



## REVIEW

# Effectiveness of mesothelin family proteins and osteopontin for malignant mesothelioma

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**ABSTRACT:** Malignant mesothelioma (MM) is an aggressive tumour with poor prognosis whose early diagnosis is difficult. Mesothelin, megakaryocyte potentiating factor (MPF) and osteopontin have attracted attention as biomarkers. The aim of the present review is to provide an overview regarding these candidate biomarkers for MM, and discuss their potential role in today's clinical practice.

Mesothelin and MPF have good specificity but sub-optimal sensitivity for detection of MM, being negative both in the sarcomatoid histological sub-type and in almost half of epithelioid mesothelioma, especially in the early stages. Osteopontin is a marker of the duration of asbestos exposure, but lacks specificity for mesothelioma. Several patient characteristics influence the diagnostic accuracy of biomarkers and make the establishment of the "optimal" diagnostic threshold difficult. Mesothelin and MPF have proved useful in assessing response to treatment. Combining different markers together may lead to an improvement in diagnostic accuracy, but there is still need for research in this area. Extensive validation and further research is required to improve the use of serum markers in mesothelioma management. In the near future, their application in clinical practice is probably in monitoring response to therapy, rather than in guiding diagnostic decisions and risk assessment of asbestos-exposed populations.

**KEYWORDS:** Asbestos, megakaryocyte potentiating factor, soluble mesothelin-related peptides

**M**alignant mesothelioma (MM) is a highly aggressive tumour mainly attributed to asbestos exposure [1]. Despite a ban of asbestos in many industrialised nations, the present high incidence of MM is expected to continue, due to the continued use of asbestos in several developing countries and the long latency period between first asbestos exposure and tumour presentation, making it an important health issue for the next decades [1, 2]. MM is responsible for approximately 15,000–20,000 deaths annually worldwide [3]. Although pemetrexed in combination with a platinum agent improves survival of unresectable MM patients, the overall survival after the diagnosis is 9–12 months [4]. However, patients with early-stage disease can survive for  $\geq 5$  yrs if the tumour is promptly resected [5].

Early diagnosis offers the best hope for a favourable prognosis. However, the early and reliable diagnosis of MM is notoriously difficult and unfortunately  $<5\%$  of patients with pleural MM

present with stage IA disease. Furthermore, it is not unusual for patients to undergo several medical investigations without definitive diagnosis early in the disease [6]. There is therefore a growing need for sensitive biomarkers to assist with the early diagnosis and management of MM.

An ideal biomarker would identify patients with MM, predict its development in asbestos-exposed subjects, differentiate MM from benign pleural disease or metastatic cancer, be useful for all pathological subtypes, and correlate with disease extent in order to monitor treatment response and predict prognosis. The biomarker should be measurable in biological samples collected using non- or minimally invasive tests and it should have an acceptable cost [7].

"Classical" biomarkers such as hyaluronic acid, various cytokeratin fragments and other cancer antigens found in serum and/or in effusions are not sensitive or specific enough, and none currently

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provides satisfactory reliability [8]. Recently, mesothelin, megakaryocyte potentiating factor (MPF) and osteopontin have attracted attention as potential candidates for MM tumour markers. The aim of the present review is to provide a current overview of the literature regarding these candidate biomarkers for MM and discuss their potential role in today's clinical practice.

## METHODS

The PubMed database was searched to identify papers using the keywords: "mesothelin", "soluble mesothelin related peptides", "megakaryocyte potentiating factor (MPF)", "osteopontin" and "MM". No lower date limit was applied. Reference lists of papers were searched manually to identify relevant publications. Articles were also identified by use of the related-articles function in PubMed. The last literature search was performed on March 15, 2012. Only articles written in English were reviewed. 71 articles were found to be relevant and were included in this non-systematic review.

## MESOTHELIN

Mesothelin is normally expressed at low levels in mesothelial cells and overexpressed in several human tumours, including MM, ovarian and pancreatic adenocarcinoma [9, 10]. The mesothelin gene encodes a precursor protein that is processed to yield mesothelin, which is attached to the cell membrane by a glycosylphosphatidyl inositol linkage and a soluble shed fragment named MPF [9]. Mesothelin has three isoforms that can enter the blood circulation, either by shedding of the membrane-bound portion (variants 1 and 2), or by a frameshift mutation (variant 3). Serum mesothelin refers to all isoforms that are present in the circulation, although variant 1 is predominantly expressed and released from the membrane [11].

Mesothelin may facilitate metastasis of mesothelin-expressing cancers by binding cancer antigen (CA)125 [11]. It promotes proliferation of pancreatic cancer cells through alteration of cyclin E as a result of constitutive activation of signal transducer and activator of transcription (STAT) protein 3 [12]. In addition, mesothelin overexpression results in upregulation of growth/survival pathways through autocrine production of growth factors such as interleukin (IL)-6 [13]. Mesothelin also induces an increase in nuclear factor (NF)- $\kappa$ B activation, which leads to resistance to tumour necrosis factor- $\alpha$ -induced apoptosis [14], indicating a mechanism through which mesothelin may help increase survival of tumour cells in the highly inflammatory milieu, evident in pancreatic cancer through Akt/phosphoinositide 3-kinase/NF- $\kappa$ B activation and IL-6 overexpression. Mesothelin overexpression results in secretion of high levels of IL-6, which could be responsible for the cells' increased viability and proliferation under serum-reduced conditions through an IL-6/soluble IL-6 receptor trans-signalling mechanism and the induction of the IL-6-STAT3 pathway [12, 13].

Multiple assays are available today for the measurement of mesothelin. However, the majority of studies use the US Food and Drug Administration-approved Mesomark™ (Fujirebio Diagnostics Inc., Malvern, PA, USA) kit, which remains the most studied assay available. It uses a sandwich ELISA format for the quantitative measurement of mesothelin in human serum and pleural fluid, which detects variants 1 and 3. The assay has not been approved for diagnostic use, but as an aid in the monitoring of epithelioid and biphasic MM [1].

## Diagnostic performance

Concentrations of mesothelin in serum of MM patients are significantly higher than those of healthy individuals [15–21]. Furthermore, it has been demonstrated that its levels in patients with MM are significantly higher when compared with those of patients with other cancers (including lung cancer) or other inflammatory lung or pleural diseases [16–27]. To date, there is no established cut-off value, but various cut-off points have been suggested for distinguishing between MM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. Table 1 shows sensitivity, specificity and the area under the receiver operating characteristic curves (AUC) at several cut-off points. The wide range of the reported "optimal" thresholds depends on the different study populations and the effect of patient characteristics [21].

Significantly increased serum levels of mesothelin were found in sera from patients with MM of the epithelial sub-type, but not in the sera from patients with sarcomatoid subtype, as the mesothelin gene is only expressed in epithelioid MM [15, 16, 19, 20, 24, 26, 28]. Some studies have also reported increased mesothelin values in mixed subtypes [16, 19]. Furthermore, whenever the sarcomatoid subtype was excluded, the diagnostic accuracy of mesothelin increased [29].

Although differences in mesothelin levels between the early and late stages of MM were evident in most studies, they did not always achieve statistical significance [15, 16, 26, 28]. However, four studies showed that patients with advanced-stage MM had significantly higher concentrations compared to those with stage I disease [19, 21, 22, 27].

## Treatment monitoring performance

Mesothelin measurement may be a useful way to monitor tumour growth as mesothelin concentrations have been correlated with tumour size and increase during tumour progression [17, 23]. Mesothelin levels fall with tumour resection and rise with its progression [17]. Measurement of mesothelin may be useful in monitoring treatment response [26, 35–37]. Two recent studies suggested that a 10% change in mesothelin levels above baseline values is significant [36, 37], whereas others proposed a threshold of 25% [38]. HOLLEVOET *et al.* [39] suggested a 15% threshold to avoid interference with ELISA variance, which can be up to 12%.

A significant association was observed between the response outcome and the relative change in mesothelin levels in 21 patients receiving chemotherapy [37]. This was further supported by CREANEY *et al.* [38], who demonstrated that a decrease in serum mesothelin levels following chemotherapy increases the likelihood that the tumour is responding to therapy and that it also predicts for improved survival independent of age, sex, histology and treatment. In addition, GRIGORIU *et al.* [36] reported that MM patients that responded to chemotherapy exhibited decreasing, or at least stable, serum mesothelin levels, whereas patients with progressive disease exhibited increasing mesothelin values. Moreover, they showed that survival of patients with decreasing/stable mesothelin values during follow-up was significantly greater than in patients with increasing mesothelin.

**TABLE 1** Efficacy of mesothelin for the detection of malignant mesothelioma (MM) in several studies

First author [ref.]	Cases n	Sample	AUC/comparison	Cut-off point	Sensitivity %	Specificity %
<b>ROBINSON [23]</b>	44 MM	Serum	NA/MM <i>versus</i> other pleural diseases	NA	84	100
	Control group		NA/MM <i>versus</i> other lung tumours	NA	84	95
	40 HAE		NA/MM <i>versus</i> other asbestos-exposed subjects	NA	84	83
	28 H non-AE					
	92 LID					
	30 C non-PLI					
	20 non-MMPLE					
<b>SCHERPEREEL [24]</b>	60 MM	Serum	0.872/MM <i>versus</i> BPDAE	0.93 nM	80	82.6
	Control group		0.693/MM <i>versus</i> Mets	1.85 nM	58.3	73.3
	23 BPDAE		0.771/MM <i>versus</i> control	1.1 nM	71.7	69.8
	30 Mets					
	43 MM PLE	Pleural	0.831/MM <i>versus</i> BPDAE	10.4 nM	76.7	76.2
Control group	0.793/MM <i>versus</i> Mets		11.4 nM	76	64	
<b>CRISTAUDO [16]</b>	21 BPDAE		0.809/MM <i>versus</i> control	11.4 nM	76.7	69.4
	28 Mets					
	107 MM	Serum	0.77/MM <i>versus</i> control	1.00 nmol·L <sup>-1</sup>	68.2	80.5
<b>GRIGORIU [28]</b>	262 healthy controls exposed or not to asbestos					
	96 MM	Serum	0.866/MM <i>versus</i> HAE	NA	NA	NA
	Control group					
	112 HAE					
33 BPDAE						
<b>CREANEY [29]</b>	43 Mets					
	52 MM PLE	Pleural and peritoneal effusions	0.898/MM <i>versus</i> all patients with benign effusions	20 nM	67	98
	Control group					
	84 PLE of benign aetiology					
	56 PLE of malignant non-MM					
7 MM PF						
<b>CREANEY [30]</b>	14 PF of malignant non-MM					
	21 PF of benign aetiology					
	117 MM	Serum	0.790/MM <i>versus</i> control	NA	52	95
	Control group					
33 HAE						
53 BPDAE						
<b>CREANEY [20]</b>	30 benign PLE					
	66 MM	Serum	0.915/MM <i>versus</i> healthy controls and BPDAE	NA	73	95
	Control group					
	10 HAE					
	10 H non-AE					
	21 BPDAE					
	30 PLE of benign aetiology					
20 PLE of malignant non-MM						
<b>PASS [22]</b>	10 LC					
	90 MM	Serum	0.810/MM <i>versus</i> AE	1.9 nM	60	89.2
	Control group		0.820/MM <i>versus</i> LC	1.1 nM	78.9	76.4
	170 LC		0.741/MM stage I <i>versus</i> AE	2.0 nM	58	91
	66 AE		0.891/MM <i>versus</i> control	1.075 nM	81.1	87.1
	409 H non-AE					
	45 MM PLE		Pleural	0.78/MM <i>versus</i> benign PLE	12.6 nM	75.6
Control group	0.76/MM <i>versus</i> control			12.6 nM	75.6	82
30 PLE of benign aetiology						
20 PLE of malignant non-MM						

**TABLE 1** Continued

First author [ref.]	Cases n	Sample	AUC/comparison	Cut-off point	Sensitivity %	Specificity %
<b>SCHNEIDER [26]</b>	129 MM Control group 139 LC 75 BPDAE	Serum	0.72/MM <i>versus</i> control	1.35 nM	53	82.7
<b>AZIM [27]</b>	50 MM Control group 33 breast cancer 22 H non-AE	Serum	0.765/MM <i>versus</i> control	7.22 nM·L <sup>-1</sup>	66	70.9
<b>IWAHORI [31]</b>	27 MM Control group 47 LC 35 other cancers 9 HAE 38 H non-AE	Serum	0.713/MM <i>versus</i> control	93.5 ng·mL <sup>-1</sup>	59.3	86.2
<b>AMATI [32]</b>	22 MM Control group 54 H non-AE 94 HAE	Serum	0.927/MM <i>versus</i> control	1.9 nM	72	90
<b>RODRIGUEZ PORTAL [18]</b>	36 MM Control group 48 H non-AE 177 HAE 101 BPDAE	Serum	0.75/MM <i>versus</i> control	0.55 nmol·L <sup>-1</sup>	72	72
<b>DAVIES [33]</b>	24 MM PLE Control group 67 PLE of malignant non-MM 75 PLE of benign aetiology	Pleural	0.878/MM <i>versus</i> control	20 nM	71	90
<b>HOLLEVOET [19]</b>	85 MM Control group 101 H non-AE 89 HAE 123 BPDAE 46 benign respiratory disease 63 LC	Serum	0.871/MM <i>versus</i> control	2.00 nmol·L <sup>-1</sup>	64	95
<b>CREANEY [25]</b>	70 MM Control group 111 BPDAE 20 LC 19 IPF 19 sarcoidosis 7 non-MM Exudative PLE	Serum Urine	0.881/MM <i>versus</i> all patients with benign disease 0.787/MM <i>versus</i> all patients with benign disease	1.62 nM NA	67 53	95 95
<b>CRISTAUDO [34]</b>	31 MM (epithelioid) Control group 93 H non-AE 111 benign respiratory disease associated or not with AE	Serum	0.762/MM <i>versus</i> control	1.32 nM	51.6	90

AUC: area under the receiver operating characteristics curve; HAE: healthy asbestos exposure; H non-AE: healthy non-asbestos exposure; LID: lung inflammatory diseases; C non-PLI: cancer without pleural involvement; non-MMPL: non-malignant mesothelioma pleural effusions; BPDAE: benign pulmonary/pleural disease associated with asbestos exposure; NA: not available; Mets: pleural metastasis of carcinomas; PLE: pleural effusions; PF: peritoneal fluid; LC: lung cancer; AE: asbestos exposure; IPF: idiopathic pulmonary fibrosis.

### Prognostic performance

Concentration of mesothelin may be an independent negative predictor of overall survival of MM patients [28]. In a study with 107 patients with MM, median survival was 11.7 months more if mesothelin values were lower than the cut-off point of  $1 \text{ nmol}\cdot\text{L}^{-1}$  compared to those with higher mesothelin levels [16]. Although with a higher cut-off point ( $3.5 \text{ nmol}\cdot\text{L}^{-1}$ ), these results were further supported by SCHNEIDER *et al.* [26], who reported that mesothelin levels differed significantly between patients with a favourable prognosis (median survival 17.1 months) and those with a worse prognosis (median survival 8.4 months). At 1-yr follow-up, survival rates were 63.1% and 32% ( $p=0.003$ ), respectively [26]. In contrast, HOLLEVOET *et al.* [21] found no prognostic value for mesothelin, even when corrected for the associated covariates. However, the general lack of a standard treatment regime for MM makes comparison of data difficult and the small study populations limit the statistical power of these studies. Large-scale validation of the prognostic value, in combination with standard clinical prognostic factors, is therefore needed.

### Screening

ROBINSON *et al.* [23] reported that of seven asbestos-exposed individuals who had increased blood concentrations of mesothelin, three developed MM later and one developed lung cancer within 1–5 yrs. None of the 33 asbestos-exposed participants whose blood samples had normal concentrations of mesothelin developed MM in the 8 yrs of follow-up. These findings have not been confirmed by other studies. Recently, a large-scale prospective study evaluated mesothelin as a potential screening tool for workers in a high-risk population with occupational exposure to asbestos. Of 538 occupationally asbestos-exposed individuals followed for 12 months, PARK *et al.* [40] found that 15 individuals had absolute values of soluble mesothelin  $>2.5 \text{ nmol}\cdot\text{L}^{-1}$ . Of those, one had chronic renal failure but no malignancy, another had early-stage lung adenocarcinoma and a third patient had a suspected cardiac tumour. No malignancy was noted in the remaining 12 patients. As a result, mesothelin is unlikely to prove useful for screening, and the false-positive rate for mesothelin screening will be high [40]. However, although the study sample was large, patients were followed for only 1 yr and a longer follow-up may have altered the results. Retrospective studies showed similar results to the study by PARK *et al.* [40]. Mesothelin levels were not elevated in serum collected 1–30 yrs prior to MM diagnosis [41]. Most recently, GUBE *et al.* [42] found that only two (10%) out of 20 patients with MM had a pre-diagnostic mesothelin level above a threshold of  $1.5 \text{ nmol}\cdot\text{L}^{-1}$ .

HOLLEVOET *et al.* [43] suggested that the use of a single baseline biomarker measurement alone is unlikely to be effective for screening asbestos-exposed individuals and that screening can benefit from incorporating serial biomarker measurements. The authors prospectively examined the longitudinal behaviour of mesothelin at 12 and 24 months in a total of 215 asbestos-exposed individuals, with no malignant disease and, in contrast to other studies, took into account the influence of age and glomerular filtration rate (GFR) on mesothelin levels. Mesothelin levels were strongly correlated across the sampling points but increased during follow-up. This was attributed to ageing, as GFR changes little in 2 yrs. The authors speculated that the use of

age- and GFR-adjusted biomarker reference values could act as a first triage and risk stratification. After this initial triaging, further follow-up would be guided by changes in serial biomarker measurements, accounted for ageing and changes in GFR. Biomarker measurements are expected to increase relatively more in patients who will develop MM compared with those who will remain disease-free [43].

CREANEY *et al.* [44], who evaluated mesothelin levels in pre-diagnosis longitudinally collected serum samples, reported that mesothelin concentrations were greater than the threshold value of  $2.5 \text{ nmol}\cdot\text{L}^{-1}$  in only 15% (17 out of 106) of asbestos-exposed individuals. When the authors examined relative increases in mesothelin levels rather than absolute increases, they identified that almost 40% of asbestos-exposed individuals developed MM. However, in this study, patient characteristics were not taken into account and this could have led to a better sensitivity.

Although an increased release of mesothelin in the serum may occur as a consequence of asbestos exposure, no correlation was observed between time of exposure and mesothelin levels [16, 22, 44], except in one report that suggested an association with asbestos exposure. The study was based on a difference in mesothelin levels between healthy and asbestos-exposed individuals [18].

The Early Detection Research Network is presently sponsoring the investigation of serum samples from CARET (Carotene and Retinol Efficacy Trial) and the North American Prostate Lung Colon and Ovarian Cancer Screening Trial to compare mesothelin and osteopontin for the screening of MM. Hopefully, either or both of these markers will be able to distinguish pre-diagnostic sera from diagnostic sera with sufficient sensitivity and specificity. If this happens, a larger prospective trial is planned using these markers to prospectively monitor serum levels from villagers in towns from Cappadocia, Turkey, who have a high incidence of MM because of environmental exposure to erionite [45].

### Pleural fluid mesothelin

Similar to serum mesothelin, median pleural fluid levels of mesothelin were significantly higher in patients with MM compared with either patients with pleural metastasis of carcinomas or patients with benign pleural lesions. Similar diagnostic power of serum mesothelin was also exhibited [22, 24, 29, 33, 46]. However, pleural levels of mesothelin were much higher than the respective serum values. Pleural levels of mesothelin were also significantly higher in epithelioid MM compared with sarcomatoid and mixed MM sub-types [24, 29, 33].

It noteworthy that in the diagnosis and exclusion of MM, pleural fluid mesothelin measurement was superior to cytological examination (sensitivity 71% *versus* 35%; specificity 89% *versus* 100%; negative predictive value (NPV) 95% *versus* 82%, respectively) [33]. For patients who had “suspicious” cytological features, pleural fluid mesothelin was 100% specific for MM. In addition, the NPV of mesothelin was 94% for 105 effusions in which cytological examination results were negative [33].

Moreover, pleural mesothelin levels can prognosticate survival, as the difference in overall survival between the groups

with pleural effusion mesothelin levels lower and higher than a cut-off point of 10 nmol·L<sup>-1</sup> was significant [46]. In contrast, a study by CREANEY *et al.* [29] indicated that pleural mesothelin levels provide no prognostic information in patients with MM given that a low concentration of mesothelin in an effusion may either reflect a small tumour burden (which may have a better prognosis) or a less differentiated tumour such as sarcomatoid MM (which has a worse prognosis). Interestingly, in the same study, pleural mesothelin levels were increased before diagnosis of MM in the effusions of four out of eight patients whose serum mesothelin levels were normal, and thus measurement of mesothelin in the pleural fluid of patients with suspected MM may be of some use when serum mesothelin levels remain normal [29]. Larger studies are needed to confirm the exact role of pleural fluid mesothelin in the diagnosis of MM.

### Mesothelin in urine

As urine is a more convenient biological sample than serum or pleural fluid and is considerably less invasive to collect, CREANEY *et al.* [25] evaluated whether measurement of mesothelin levels in the urine would improve sensitivity and specificity for the diagnosis of MM, as had been previously reported for patients with early-stage ovarian cancer [47]. At a specificity of 95% relative to individuals with benign lung or pleural disease, serum mesothelin had a sensitivity of 66% and AUC of 0.882, whereas urinary mesothelin corrected for urine creatinine concentration had a sensitivity of 53% and AUC of 0.787. The authors concluded that the low sensitivity of urinary mesothelin cannot allow its use as a biomarker specimen for MM diagnosis.

### MPF

Although mesothelin is strongly expressed in epithelioid MM, elevated serum mesothelin levels are present in only 50–75% of the patients with epithelioid MM [48]. In an attempt to discover a marker of MM with a better sensitivity, researchers hypothesised that MPF would be at least as sensitive as serum mesothelin. MPF, also referred to as N-ERC/mesothelin, originates from the same precursor protein as mesothelin [49]. MPF was originally identified as a cytokine with megakaryocyte-stimulating activity [50]. WANG *et al.* [51] found that N-ERC had a cytokine-like function and could stimulate tumour growth by suppressing cell death. ONDA *et al.* [52] established monoclonal antibodies against MPF and, ultimately, a sandwich ELISA using monoclonal antibodies to two different epitopes to measure the presence of MPF in the media of various mesothelin-expressing cancer cell lines and in human serum.

MPF is significantly higher in the serum of patients with MM compared with healthy donors [19, 20, 21, 31, 49, 52, 53] and patients with other lung or pleural diseases [19, 20, 21, 49, 53], such as patients with lung cancer [19, 21, 31], individuals with other cancers [21, 31] and healthy asbestos-exposed subjects [19, 21, 31, 53]. However, the few MPF validation studies used different MPF ELISA kits, making comparison difficult. Similar to mesothelin, MPF levels were significantly lower in patients with sarcomatoid MM, compared with those with epithelioid and mixed histology, as the mesothelin gene is only expressed in epithelioid MM [19, 53].

When MPF was compared to mesothelin for differentiating MM patients (n=27) from controls (n=129, including lung cancer patients, asbestos-exposed individuals and normal volunteers), MPF appeared to be more effective for the detection of MM. MPF with a cut-off value of 19.1 ng·mL<sup>-1</sup> achieved 74.1% sensitivity with 90.4% specificity, while mesothelin with a cut-off value of 93.5 ng·mL<sup>-1</sup> had 59.3% sensitivity and 86.2% specificity [31]. However, it should be kept in mind that the authors did not use the Mesomark™ assay for measurement of mesothelin, but they developed a new ELISA system [31]. A large, prospective multicentre study which used the Mesomark™ assay demonstrated that mesothelin and MPF have an equivalent diagnostic performance [19].

It has also been suggested that MPF levels can differentiate between early- and late-stage disease [19, 21, 53]. In a large prospective multicentre study, patients with stage I MM had significantly lower MPF levels compared with those with stage II, III and IV [19].

Furthermore, some studies report an association of MPF with disease course and suggest that measurement of MPF in the blood of patients with MM may be useful for monitoring the response of MM to treatment [34, 52, 54]. However, safe conclusions cannot be extracted as MPF has not yet been extensively studied as a marker of response.

MPF was reported to be an independent negative prognostic factor, but only if adjusted for the effects of age, GFR and body mass index (BMI). However, no prognostic MPF threshold was found, probably because of the relatively low number of patients and events. The role of MPF as marker of outcome consequently requires further validation in larger study populations, ideally with adjustment for the biomarker-associated covariates [21].

### OSTEOPONTIN

Osteopontin is an extracellular cell adhesion protein that is not only involved in non-mineral bone matrix formation, but is also a primary cytokine in mediating type I immune responses, and has been implicated in regulating metastatic spread by tumour cells. Osteopontin is a secreted glycoprotein implicated in cell-signalling pathways that are associated with asbestos-induced carcinogenesis [55]. Questions regarding its effectiveness as a biomarker are related to the fact that it is expressed in various non-pleural malignant diseases. Serum osteopontin levels are elevated in breast, ovarian, lung, colorectal, gastric, melanoma and prostate cancer [56]. Osteopontin can be detected in serum, plasma, urine and other bodily fluids and in tumour tissue.

Serum osteopontin levels have been reported to be higher in MM patients compared with healthy asbestos-exposed subjects and have a good capability in distinguishing these two populations [20, 28, 55]. Several studies have demonstrated that osteopontin levels can also be used to distinguish patients with pleural MM from those who have benign pleural disease associated with asbestos exposure [20, 57]. Osteopontin also reflects the extent of radiographic abnormalities as in the subgroup of asbestos-exposed patients; the highest levels of serum osteopontin are usually found in subjects who have both plaques and fibrosis [28, 55, 57]. In addition, there is a

relationship between osteopontin serum levels and duration of asbestos exposure [28, 55, 57].

Mean osteopontin levels do not vary according to the histological characteristics of the tumour and thus osteopontin, in contrast to mesothelin and MPF, can identify sarcomatoid patients and patients with a mixed histological sub-type too [20, 55, 57].

Conversely, osteopontin seems to be unable to distinguish between MM and pleural metastatic carcinoma and neither plasma nor pleural fluid osteopontin is more powerful in this respect [28]. Furthermore, no significant differences in osteopontin levels between patients with MM, lung cancer or effusions of a transudate or malignant nature were observed [20].

Osteopontin could discriminate between asymptomatic asbestos-exposed individuals and early-stage MM patients [55, 57]. In a study by PASS *et al.* [55] osteopontin differentiated asbestos-exposed patients from stage I MM patients with a sensitivity of 84.6% and a specificity of 88.4% at a cut-off value of 62.4 ng·mL<sup>-1</sup>. However, the utility of osteopontin as a screening marker is hampered by an insufficient specificity, which would result in a very high number of false-positive tests [28].

Regarding response to treatment, in contrast to mesothelin and MPF which decrease shortly after resection, osteopontin increases [39]. This probably highlights the effect of wound healing and tissue remodelling on osteopontin levels. When radiological response was compared with the changes in serum mesothelin, MPF and plasma osteopontin levels demonstrated that osteopontin levels were less closely associated with the radiological responses compared to those of mesothelin and MPF. However, a link between higher blood osteopontin level and patient's shorter survival has been reported, but extensive validation is absent [28, 39].

As osteopontin is cleaved by thrombin, plasma is more appropriate to measure osteopontin than serum. A French study reported higher osteopontin levels in plasma compared to serum, but showed that the ability of osteopontin to discriminate between patients with MM and healthy asbestos-exposed subjects was similar using either plasma or serum [28]. However, other studies suggest that plasma is superior to serum when assaying osteopontin levels with a view to discriminating between MM and control patients [58]. On the contrary, the choice of blood sample type has limited effect on soluble mesothelin sensitivity [59].

### COMBINATION OF BIOMARKERS

Historically, hyaluronic acid was the first proposed serum diagnostic marker for MM. When it was compared with mesothelin in 76 patients with MM, 33 patients with pleural metastases of carcinomas and 27 patients with benign pleural effusion related to asbestos exposure, mesothelin was more sensitive than hyaluronic acid in diagnosing MM and there was no benefit in combining both markers [60].

GUBE *et al.* [42] studied the value of mesothelin, CA125 and cytokeratin 19 fragment (CYFRA) 21-1 as markers for lung cancer and MM in a cohort of asbestos-exposed workers (n=626). The biomarkers were retrospectively analysed with an average time between sample collection and diagnosis of either lung cancer (n=12) or MM (n=20) of 4.7 yrs. Individually, the biomarkers showed low sensitivity and positive predictive

values, and combinations of the biomarkers investigated did not improve test sensitivity.

Moreover, combination of serum mesothelin and CA125 using a logistic regression model did not improve the sensitivity of MM diagnosis over mesothelin alone [30]. In contrast, combination of mesothelin and carcinoembryonic antigen increased accuracy in differentiating MM from nonsmall cell lung cancer [61].

In a prospective multicentre study, mesothelin and MPF had an equivalent diagnostic performance and combination of both markers did not improve detection of MM [19]. Unexpectedly, no improvement was observed in combining serum osteopontin and MPF with mesothelin in determining the accuracy of diagnosis of MM over the mesothelin marker used alone [20]. In addition, the combination of osteopontin and mesothelin did not improve diagnosis of MM in contrast to mesothelin alone [28].

In a recent study, combination of mesothelin and plasma osteopontin, through the application of a logistic regression formula, increased both sensitivity and specificity in MM diagnosis. The AUC increased from 0.795±0.05 for plasma osteopontin and 0.762±0.05 for mesothelin to 0.873±0.043 for the combination of mesothelin and plasma osteopontin [34].

### EFFECT OF PATIENT CHARACTERISTICS ON MESOTHELIN, MPF AND OSTEOPONTIN

The concentration of mesothelin appears to be influenced by the degree of renal dysfunction, which can produce falsely increased mesothelin concentrations [35, 62, 63]. This is a very important limitation as most patients with MM are elderly with various degrees of renal dysfunction, and their renal function sometimes becomes worse during chemotherapy.

Several studies have proposed that mesothelin values are positively associated with age and inversely associated with weight, BMI, performance status, blood glucose, % predicted single-breath carbon monoxide diffusing capacity and single-breath carbon monoxide diffusing capacity per unit alveolar volume [27, 40, 64]. Moreover, although there is no clear explanation, a correlation between elevated mesothelin and high serum alkaline phosphatase has been observed [27].

In addition, like mesothelin, MPF is influenced by renal dysfunction [21]. Recently, a large multicentre study demonstrated that mesothelin and MPF levels were independently associated with age, GFR and BMI in control subjects and with GFR and tumour stage in patients with MM. Neither age, BMI, sex, smoking history, tumour histology, C-reactive protein nor any of the blood count parameters displayed a significant association in MM patients. Mesothelin and MPF had either a high or a poor diagnostic accuracy, depending solely on the age, GFR and BMI of the control subjects, or the tumour stage of the patients with MM. Patients with MM were best distinguished from the control subjects with the youngest age, the highest GFR or the largest BMI. Furthermore, the control subjects were significantly better differentiated from stage II to IV than from stage I MM. MPF was an independent negative prognostic factor, but only if adjusted for the effect of age, GFR and BMI. However, the prognostic impact of MPF was relatively low, compared with other factors such as tumour stage, histology, and performance status [21].

Individual basal mesothelin levels can be affected by a genetic polymorphism within the 3' untranslated region of the mesothelin gene. The genetic polymorphism rs1057147 [G>A] can affect mesothelin expression and may account, at least in part, for increased levels of mesothelin in healthy subjects [65].

Furthermore, it has been suggested that in the normal state, the mesothelin gene within the pleura is methylated and this explains the low levels of mesothelin observed in non-diseased individuals. In contrast, the mesothelin gene is hypomethylated in MM tumours. In a study by TAN *et al.* [66], hypomethylation of the mesothelin gene in MM patients was observed in both the epithelioid type with positive mesothelin expression and the sarcomatoid type with negative expression. This result suggests that hypomethylation of the mesothelin gene occurs and might be involved in an earlier stage of MM before bifurcation to the epithelioid and sarcomatoid types. In a study by NELSON *et al.* [67], it was demonstrated that mesothelin gene methylation was significantly higher among tumours from patients testing negative for mesothelin (<1.5 nM) *versus* those that were positive ( $p < 0.03$ ). However, in a subset of tumours methylation is retained, and this mechanism explains the poor sensitivity of the mesothelin assay.

Osteopontin has some limitations too. Its concentration is also influenced by renal dysfunction, but to a lesser extent than that of mesothelin [68]. In a relatively small population previously exposed to asbestos, age, restrictive respiratory function and smoking habit could affect the result of serum osteopontin [69]. In addition, some common nonmalignant, non-pleural effusion associated conditions, including coronary artery disease, interstitial pneumonia and other benign pulmonary disease, can result in increased levels of osteopontin in the serum [20]. Conversely, CRISTAUDO *et al.* [58] found, in a large number of cases, that the mean levels of serum osteopontin were not correlated with personal anamnestic variables (age, duration of asbestos exposure and smoking cigarettes) or instrumental variables (spirometry, diffusion capacity, chest radiography and computed tomography).

Currently, there is no agreement on the diagnostic thresholds of any of the above-mentioned biomarkers, because the reported "optimal" thresholds range widely [70]. The establishment of the "optimal" diagnostic thresholds is difficult as it depends on the distribution of the aforementioned characteristics of the study population. Thus, mesothelin assays should be interpreted in consideration of clinical and genetic findings. The application of covariate-specific biomarker reference intervals, instead of a single threshold, might therefore improve the diagnostic use of mesothelin.

## DISCUSSION

The clinical application of soluble biomarkers of MM is still under debate. Osteopontin is a marker of the duration of asbestos exposure but lacks specificity for MM. Low levels of specificity combined with low pre-test probabilities make positive predictive values a barrier to widespread utility. The insufficient specificity of osteopontin limits its utility as diagnostic marker.

In contrast, soluble mesothelin family proteins have a good specificity but a sub-optimal sensitivity. However, a positive mesothelin test at a high-specificity threshold would provide a strong incentive to urge ensuing diagnostic steps [19]. By contrast

with the higher specificity, mesothelin will have low sensitivity. The low sensitivity will not allow exclusion of non-MM patients even if they have mesothelin concentrations lower than the cut-off value. Thus, it would not be advisable to use a negative mesothelin test to exclude MM, even at a high-specificity threshold [70]. However, for the establishment of the optimal threshold, it should be taken into account that the diagnostic accuracy of the biomarkers depends on the distribution of individual characteristics, such as GFR, age, tumour stage and histology. The incidence of the disease in the studied population is also important as it will strongly influence the results.

Increased effusion levels of mesothelin before a definitive cytological and/or histological diagnosis may provide an early suggestion of the presence of malignancy and indicate the need for active invasive investigation such as thoracoscopy to establish a diagnosis. Pleural mesothelin levels can also be used as an adjunct to cytological examination for the diagnosis of MM as reported by DAVIES *et al.* [33]. In cases where fluid cytology shows MM cells with a high degree of certainty, measurement of mesothelin adds nothing. On the contrary, a high level of mesothelin in the fluid, especially in the absence of malignant cells, suggests a diagnosis of malignancy, particularly MM and the need for early biopsy.

The more accurate association with tumour debulking and radiological response indicates that mesothelin and MPF are more suitable for routine monitoring, when compared with osteopontin. The findings of increased mesothelin concentrations in patients at recurrence or progression after initial therapy suggest that mesothelin measurement might be a useful tool for monitoring patients' response. Consequently, systemic therapy may be started or an ineffective treatment changed. Response evaluation after chemotherapy or chemoradiotherapy is known to be difficult as Response Evaluation Criteria in Solid Tumors, the standard method for radiological response assessment, is not adequate in pleural MM as it often presents as a rind around the lungs, rather than as a spherical mass [71]. Mesothelin measurement may be helpful to overcome this problem. If confirmed in larger series, mesothelin or MPF could be used as an adjunct to radiological monitoring in patients with epithelioid or mixed MM [39].

Mesothelin, MPF and osteopontin are all promising for the early detection of MM, but none of these has had rigorous validation in a blinded fashion using retrospective specimens that were collected in a prospective manner during trials in which MM developed. Furthermore, the limited sensitivity of these markers for early stages might limit their use in screening. However, it should be kept in mind that in asbestos-exposed individuals, the positive predictive value of any diagnostic tool is also limited due to the low prevalence of MM. This makes a screening-based early detection strategy only worthwhile if the target population is better selected. If found to be sufficiently accurate, biomarker levels, alone or in combination with radiographic findings, could triage asbestos-exposed individuals, resulting in an enriched population with an increased risk of developing MM. The resulting sub-group could thereby be subject to a more dedicated medical follow-up (*i.e.* more frequent diagnostic exams) [32, 43].

Larger studies are needed to establish the appropriate cut-off point for each biomarker as a highly sensitive cut-off would be



responsible for a high number of false positives, and consequently a high number of unnecessary potentially harmful radiological tests. However, a more specific cut-off would reduce the sensitivity, thereby clearly limiting the added value of these biomarkers in diagnostic practice [70]. Maximising specificity at the cost of sensitivity will be helpful in confirming diagnosis of MM. Combining different markers together may lead to an improvement in diagnostic accuracy, but there is still a strong need for research in this area. Research should also focus on the patients that lack elevated mesothelin levels, including patients with epithelioid histologies.

In conclusion, extensive validation and further research is required to improve the use of serum markers in MM management. In the near future, their application in clinical practice is most probably situated in monitoring response to therapy, rather than in guiding diagnostic decisions and risk assessment of asbestos-exposed populations. Discovery of new soluble biomarkers that may help in the diagnosis and management of MM will be watched with great interest.

#### STATEMENT OF INTEREST

None declared.

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