissues, including MPM.[205–207] VEGF levels in PE were significantly higher in patients with MPM compared with those with PEs related to nonmalignant pleural diseases or lung cancer.[208] Hirayama and colleagues[208] determined 2000 pg/mL to be the optimal cutoff value between low and high VEGF-A levels in MPM PE, showing a significant correlation with survival. The median survival was 12 and 4 months in the low- and high-level groups, respectively.[208] Similarly, higher levels of serum VEGF were found in patients with MPM compared with those with nonmalignant asbestos-related diseases.[209] At a cutoff at 460 pg/mL, there was a strong correlation between high serum VEGF level and shorter survival. Another study showed that VEGF staining correlates with short survival, tumor stage, and prognosis in MPM.[207] Nowak and colleagues[210] found that, in patients with MPM treated with multitarget tyrosine kinase inhibitor sunitinib malate, baseline serum levels of VEGF-A and VEGF receptor 2 (VEGFR-2) correlated with radiological response.

Miscellaneous Potential Biomarkers

Stockhammer and colleagues[211] found strong prognostic value for PE transforming growth factor β (TGF-β) levels in patients with MPM. Subgroup analysis showed that PE TGF-β levels were highly prognostic in epithelioid histology, but there was only a trend in the nonepithelioid group.[211] Conversely, the study showed no diagnostic and prognostic power for circulating TGF-β levels in patients with MPM. In addition, there was no correlation between PE and circulating TGF-β levels.

Serum matrix metalloproteinase 9 (MMP-9) is an extracellular protein that has a role in various physiologic and pathologic processes, including development, wound healing, cell migration, and metastasis.[212] One study showed that MMP-9 overexpression in correlation with MSLN overexpression was associated with increased tumor invasion and decreased survival in patients with MPM.[41] Štrbac and colleagues[213,214] identified certain MMP-9 genotypes that were associated with significantly shorter OS and time to progression compared with other alleles. A later study showed that although serum MMP9 concentration was different in patients with complete versus partial response and in patients with stable versus progressive disease, the concentration differences did not reach statistical significance and thus not associated with survival or treatment response.[215]

Bridging integrator 1 (BIN1) is a member of BAR domain superfamily involved in endocytosis, cell division, and migration.[216] In a study of 67 patients with MPM, Ahmadzada and colleagues[217] found high BIN1 expression to be a favorable prognostic biomarker for MPM and associated with tumor-infiltrating lymphocytes (TILs).

Metallothionein (MT) is a family of cysteine-rich low-molecular-mass proteins that have capacity of binding heavy metals through thiol groups of their cysteine residues.[218] In a retrospective study of 105 patients with MPM, both OS and progression-free survival negatively correlated with detectable MT expression, suggesting a possible resistance to platin-based chemotherapy associated with MT expression upregulation, found exclusively in progressive MPM samples.[219]

Aquaporin-1 (AQP1) is a cell membrane water channel protein found throughout the body that plays a role in transcellular water transport.[220] A study by Angelico and colleagues[221,222] showed that patients with AQP1 overexpression (defined as $\geq$50% of tumor cells showing membranous staining) had a significantly longer median OS compared with those with an AQP1 score of less than 50% (26.3 months compared with 8.9 months, respectively).

Marcq and colleagues[223] investigated immune cell composition of PE in patients with MPM and identified 2 factors with clinical value. Percentage of PD-L1+ podoplanin (PDPN)+ tumor cells was a significant prognostic factor for worse outcome, whereas CD4+ T cells were associated with better response to chemotherapy.

Later studies corroborated earlier results indicating that higher expression of peritumoral TILs correlated with improved OS, whereas PD-L1 expression inversely correlated with clinical outcomes.[224–226]

Combined Panels

Many studies have investigated various combination of biomarkers to achieve higher accuracy for diagnosis of MPM.[183,227] Jimenez-Ramirez and colleagues[227] used a mesothelin-calretinin-MPF combination and were able to obtain sensitivity of 82% in men (AUC, 0.944), and 87% in women (AUC, 0.937). In a unique study, Bonotti and colleagues[228] assessed various combinations of biomarkers in MPM and identified the 2 best 3-marker combinations as IL6-OPN-SMRP (AUC, 0.945) and IL6-OPN-Desmin (AUC, 0.950). In addition, they found the best 4-marker combination to be SMRP-OPN-IL6-Vimentin (AUC, 0.962).

In a recent study, Doi and colleagues[229] developed a novel prognostic risk classification system for MPM. Significant independent predictors of poor survival were an NLR of $\geq$5.0, along with non-epithelioid histologic type, increased serum lactate dehydrogenase levels, and a total lesion glycolysis of $\geq$525 g.

Breath analysis

Exhaled breath is composed of 2 major phases: a liquid phase containing water vapor, and a gaseous phase consisting of oxygen, carbon dioxide, nitrogen, inert gases, and a small fraction of volatile organic compounds (VOCs).[230] VOCs' origin can either be exogenous via inhalation and dermal absorption, or endogenous as a result of physiologic and pathophysiological processes, including inflammation, metabolism, and oxidative stress.[231,232] More than 3000 different VOCs have been described so far, but a single breath sample commonly contains about 200 different VOCs.[230] Because VOCs are influenced by various pathophysiological states, they have been studied as potential biomarkers for diagnosis of various benign and malignant diseases, including MPM.[30,233–235] Although gas chromatography-mass spectrometry continues to be the gold standard method for breath analysis, various other methods have been successfully used for molecular assessment of breath components, including multicapillary column ion mobility spectrometry (MCC-IMS),[236] selected ion flow tube-mass spectrometry,[237] proton transfer reaction-mass spectrometry,[187] electronic noses (eNoses), and canine scent test.[232] In a meta-analysis of various breath analysis methods, eNose and MCC-IMS were found to have the highest and the lowest accuracies, respectively (95% versus 65%).[234,238]

SUMMARY

Over the last 2 decades there have been numerous investigations on diagnostic, monitoring, and prognostic biomarkers for MPM, but thus far, only 1 biomarker has been validated as a blood-based test in North America, Europe, and Australia. The main grounds for failure of validations of other marker are scarcity of large archives of prospectively collected high-quality specimens, limited funding for performance of large-scale validation trials, and necessity of a more cohesive approach by mesothelioma investigators. The relative lack of industrial interest and support for MPM biomarker development and validation, as compared with lung cancer, is likely secondary to its smaller market and the common misconception that MPM will evade over the next few decades. There is a continued need for accurate diagnosis and early detection of the disease particularly with an increasing rate of cases secondary to familial BAP1 germline mutations and recognition of other carcinogenic fibers, such as erionite in new locations.

CLINICS CARE POINTS

- Poor prognosis of advanced staged MPM, along with cost and morbidities associated with pleural biopsy, has developed high interest in investigating biomarkers for diagnosis and monitoring of therapy in MPM.
- Studies suggest that MPM has a low mutation burden, with tumor suppressors BAP1, NF2, and CDKN2A being the most frequently mutated genes.
- Certain biomarkers have strong diagnostic values, whereas others exhibit better prognostic values.
- Combined panels have shown the most promising results in improving accuracy.
- Large coordinated validation studies are required for further assessment of practical utility of biomarkers.

DISCLOSURE

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BAP1. M. Carbone and H. Yang have a patent issued for "Using Anti-HMGB1 Monoclonal Antibody or other HMGB1 Antibodies as a Novel Mesothelioma Therapeutic Strategy," and a patent issued for "HMGB1 As a Biomarker for Asbestos Exposure and Mesothelioma Early Detection." Michele Carbone is a board-certified pathologist who provides consultation for mesothelioma expertise and diagnosis. The remaining authors report no disclosures.

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