Mesothelioma Biomarkers: A Review Highlighting Contributions from the Early Detection Research Network

Harvey I. Pass[1], Marjan Alimi[1], Michele Carbone[2], Haining Yang[2], and Chandra M. Goparaju[1]

ABSTRACT

Malignant pleural mesothelioma (MPM) is an asbestos-related neoplasm, which can be treated successfully only if 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. This review details the recent work with biomarker development in MPM and the contributions of the NCI Early Detection Research Network Biomarker Developmental Laboratory of NYU Langone Medical Center. The literature of the last 20 years was reviewed to comment on the most promising of the blood- and tissue-based biomarkers. Proteomic, genomic, and epigenomic platforms as well as novel studies such as "breath testing" are covered. Soluble mesothelin-related proteins (SMRP) have been characterized extensively and constitute an FDA-approved biomarker in plasma with diagnostic, monitoring, and prognostic value in MPM. Osteopontin is found to be a valuable prognostic biomarker for MPM, while its utility in diagnosis is slightly lower. Other biomarkers, such as calretinin, fibulin 3, and High-Mobility Group Box 1 (HMGB1), remain under study and need international validation trials with large cohorts of cases and controls to demonstrate any utility. The EDRN has played a key role in the development and testing of MPM biomarkers by enlisting collaborations all over the world. A comprehensive understanding of previously investigated biomarkers and their utility in screening and early diagnosis of MPM will provide guidance for further future research.

See all articles in this *CEBP Focus* section, "NCI Early Detection Research Network: Making Cancer Detection Possible."

Introduction

Malignant pleural mesothelioma (MPM) is a relatively rare malignancy with an estimated incidence of 3,200 cases per year in the United States, and a minimum 34,000 deaths in 2013 worldwide (1–3). Exposure to asbestos is believed to be the main cause, with a long latency period of approximately 20 to 40 years between initiation of exposure and clinical diagnosis (4–6). Asbestos continues to be used worldwide and still exists in previously constructed buildings' walls and ceilings, resulting in occasional exposure of the residents and construction workers; therefore the incidence of MPM is not expected to decrease (7–9). Additional risk factors include high-dose radiation exposure and other mineral fibers; the role of SV40 infection remains controversial (10–14).

Prolonged asbestos exposure induces a sustained inflammatory response (the inflammasome), resulting in the release of cytokines and an increase in reactive oxygen species (ROS) that can initiate MPM carcinogenesis (15–19). Current data suggest that MPM has a low mutation burden and tumor suppressors *BAP1*, *NF2*, and *CDKN2A* are the most frequently mutated genes involved in pathogenesis of MPM (20–24).

Despite aggressive multimodality therapy with surgery, chemotherapy, and radiotherapy, MPM's prognosis continues to be poor due to its unpredictable growth and minimal response to current treatments (4, 25–28). Diagnosis and treatment of MPM at an early stage (stage I) is shown to have a significantly better outcome and overall survival (29, 30); however, this constitutes only 5% of the patients. Average survival for all patients is close to 13 months, largely due to late diagnosis (1).

Several strategies have been explored to screen the population at high risk for developing MPM (31, 32). The ideal biomarker-based screening test should be concomitantly highly sensitive and specific (highly accurate), preferably noninvasive, easily accessible and also cost effective. While both sensitivity and specificity are essential elements of an effective screening test, sensitivity has higher priority to ensure the lowest false-negative rate. While there are currently no accurate evidence-based cutoff values, based on MPM incidence rate, a minimum sensitivity of 85% with a concurrent specificity of 75%–80% is desired. Imaging modalities are nonspecific in differentiating between MPM and benign pleural pathologies, and CT screening trials for the disease have been disappointing (33). There is a critical need for a sensitive and specific noninvasive screening method of high risk, asbestos-exposed populations to potentially diagnose and treat mesothelioma at earlier stages. In the absence of strong and reliable noninvasive diagnostic tests, currently, pleural biopsy remains the only standard diagnostic method for MPM, associated with considerable morbidity and costs (34). The complication rate and morbidity associated with pleural biopsy depends on various patient and procedure-related factors. The total complication rate is around 20%, with major morbidities including pneumothorax requiring chest tube placement and hemorrhage being 7.3% and 2.8%, respectively (35). Similarly, costs depend on the procedure utilized to attain the specimen (thoracoscopic surgery vs. transthoracic imaging-guided biopsy) and the potential hospital stay

[1]Department of Cardiothoracic Surgery, NYU Langone Medical Center, New York, New York. [2]John A. Burns School of Medicine, Department of Thoracic Oncology, University of Hawaii Cancer Center, Honolulu, Hawaii.

Corresponding Author: Harvey I. Pass, NYU Langone Medical Center, 530 First Avenue, 9V, New York, NY 10016. Phone: 212-263-7417; Fax; 212-263-2042; E-mail: harvey.pass@nyumc.org

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associated with each procedure. In one study with approximately 9,000 patients, the average cost of lung biopsy was $3,784, with a mean overall health care costs of $14,634 per patient (the average cost of a lung biopsy with complications was $37,745, observed in 19.3% of patients with biopsies; ref. 36). Although certain imaging modalities and blood tests can be used as helpful adjuncts, they lack sufficient sensitivity or specificity to be utilized as solitary tests. Moreover, accurate biomarker prognostication could potentially spare patients with MPM from having potentially morbid resections for disease with a high propensity for early recurrence.

Given that MPM is a relatively rare malignancy with a low incidence rate, majority of which occurs in the high-risk asbestos-exposed population, the role of screening the general population is currently unclear. While at this time it is unlikely that it would yield in significantly higher rate of early diagnosis to potentially improve the overall survival, this topic needs to be viewed in the context of accuracy, availability and cost effectiveness of the test/tests utilized

Materials and Methods

Role of the EDRN

The contributions of the EDRN in the investigation of diagnostic and prognostic biomarkers for mesothelioma cannot be overstated. The first Biomarker Discovery Laboratory at NYU Langone Medical Center specifically devoted to MPM was funded in 2005, and this was facilitated through the EDRN Associate Membership Program in 2003–2004. This early funding resulted in one of the first papers to use the Affymetrix U95 gene chip for a 27-gene prognostic model, which was validated by the group at Brigham and Women's Hospital (37). Since then, the NCI has continuously funded the MPM Biomarker Discovery Laboratory, and has contributed to the discovery and attempted validation of numerous blood-based biomarkers for asbestos-related malignancy. This manuscript will highlight the contributions of the EDRN to mesothelioma biomarker discovery as well as comment on other potential biomarker platforms.

Results

Mesothelin, soluble mesothelin-related proteins, and megakaryocyte potentiating factor

The first biomarker with any diagnostic credibility for MPM actually evolved from the work of Pastan and colleagues with the discovery of mesothelin (38). Mesothelin is a protein thought to play a role in cellular adhesion. It is produced at low levels by normal mesothelial cells and is overexpressed in certain cancers including MPM, pancreatic adenocarcinoma, ovarian, and lung cancers (39, 40). Mesothelin has been shown to promote tumor cell survival and proliferation via activation of the NFκB pathway (41). It is initially translated into a preprotein form and then cleaved into mesothelin and MPF (42–44). Soluble mesothelin-related peptide (SMRP) is a soluble form of mesothelin released by tumor cells into the circulation originally described by the Hellstroms (43), which was commercialized by Fujirebio (45), and remains the only FDA-approved biomarker for diagnosis of MPM (46, 47). Robinson and colleagues (43) were the first to detail the diagnostic capabilities of SMRP in MPM, and samples from the EDRN funded MPM tissue bank were used in the technical validation of the MESOMARK assay (45), as well as clinical validation in serum and pleural effusion in a North American population (48). These early studies revealed that the serum level of SMRP was able to identify patients with MPM from asbestos-unexposed and asbestos-exposed individuals, as well as those with benign pleural diseases (49–52). The EDRN sponsored an international blinded trial of MESOMARK levels in 652 control (asbestos exposed) and 165 cases (MPM) serum samples from Australia and North America which were independently measured by Fujirebio and the UCLA Biomarker Reference Laboratory. As seen in **Fig. 1**, both sites validated the discrimination between cases and controls, with very similar sensitivities and specificities. Subsequent studies have validated these findings. A systematic review and meta-analysis of 12 studies with 717 MPM patients and 2,851 controls (healthy individuals and patients with non-MPM diseases) revealed 64% sensitivity and 89% specificity for serum SMRPs in diagnosis of MPM (53). Meta-analysis of 16 studies with 1,026 patients with MPM and 4,491 controls showed 32% sensitivity and 95% specificity for mesothelin in diagnosis of MPM (54). There was also a proposed ability for differentiation between MPM and pleural metastases of other carcinomas (45, 50, 52).

Whether SMRP could be used for the screening of high risk for MPM asbestos-exposed patients was also addressed by the EDRN. The beta-Carotene and Retinol Efficacy Trial (CARET), a study of vitamin supplementation for chemoprevention of lung cancer, followed 4,060 heavily asbestos-exposed U.S. men for 9–17 years, and 49 individuals who, while on the CARET trial, developed MPM. The EDRN sponsored an investigation of the 49 malignant MPM cases and 96 matched controls to determine whether SMRP could predict MPM development years before clinical presentation of the disease with the marker measured blindly at two separate sites. It was not surprising that the overall ROC curve AUC was 0.604 with 95% confidence interval (95% CI) of 0.489–0.699 since a marker for malignant MPM may not be elevated many years prior to diagnosis. However, the AUC for SMRP measured less than one year before diagnosis was 0.720 with 95% CI of 0.562–0.853, so there is statistically significant evidence that SMRP can be elevated in the year prior to diagnosis (**Fig. 2**). The AUC was not statistically significant for the 1–2 years' time interval since the number of cases was small. Other studies have also revealed that pre diagnostic SMRP levels rise in the year before diagnosis; the sensitivity is not suitable for accurate MPM screening (55, 56).

SMRP is useful for the monitoring of therapy. Early studies sponsored by the EDRN in collaboration with Fujirebio were reported at the International MPM Interest Group Meeting in 2008. MESOMARK was used to monitor the response of 28 patients to therapy with a copper reducing agent tetrathiomolybdate (57) and the time to progression (**Fig. 3**). MESOMARK assay is a two-step immunoassay test utilized to quantitate SMRP in human serum using enzyme immunoassay technology with colorimetric detection in a standard ELISA microplate sandwich assay format. MESOMARK levels decreased immediately postsurgery and increased over time during disease progression in 82.4% of patients with progressive disease. Patients with stable disease did not exhibit such increases in 45.5% of cases. The difference of MESOMARK concentration increases between the group with progressive disease and nonprogressors was statistically significant. Subsequent studies have validated a role for the monitoring of therapy in patients with MPM (58–62).

Overall, current evidence suggests high specificity but low sensitivity for mesothelin limiting its application as a screening test (63), but it remains the most used blood-based biomarker for diagnosis or monitoring of MPM treatment.

Serum MPF level was also found to be higher in patients with MPM, compared with healthy individuals, patients with benign

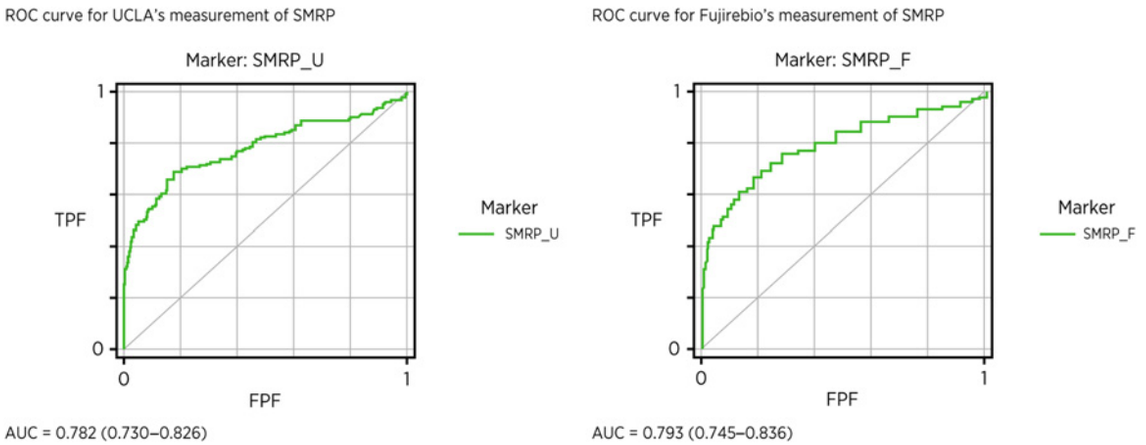

Figure 1.
EDRN validation trial results for SMRP as an MPM biomarker.

asbestos-related diseases, and those with lung cancer (64, 65). Further studies suggested equivalent diagnostic performances of SMRPs and MPF in distinguishing MPM from other diseases (46, 66). However, it must be noted that SMRPs and MPF levels can be significantly affected by covariates including age, renal function, and body mass index (BMI; refs. 59, 67–69).

Osteopontin

Osteopontin (OPN) is an extracellular protein involved in various biological processes including cell–matrix interaction, immunologic regulation, tumor development, and cell migration (70–74). OPN upregulation has been observed in asbestos-exposed cells *in vitro*, and in rat models for asbestos-induced carcinogenesis (75). Serum OPN levels are elevated in several cancers, including MPM, lung, colon, ovarian and breast cancer making it a potential diagnostic biomarker for MPM (76–81).

Discovery of OPN in MPM was completely funded through the EDRN grant mechanisms, both for diagnostic and prognostic studies. Its role in MPM pathogenesis was suggested by a 9-fold RNA expression elevation in in 48 MPMs over matched normal peritoneum using the HG1 Affymetrix array. Using a commercially available OPN ELISA, serum OPN levels were significantly higher in MPM patients compared with asbestos-exposed and asbestos-unexposed individuals, whereas levels were not significantly different between the latter two (79). Serum OPN levels had sensitivity and specificity for diagnosis of stage I MPM patients

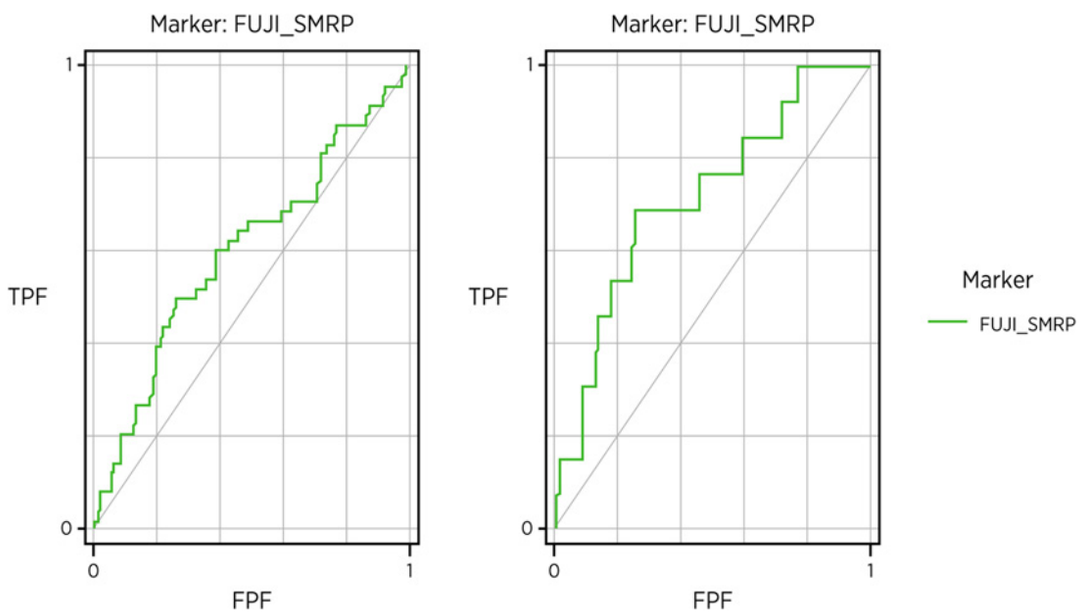

Figure 2.
ROC curve for SMRP, all cases [AUC = 0.604; 95% CI = (0.489–0.699)]; ROC curve for SMRP, cases < 1 year prior to diagnosis [AUC = 0.720; 95% CI = (0.562–0.853)].

from asbestos-exposed individuals, but failed to differentiate MPM from other pathologies associated with asbestos exposure (82). Utility of OPN in early diagnosis of MPM has remained controversial due to its lack of specificity, and the original results were validated by certain studies (83–85), and not by others (82, 86). Diagnostic performance of OPN was found to be higher when performed in plasma as compared with serum (83, 87). Hu and colleagues reported a meta-analysis of 6 studies with 360 patients with MPM and 546 patients with non-MPM, which revealed an overall sensitivity of 65% and specificity of 81% for OPN, with AUC of ROC curve equal to 0.83 (88). Overall, the data suggest that OPN can be a helpful biomarker for diagnosis of MPM; however, the primary role of OPN in MPM may be as a prognostic biomarker. Grigoriu (82) reported on the prognostic capability of OPN in MPM along with SMRP, and Hollevoet (89) confirmed the unfavorable prognosis of elevated OPN in multivariate analysis. An international EDRN-sponsored blinded study with a discovery set of 83 MPMs from the US and a validation set of 111 MPMs from Canada remains as one of the few studies to reveal how a blood-based biomarker can improve prognostication accuracy (90). Higher levels of OPN and mesothelin were individually associated with worse prognosis in both sets. Incorporating either plasma OPN or mesothelin into a baseline predictive prognostic index model substantively and statistically significantly improved Harrell C-statistic. A final model consisting of log-OPN, the EORTC clinical prognostic index, and hemoglobin remained as independently significant predictors of survival, and the combined biomarker and clinical model improved the Harrell C-index significantly from the clinical model, from 0.718 (0.67–0.77) to 0.801 (0.77–0.84). Most recently, OPN has been reported to have prognostic impact in peritoneal MPM (91).

Fibulin-3

Fibulin-3 is a member of extracellular glycoprotein fibulin family, encoded by EGF-containing fibulin-like extracellular matrix protein-1 (EFEMP-1) gene, and is implicated in regulation of cell proliferation and migration (92, 93). In studies sponsored by the EDRN, EFEMP1 was found to have a 7-fold increase in RNA expression ($P = 10^{-9}$) in 48 MPMs with matched normal peritoneum out of 32,000 probe IDs studied with the HG1 Affymetrix array. Using the only available ELISA in 2012, the MPM BDL reported that the plasma Fibulin-3 level was able to distinguish patients with MPM from healthy asbestos-exposed controls and patients affected by other cancers (94). Moreover, PE Fibulin-3 level was able to differentiate between patients with MPM and those with pleural effusion (PE) unrelated to MPM, and that PE fibulin-3 levels were prognostic. Plasma Fibulin-3 level cutoff of 52.8 ng/mL showed 96.7% sensitivity and 95.5% specificity in distinguishing patients with and without MPM, although slightly lower accuracy was observed in a blinded validation cohort from the Princess Margaret Cancer Center. Transfection of EFEMP1 into tert-transformed mesothelial cells was associated with increased migration, colony formation, and proliferation, and the opposite functional characteristics were seen with siRNA FBLN3 transfection into two MPM cell lines. This marker, however, has been one of the most controversial for MPM. Despite studies with strong supportive evidence (95), other studies from Europe and Australia indicated that Fibulin-3 was unable to distinguish patients with MPM from patients with other pathologies (96), and that its diagnostic performance was lower than that of mesothelin (97). Pei and colleagues meta-analysis of 7 studies with 468 MPM showed a pooled sensitivity of 62% (95% CI, 45%–77%) and specificity of 82% (95% CI, 73%–89%) for serum Fibulin-3 level in diagnosis of MPM (AUC of ROC: 0.81; ref. 98). Concerns regarding lot inconsistencies associated with the sole FBLN3 USCN ELISA have led to a series of investigations funded by the EDRN for the development of alternative assays for FBLN3. The EDRN sponsored MPM BDL has reported at EDRN scientific meetings that a custom MRM mass spectroscopy assay for FBLN3 distinguished 15 MPMS from 15 asbestos-exposed controls with an AUC of 0.82, significantly better than that reported from Australian investigators (0.69) using the USCN ELISA. Further studies presented at EDRN scientific meetings comparing asbestos-exposed plasma to MPM plasmas have reported MPM data from newer fibulin 3 ELISAs, including the LS-Biosciences Chemoluminscent human EFEMP1 ELISA, with an AUC of 0.93. In addition, the MPM BDL has developed a one of a kind slow off rate modified aptamer (somamer) Luminex assay using a FBLN3 Somamer with AUCs of 0.98. The SomaMer assay could also separate plasma from patients with non-MPM pleural effusion from those with MPM effusion with AUCs of 0.93. Finally, in collaboration with researchers who first described EFEMP1 expression in glioblastoma (99), a novel sandwich ELISA has been constructed in the MPM BDL using mAB382 developed at Brigham and Women's Hospital and results are encouraging. Further blinded validations of FBLN3 with other MPM and asbestos-exposed cohorts are underway.

Proteomics (multiplex protein signature) and glycomics

The proteome is the entire set of proteins that can be expressed by a given type of cell or organism, at a given time, under defined conditions. Proteomics have yielded valuable protein signatures that can help in diagnosis of various malignancies including MPM (100–102). One of the most intriguing of the proteomic platforms for MPM was sponsored by the EDRN, and was developed by Somalogic using over 1100 Slow Off-Rate Modified Aptamers (SOMAmers). SOMAmers are short, single-stranded deoxynucleotides that are designed to bind specific protein targets and are modified to be selectively eluted from the protein during steps to concentrate and quantitate the proteins that they bind (103, 104). Multiplexing of these SOMAmers allows many proteins to be quantitated with very small amounts of sample. In a collaboration between the MPM BDL and Somalogic, SOMAmers were used in a multicenter case–control study on 117 patients with MPM and 142 controls with asbestos exposure (102). This study screened over 1,000 proteins, identified 64 candidate biomarkers, and generated a 13-marker random forest classifier that could distinguish patients with MPM from controls with both sensitivity and specificity of >90% (AUC of 0.99), superior to performance of mesothelin. Moreover, the 13 SOMAmer panel was validated in two other cohorts.

In a recent study, quantitative mass spectrometry was used to explore MPM proteomic profile and was able to identify a specific group of proteins upregulated in MPM effusions that could distinguish MPM PE from benign reactive and adenocarcinoma PEs (105).

Glycomics with the quantification of serum glycosylated moieties was pursued by the BDL over a 4-year period, but early promising signatures could not be validated either for diagnosis or prognosis (106). Cerciello and colleagues identified a seven glycopeptide signature as a potential biomarker for diagnosis of MPM (107). Further studies confirmed earlier findings (105, 108).

Genomics and epigenomics

With the rapid technical advances in next-generation sequencing, biomarker discovery and validation for MPM should accelerate.

The EDRN BDL has had a long-term interest in genomic events in MPM. Prior to the routine use of next-generation sequencing, the EDRN BDL used Representative Oligonucleotide Microarray Analysis (ROMA) to study copy number abnormalities (CNA) in patients with MPM who recurred at variable interval with the disease after surgery (109). A significantly greater increase in CNA was noted in the patients with early recurrence, with deletions in chromosomes 22q12.2, 19q13.32, and 17p13.1 as the most frequent events (55%–74%). This was one of the first presentations that CNA in MPM was associated with prognosis. The EDRN sponsored MPM BDL was also one of the first laboratories to publish whole-exome sequencing of pleural MPM in collaboration with the Broad Institute (Cambridge, MA) and identified 517 somatic mutations across 490 mutated genes (22). Frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 were detected and these findings have been validated and expanded by larger studies from Brigham and Women's (110) and the TCGA (111).

Tissue and circulating miRNAs

miRNAs are small noncoding RNAs involved in regulation of gene expression and modulation of many cellular activities, such as proliferation, differentiation, apoptosis, angiogenesis, and invasion (112–116). Tumor cells demonstrate a specific signature of miRNAs and are able to release miRNAs through active secretion or after cell death (117, 118). MPM miRNA expression profiles have been widely reported (119–131). One of the earliest tissue-based studies was published by the EDRN BDL in collaboration with Rosetta Genomics, Israel demonstrating that mir-29c* was a prognostic miRNA-independent of MPM histology (124). Moreover, the mechanism of mir-29c* was through epigenetic regulation of the tumor via downregulation of DNA methyltransferases as well as upregulation of demethylating genes. The prognostic impact of mir-29c* in MPM has been validated by the TCGA (**Fig. 4**; Gordon Robertson; personal communication).

Many other studies have investigated the role of miRNAs in MPM with little overlap of prognostic or diagnostic miRNAs between studies, and there has been a lack of validation of models. Difference in normalizers/housekeepers may be the etiology for some of the discrepancies. An early study showed over-expression 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 in MPM (123). Another study showed overexpression of miR-17-5p, miR-21, miR-29a, miR-30c, miR-30e-5p, miR-106a, and miR-143 (122). Both studies found correlation between expression of certain miRNAs and specific tumor histologic subtypes. Tomasetti reported that miR-126-3p was able to distinguish MPM patients from healthy controls (126), whereas another study found it unable to differentiate patients with MPM from asbestos-exposed controls (130). Loss of miR-31 was found to be associated with tumor suppressor activity (126). Other miRNAs found upregulated in MPM are miRNA 197-3p, miRNA-1281, miRNA 32-3p (132, 133), miR-625-3p (121), miR-103a-3p (120, 134), miR-30e-3p (133), and

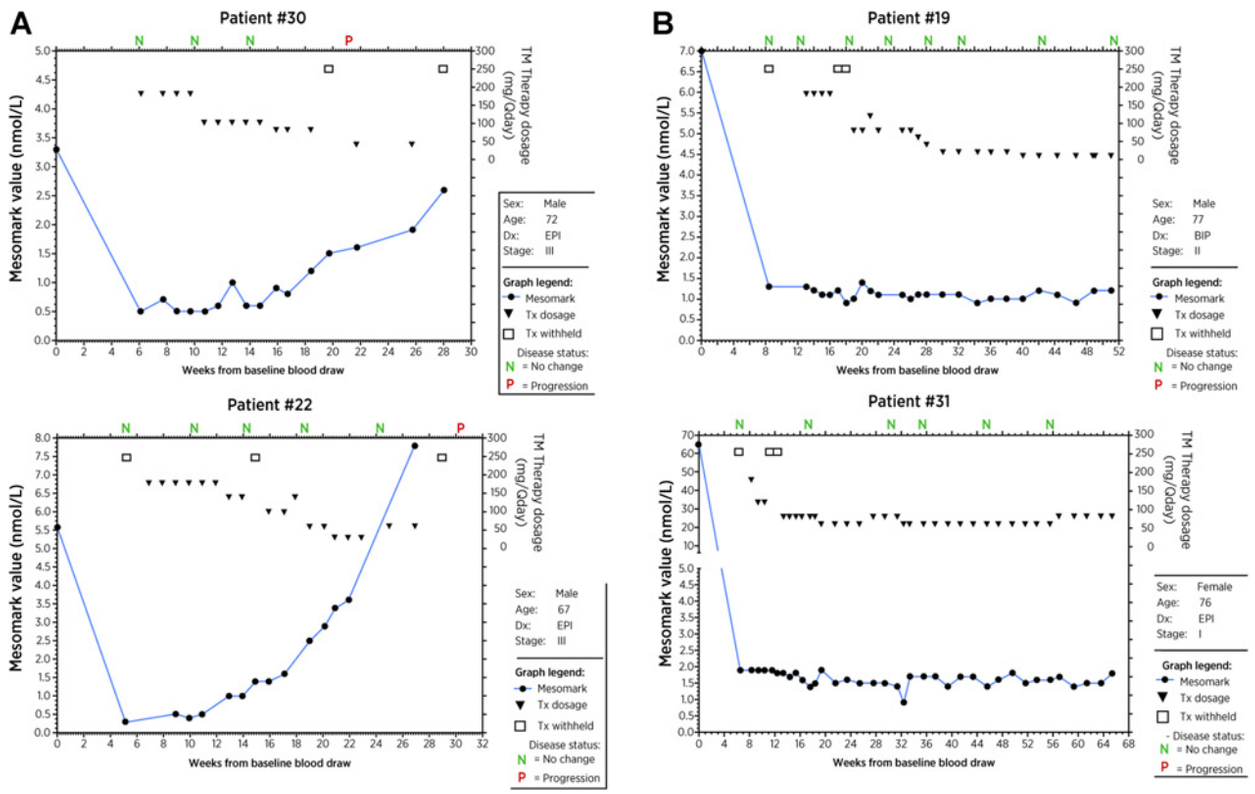

Figure 3.

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 prior to surgery and during the course of treatment postsurgery.

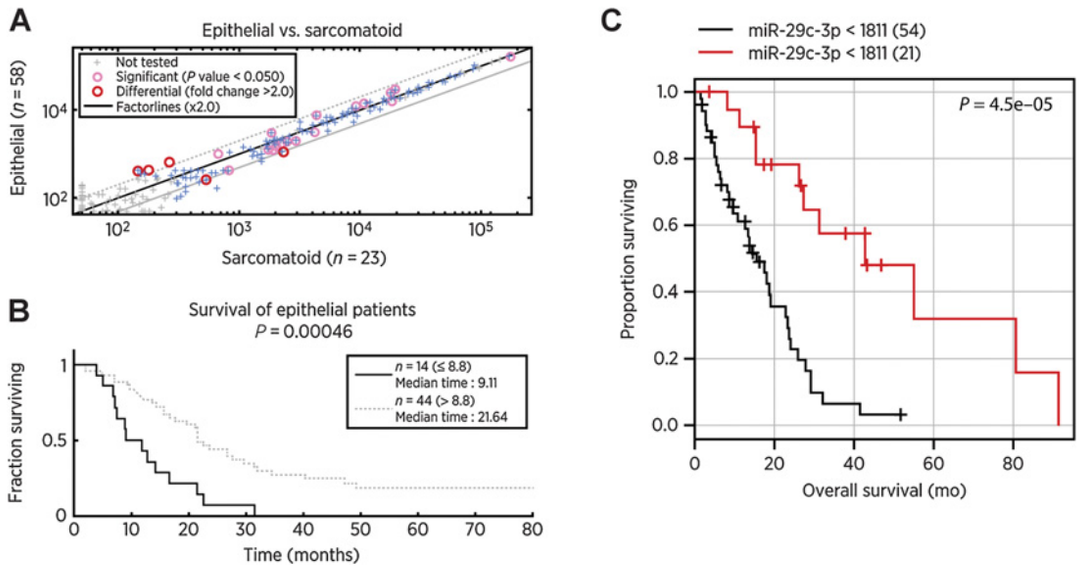

Figure 4.

mir-29c* and MPM. **A** and **B,** Significant elevation of the miRNA in epithelial mesothelioma and loss of the miRNA associated with poor prognosis. **C,** Validation in 75 patients with TCGA MPM.

miR-2053 (135). Investigators at Brigham and Women's Hospital have concentrated on discovery and validation of miRNA ratios in the prognostication of mesothelioma (136–139).

Evaluation of newer platforms has been a characteristic of the EDRN BDL, and the HTG EdgeSeq miRNA Whole Transcriptome Assay measured the expression of 2,083 human miRNA transcripts. This platform when combined with next-generation sequencing (NGS) uses only 15 μL of serum or plasma. Preliminary data using 7 serum miRNAs separated 93 MPMs from 53 asbestos-exposed pipe fitters with an AUC of 0.953 (140).

ctDNA

Circulating tumor (ctDNA) is the fraction of circulating cell-free DNA (cfDNA) released by the tumor apoptosis, necrosis, or active secretion (141, 142). Higher concentration of cfDNA-containing tumor mutations are detected in patients with cancer (143) and reflect the mutations that are present in the tumor. As opposed to other malignancies, there are only a few reports of ctDNA with mesothelioma, and these at least show proof of principle that the technique can be performed but with low sensitivity. In 10 patients with MPM having whole-exome sequencing with validation of the tumor-specific variants by digital droplet PCR, patient-specific, selected variants could be detected in circulating DNA from only three treatment naïve MPM patients, either in one or both independent droplet digital PCR runs (144). Japanese investigators used a different approach by specifically looking at the degree of miR-34b/c methylation in serum-circulating DNA using a digital methylation–specific PCR assay (MSP), since this miRNA is downregulated in MPM in 90% of the cases (145). Their technique evolved from MSP to the use of digital droplet PCR methods with MSP (146). Using a discovery and validation set of MPM, patients with pleural plaques and healthy volunteers' sensitivity and specificity comparisons between discovery and validation sets were promising: 76.9% and 59.1% sensitivities and 90% and 100%, specificity, respectively. Accuracy improved even further with advancing stage of disease. Further refinement in larger cohorts are expected with these ctDNA platforms in the future.

DNA methylation

DNA methylation is an epigenetic modification on regulatory regions of genes, the pattern of which can be modified by factors such as environmental exposure, aging, disease, and therapy (147). Initial studies show that the DNA methylation profile in tissue can differentiate between MPM cells and normal pleural cells (148). Further studies have suggested that DNA methylation profile in peripheral blood, isolated or in combination with other biomarkers, may be helpful in diagnosing MPM (127, 149). A genome-wide methylation array was used in a discovery set and a test set of MPMs and asbestos-exposed controls to identify novel DNA methylation markers from whole blood. Considering the top differentially methylated signals, seven single- cytosine-guanine dinucleotides, and five genomic regions of coordinated methylation were seen in both cohorts and a model using clinical characteristics of age, sex, asbestos exposure levels, and cytosine-guanine dinucleotides (CpG) methylation levels had an AUC of 0.89 (149). The next step for such studies would be to see whether these methylation profiles change over time in a known at-risk asbestos-exposed population.

Circulating tumor cells

Circulating tumor cells (CTC) are intact cells released from primary or metastatic tumor sites, the number of which increases in blood as cancer progresses to more advanced stages. Although methods of counting these cells has been helpful in diagnosis of other cancers such as lung cancer, the diagnostic value of CTCs for MPM has been low (150–152). Recently, improvements in CTC capture with microfluidics has demonstrated increased efficacy for CTC quantitation in MPM. Chikaishi and colleagues developed a "CTC-chip" that had better diagnostic value for MPM by developing a novel microfluidic device (CTC-chip) in which the capture antibody was one against

podoplanin which is abundantly expressed on MPM (153). Few cells were detected in earlier stages and in clinical samples only 68% were positive for CTCs. The best discrimination was when the number of CTCs for stages IIIB and IV were compared with earlier stages (AUC = 0.851) and a CTC count ≥2 cells/mL was significantly associated with a poor prognosis ($P = 0.030$).

Inflammatory and angiogenic factors

High-mobility group box 1

High-Mobility Group Box 1 (HMGB1) is a damage-associated molecular pattern (DAMP) involved in various biological processes including transcription, proliferation, DNA repair, and inflammation (154, 155). While in physiologic conditions, HMGB1 functions as a chromatin-binding protein and is released by cells undergoing necrosis, in pathologic states a hyperacetylated form of HMGB1 can be secreted by inflammatory and cancer cells (156–158). Human mesothelial cell exposure to asbestos induces programmed necrosis and release of HMGB1, activation of Nalp3 inflammasome, and eventually cell transformation (15, 18, 19, 159, 160). Serum HMGB1 level was found to be higher in patients with MPM compared to non-MPM asbestos-exposed individuals (161). Studies showed a significantly higher total blood HMGB1 level in patients with MPM and asbestos-exposed patients, compared with healthy controls (162). Moreover, significantly higher levels of hyper-acetylated HMGB1 was detected in patients with MPM, compared with asbestos-exposed patients and healthy controls.

The EDRN BDL is actively pursuing new techniques for measuring and validating whether acetylated isoforms of HMGB1 are sensitive and specific for detection of MPM in serum or plasma. Preliminary data have revealed that the combination of HMGB1 and fibulin-3 could provide better diagnostic performance (162). A systematic review and meta-analysis by Wu and colleagues recognized HMGB1 as a prognostic marker for MPM (163).

Calretinin

Calretinin is a calcium-binding protein encoded by the *CALB2* gene that was originally found in neurons, but is also expressed on mesothelial cell surface and overexpressed in MPM (164, 165). Raiko and colleagues developed a calretinin assay that was able to distinguish MPM patient from asbestos-exposed and healthy controls, and also differentiate between the latter two (166). A later study by Johnen and colleagues confirmed prior results and revealed a sensitivity of 71% for a predefined specificity of 95%, and comparisons of controls compared with MPMs had AUCs between 0.77 and 0.95 depending on the county of origin and gender of the specimens (167–169). Moreover, blood calretinin levels were able to prediagnose mesothelioma in an asbestos-exposed population with an AUC of 0.77 one to 15 months prior definitive diagnosis (170) and the AUC could be improved to 0.85 with combination with serum mesothelin. These results are some of the most encouraging in the literature, and validation studies for both Calretinin and FBLN3 are being planned.

ENOX2

ENOX2 is a cell surface protein involved in oxidization of reduced pyridine nucleotides and is essential for cell growth (171). Various tissue specific patterns of ENOX2 transcript variants have been discovered corresponding to specific cancer cell origins (172). ENOX2 proteins are released in circulation and can be detected at an early stage in certain cancers, including breast, lung, colon, prostate, and ovarian cancer (172). Morré and colleagues identified specific ENOX2 protein transcript variants characteristic of MPM that were present in sera of patients 4–10 years prior to development of clinical symptoms (173).

Thioredoxin-1

Thioredoxin-1 (TRX) is a small redox-regulating protein that has a role in decreasing ROS levels (174). Its expression was found to be increased in patients with MPM (18). Demir and colleagues indicated that TRX, as well as SMRP levels, increase in a graded fashion among controls, asbestos-exposed individuals, and patients with MPM, respectively, and they observed a sensitivity of 92.9 % and specificity of 77.6% for TRX in diagnosing MPM (175).

VEGF

VEGF is a key neoangiogenesis stimulator and is overexpressed in various malignant tissues including MPM (176–178). VEGF level is also elevated in PE of patients with MPM compared with those with PE due to nonmalignant pleural diseases or lung cancer (179). Serum VEGF level was found to be higher in patients with MPM compared with patients with nonmalignant asbestos-related diseases (180).

Combined panels

Various combinations of biomarkers have been studied with the aim of achieving higher accuracy in diagnosis of MPM (162, 181). Combinations of mesothelin, calretinin, and MPF showed a sensitivity of 82% in men at a fixed specificity of 95% (AUC = 0.944), and a sensitivity of 87% in women also at a fixed specificity of 95% (AUC = 0.937; ref. 181). Bonotti and colleagues explored various combinations of biomarkers and found two best three-marker combinations as IL6-OPN-SMRP and IL6-OPN-Desmin (AUC = 0.945 and 0.950, respectively; ref. 182). The best four-marker combination was SMRP-OPN-IL6-Vimentin (AUC = 0.962).

Breath analysis

Exhaled breath consists of liquid phase containing water vapor and gaseous phase including nitrogen, oxygen, carbon dioxide, inert gases, and a small function of volatile organic compounds (VOC; ref. 183). VOCs can originate either exogenously via inhalation or skin absorption or endogenously as a result of physiologic and pathophysiologic processes in the body, such as inflammation, oxidative stress, and metabolism (184, 185). A single breath sample typically contains around 200 different VOCs, and over 3,000 different VOCs are described so far (183). VOCs have been widely studied as potential biomarkers in diagnosis of benign and malignant diseases, including MPM (32, 186–188). Gas chromatography–mass spectrometry (GC-MS) is the gold standard for breath analysis. Other methods include electronic noses (eNoses), multicapillary column ion mobility spectrometry (MCC-IMS; ref. 189), selected ion flow tube-mass spectrometry (SIFT-MS; ref. 190), proton transfer reaction-mass spectrometry (PTR-MS; ref. 191), and Canine scent test (185). Meta-analysis of various methods of breath analysis used in diagnosis for MPM showed the highest level of accuracy for eNose (95%) and the lowest level for MCC-IMS (65%; refs. 187, 192). In a recent study, Töreyin and colleagues performed a systematic review of the studies assessing various methods of breath analysis in diagnosis of MPM. There were a total of 135 MPM patient and 427 non-MPM cases including asbestos-exposed individuals, healthy controls, patients with asbestos related benign diseases, non-asbestos–exposed lung diseases, and lung cancer. While meta-analytic approaches to pool diagnostic accuracy of each biomarker was not performed due to small sample sizes and number of the studies, results were provided in a comparative

Table 1. Summary of major biomarkers for diagnosis of MPM.

Biomarker	Year	Author	N (MPM/total)	Compared groups	Method	Results	Conclusion
Mesothelin	2014	Creaney et al. (97)	82/153	Non-MPM malignant, benign	Plasma, pleural fluid; ELISA (mesothelin and Fibulin-3)	Mesothlein showed high diagnostic accuracy for MPM. Plasma AUC: 0.822 Pleural AUC: 0.815	Mesothelin is a superior diagnostic biomarker for MPM compared to Fibulin-3.
	2014	Bayram et al. (63)	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.
	2013	Creaney et al. (46)	66/213	Other malignant, benign, asbestos-exposed healthy, kidney disease	Pleural fluid, serum ELISA	Serum and pleural mesothelin was elevated 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).
	2012	Hollevoet et al. (54)	1,026/5,517	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	2017	Burt et al. (58)	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.
	2016	Demir et al. (175)	42/131	Asbestos-exposed, healthy	Serum (various markers, including SMRP, TRX, EGFR, Mesothelin, syndecan-1, and 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.
	2015	Santarelli et al. (127)	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 had 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.
	2013	Filiberti et al.(55)	—/1,704	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.
	2011	Hollovoet et al. (59)	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.
	2010	Hollovoet et al. (66)	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.
	2010	Luo et al. (53)	717/3,568	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 OR of 19.35 (95% CI, 10.95–34.17).	Serum SMRP level is a candidate biomarker for diagnosis of MPM.
	2009	Rodriguez Portal et al. (51)	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.

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Table 1. Summary of major biomarkers for diagnosis of MPM. (Cont'd)

Biomarker	Year	Author	N (MPM/total)	Compared groups	Method	Results	Conclusion
	2008	Pass et al. (48)	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 stage 2–4 MPM compared with stage 1 MPM.	Serum and pleural SMRP levels can be used in screening asbestos-exposed individuals.
	2008	Pass et al. (57)	30/85	—	MesoMark ELISAs (response to therapy with a copper-reducing agent tetrathiomolybdate, assessed by target ceruloplasmin levels and VEGF levels)	SMRP levels decreased immediately postsurgery and increased over time during progression of disease.	SMRPs can have a role in monitoring posttreatment MPM patients.
	2008	Park et al. (56)	—/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.
	2006	Scherpereel et al. (50)	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.
	2003	Robinson et al. (43)	44/272	Healthy, asbestos-exposed, other inflammatory or malignant lung and pleural diseases	Serum; ELISA	SMRP levels were elevated in vast majority of MPM patients. Elevated 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	2013	Creaney et al. (46)	66/213	Other malignant, benign, asbestos-exposed healthy, kidney disease	Pleural fluid, serum; ELISA	Serum and pleural MPF were elevated 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).
	2010	Hollovoet et al. (66)	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).
	2006	Onda et al. (65)	56/126	Healthy	Serum, ELISA (MPF and SMRP)	MPF level was elevated in 91% of patients with MPM compared with healthy controls.	MPF can aid in diagnosis of MPM.
Osteopontin	2014	Hu et al. (88)	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	Osteopotin is an effective biomarker for MPM diagnosis.
	2014	Bayram et al. (63)	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.
	2014	Felten et al. (81)	–/2262	Formerly asbestos-exposed, unknown history of asbestos exposure, non–asbestos-exposed	Blood; commercial ELISA (osteopontin and mesothelin)	Osteopontin rise was associated with age.	Age effects on biomarkers need to be taken into account.

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Table 1. Summary of major biomarkers for diagnosis of MPM. (Cont'd)

Biomarker	Year	Author	N (MPM/total)	Compared groups	Method	Results	Conclusion
	2011	Creaney et al. (87)	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 MPM patients 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.
	2011	Cristaudo et al. (83)	31/235	Healthy, benign respiratory disease	Plasma; ELISA (plasma osteopontin and serum SMRP)	Plasma osteopontin level was significantly higher in patients with MPM compared to other groups. AUC: 0.795	Combined osteopontin and SMRP panel provides a high accuracy for diagnosis of MPM. AUC: 0.873
	2010	Rai et al. (85)	205/286	Healthy, non-mesothelioma other cancer patients	Plasma; ELISA (plasma osteopontin and serum SMRP)	Osteopontin level was significantly higher in MPM patients 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.
	2007	Grigoriu et al. (82)	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, was unable to distinguish between MPM and pleural metastatic carcinoma or benign pleural lesions associated with asbestos exposure.	Insufficient specificity limits osteopontin utility as a diagnostic marker.
	2005	Pass et al. (79)	76/190	Asbestos-related nonmalignant pulmonary disease, healthy non–asbestos-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 as a biomarker for diagnosis of MPM in asbestos-exposed individuals.
Fibulin-3	2017	Pei et al. (98)	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 diagnosis of MPM. AUC: 0.81	Fibulin-3 has a relatively high diagnostic efficacy for identification of MPM.
	2016	Napolitano et al. (162)	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.
	2015	Kaya et al. (95)	43/83	Healthy	Serum; ELISA	Serum Fibulin-3 level was significantly higher in MPM patients compared with controls. AUC: 0.976	Serum fibulin-3 is a useful biomarker for diagnosis of MPM.
	2014	Creaney et al. (97)	82/153	Non-multiple myeloma 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.
	2012	Pass et al. (94)	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.

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Table 1. Summary of major biomarkers for diagnosis of MPM. (Cont'd)

Biomarker	Year	Author	N (MPM/total)	Compared groups	Method	Results	Conclusion
Proteomic	2012	Ostroff et al. (102)	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	2013	Cerciello et al. (107)	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	2018	Sun et al. (140)	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.
	2016	Bononi et al. (132)	10/30	Asbestos-exposed workers; healthy	Serum; microarray, Real-Time Quantitative (RT-qPCR)	miR-197-3p, miR-1281, and miR-32-3p upregulated in MPM.	Distinct mRNAs are potential new biomarkers for diagnosis of MPM.
	2015	Santarelli et al. (127)	45/188	Asbestos-exposed, healthy	Serum [various markers, including miR-126, SMRP, and methylated thrombomodulin (Met-TM)]	Combination of miR-126, SMRP, and Met-TM has higher diagnostic accuracy compared with isolated SMRP. AUC: 0.857 vs. 0.818	Combined 3-biomarker panel including miR-126 provides high diagnostic accuracy for MPM.
	2014	Andersen et al. (131)	45/76	Reactive mesothelial proliferations (RMP)	Tissue RT-qPCR, formaldehyde-fixed paraffin-embedded preoperative biopsy samples.	A 4-miRNA group including miR-126, miR-143, miR145, and miR-652 was able to accurately differentiate MPM. AUC: 0.96	The identified 4-miRNA group can aid in differentiation of MPM from RMPs.
	2012	Tomasetti et al. (126)	45/121	NSCLC, healthy	Serum qRT-PCR	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.
	2010	Busacca et al. (122)	24/24	—	Tissue; miRNA microarray analysis; RT-qPCR	Analysis of MPM specimen revealed over-expression 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.
	2009	Guled et al. (123)	17/17	—	Tissue; miRNA microarray analysis	miRNA microarray analysis of MPM revealed over-expression 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	2019	Guarrera et al. (149)	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	2017	Ying et al. (161)	15/497	Healthy, asbestos-exposed <10 yrs, asbestos-exposed >10 yrs, 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.
	2016	Napolitano et al. (162)	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.

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Table 1. Summary of major biomarkers for diagnosis of MPM. (Cont'd)

Biomarker	Year	Author	N (MPM/total)	Compared groups	Method	Results	Conclusion
Calretinin	2018	Johnen et al. (170)	34/170	Healthy	Plasma, ELISA	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.
	2017	Johnen et al. (167)	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.
	2010	Raiko et al. (166)	42/174	Asbestos-exposed, healthy	Plasma, ELISA	Calretinin was significantly higher in MPM compared to asbestos-exposed and healthy (no AUC provided).	Calretinin has high sensitivity in diagnosis of MPM and can be used as a biomarker for diagnosis of MPM.
Thioredoxin-1 (TRX)	2016	Demir et al. (175)	42/131	Asbestos-exposed, healthy	Serum [various markers, including thioredoxin-1 (TRX), SMRP, EGFR, mesothelin, syndecan-1, and Fibulin-3]	TRX (as well as 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.

summary table and by Forrest plots. Conclusions need to be drawn with caution given the wide variability of reported accuracy for each test in different studies. Nonetheless, studies fairly consistently show that breath analysis methods have higher diagnostic value in distinguishing MPM from other pathologic states, than from healthy controls. Results are promising for E-Nose, GC-MS, and MCC-IMS; however, larger studies are needed to further elucidate their application and role as biomarkers in the diagnosis of MPM (193).

Discussion

Further discussion, apart from that seen in the discussion of the individual biomarkers above, as well as short synopses can be seen in **Table 1**.

Conclusions

When one considers the wealth of papers in the literature investigating diagnostic, monitoring, and prognostic biomarkers for MPM as summarized in **Table 1**, it is disappointing that only one remains as a validated blood-based test for the disease in North America, Europe, and Australia. Nevertheless, MesoMark has stood the test of time as the only validatable markers despite its lack of sensitivity. Fibulin 3 remains intriguing and new assays as well as new ELISAs are showing very promising results similar to the original publication. For prognostic power, osteopontin remains the standard as it has been externally validated by another cohort.

Failure of validation is multifactorial and includes the failure to have large archives of prospectively accrued specimens of high quality, lack of funding apart from the EDRN in the performance of large-scale validation trials, and the need for a more unified approach to these studies among mesothelioma investigators. As opposed to lung cancer, enthusiasm of industry support for MPM biomarker development and their validation is lacking, possibly due to a smaller market, and due to the incorrect assumption that the disease will simply disappear. There will be a continued need for the accurate diagnosis and early detection of the disease especially with the increasing number of cases due to familial BAP1 germline mutations and discovery of other carcinogenic fibers like erionite in new locations. Future funding of such efforts hopefully will continue through peer review study sections, including the EDRN, the DOD, and the NIEHS.

Disclosure of Potential Conflicts of Interest

H.I. Pass reports funding from the NCI, the U.S. Department of Defense, the Centers for Disease Control and Prevention, Genentech, and Belluck and Fox. M. Carbone and H. Yang report grants from the NIH, the NCI, the U.S. Department of Defense, and the UH Foundation through donations to support research on "Pathogenesis of Malignant Mesothelioma" from Honeywell International Inc., Riviera United 4 a Cure, and the Maurice and Joanna Sullivan Family Foundation. M. Carbone has a patent issued for 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." M. Carbone is a board-certified pathologist who provides consultation for mesothelioma expertise and diagnosis. No potential conflicts of interest were disclosed by the other authors.

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