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Prognostication and monitoring of mesothelioma using biomarkers: a systematic review

David T Arnold*,[1], Duneesha De Fonseka[1], Fergus W Hamilton[2], Najib M Rahman[3] and Nick A Maskell[1]

[1]Academic Respiratory Unit, School of Clinical Sciences, University of Bristol, Southmead Hospital, Learning and Research Centre, Westbury-on-Trym, Bristol BS10 5NB, UK; [2]Southmead Hospital, North Bristol NHS Trust, Bristol BS10 5NB, UK and [3]Oxford Centre for Respiratory Medicine and Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford OX3 7LE, UK

Background: Radiological markers of treatment response and prognostication in malignant pleural mesothelioma have limitations due to the morphology of the disease. Serum or pleural fluid biomarkers that could act as an adjunct to radiological assessment would be of significant value. The aim of this review was to collate and summarise the literature relating to this topic.

Methods: A systematic review was performed on the databases Pubmed and EMBASE to identify relevant studies. Two independent researchers read the abstracts and used the Quality in Prognostic Studies tool to assess the quality of the evidence.

Results: Forty-five studies were identified from the current literature. Twenty studies investigated the role of serum soluble mesothelin with majority suggesting that it has variable utility as a baseline test but when measured serially correlates with treatment response and prognosis. Several studies demonstrated that serum osteopontin correlated with survival at baseline. Other biomarkers have shown prognostic utility in individual studies but are yet to be reproduced in large cohort studies.

Conclusions: From the available literature no serum or pleural fluid biomarker was identified that could be recommended currently for routine clinical practice. However, a falling serum soluble mesothelin might correlate with treatment response and improved survival.

Malignant pleural mesothelioma (MPM) is a rapidly progressive and invariably fatal malignancy. Mean survival is 9–14 months from diagnosis (Chapman *et al*, 2008). First-line palliative chemotherapy with pemetrexed and a platinum-based agent (Cisplatin or Carboplatin) has been the standard of care for over a decade (Vogelzang *et al*, 2003). However, following encouraging results from the MAPS trial, many guidelines are now advocating the addition of bevacizumab to this regimen (Zalcman *et al*, 2016). Despite this, chemotherapy has only a modest impact on survival of around 2–3 months with a possible small improvement in symptomatology (Arnold *et al*, 2015). Response to chemotherapy differs greatly between patients with a partial response rate of 30–40% (Vogelzang *et al*, 2003). Clinical trials and clinicians use radiological markers to assess treatment response and progression-free survival (PFS). The current best practice is serial thoracic computerised tomography (CT) scans reported using the modified RECIST criteria, a technique which only partially allows for the fact that MPM usually grows as a pleural rind as opposed to a spherical mass. Other challenges, such as the presence of pleural fluid (pf) and benign asbestos-related plaques, make radiological assessment of MPM difficult (Armato *et al*, 2006).

A blood or pf biomarker that could act as an adjunct to radiological assessment by giving prognostic information as well as reflecting response to treatment would be of considerable use to clinicians. The majority of literature on biomarkers in MPM focuses on their utility as a screening or diagnostic test. Most

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*Correspondence: Dr DT Arnold; E-mail: arnold.dta@gmail.com

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researched is soluble mesothelin (SM)-related peptide often called SM. Soluble mesothelin is the circulating form of a 40 kDa membrane-bound glycoprotein and is highly expressed by mesothelial cells in MPM and some other cancers (Robinson *et al*, 2003). Soluble mesothelin levels are much higher in epithelioid MPM compared with other histological subtypes, and in larger tumours. However, its diagnostic ability in both serum and pf is limited by an inability to exclude MPM with a negative result. Another biomarker is megakaryocyte potentiating factor (MPF), also called N-ERC/mesothelin as it is formed from the same precursor protein as SM (Hollevoet *et al*, 2010). Osteopontin (OPN) is a glycoprotein that mediates cell-matrix interactions and has been shown to infer a poor phenotype when raised in other malignancies, including breast, lung and colon (Pass *et al*, 2005; Shojaei *et al*, 2012). Finally, fibulin-3 is an extracellular glycoprotein, which has shown promise in the diagnosis of MPM (Pass *et al*, 2012), but information on its role in prognostication is limited. We performed a systematic review of studies that had assessed the role of biomarkers in providing prognostic and treatment response information for MPM in an attempt to guide clinicians as to the strength of evidence for use in current practice.

MATERIALS AND METHODS

Search strategy. The databases PubMed (Medline) and EMBASE were interrogated for papers related to our study question. The 30 June 2016 was used as a cut-off with no early limit date applied. The search was limited to English language papers using the search terms shown in Supplementary Table 1. The search terms were designed to limit the search to papers that provided information on prognostication and disease monitoring and have been used in previous such studies (Altman, 2001; Dretzke *et al*, 2014). Articles were also identified using the 'related articles' function of PubMed and the references of the selected papers were assessed for other relevant papers. Two reviewers (DA and FH) screened the abstracts for study eligibility; any disagreements were resolved by mutual consensus.

Study inclusion criteria

- Involved the measurement of a serum or pf biomarker in patients with proven MPM.
- Treatment response or survival data collected and correlated with biomarkers.

Study exclusion criteria

- Reported only biomarkers from tumour immunohistochemistry.
- Results duplicated from another selected study.
- Involved less than 10 patients with MPM.
- Conference abstract or letter.

Quality assessment and data extraction. Once the full set of articles were extracted, two reviewers (DA and FH) independently applied the quality in prognostic studies (QUIPS) tool to assess the quality of selected studies (Hayden *et al*, 2013). The QUIPS score of each paper has been reported in Supplementary Table 2. In addition, routine data were extracted from the studies including author, publication year, study type (prospective or retrospective), patient number, histological subtype, patient treatment and biomarkers studied.

Data extraction was dependant on the findings reported by the individual studies. Correlation of biomarkers with survival is reported using univariate or multivariate Cox-regression analysis unless otherwise stated. Any survival time comparisons are reported using hazard ratios (HR) with 95% confidence intervals (CI).

RESULTS

The search strategy generated 1325 abstracts from the Pubmed and EMBASE databases. After screening all the abstracts, 68 were read in full by the independent reviewers. A further 23 were excluded as they did not meet the review criterion (see Figure 1). Therefore, 45 studies were included in the final review. Because of considerable heterogeneity between studies in areas such as histological subtype, biomarker testing and patient therapy, no attempt to combine or meta-analyse the data was made. The selected studies are shown in Table 1 and summarised by biomarker below.

Soluble mesothelin (SM). Serum/plasma SM was the most studied biomarker with 20 studies (18 prospective, 2 retrospective), a total of 1578 patients, investigating its role as a marker of prognostication or treatment response (Robinson *et al*, 2003; Cristaudo *et al*, 2007; Grigoriu *et al*, 2007; Pass *et al*, 2008; Schneider *et al*, 2008; Grigoriu *et al*, 2009; Wheatley-Price *et al*, 2010; Creaney *et al*, 2011; Hollevoet *et al*, 2011; Yamada *et al*, 2011; Franko *et al*, 2012; Hollevoet *et al*, 2012; Kao *et al*, 2012; Creaney *et al*, 2013; Nowak *et al*, 2013; Creaney *et al*, 2014; Hassan *et al*, 2014; Linch *et al*, 2014; Hooper *et al*, 2015; Pass *et al*, 2016). Early studies were primarily aimed at SM's ability to diagnose MPM from other malignant or benign lung pathologies with its role as a prognostic indicator a secondary outcome. The earliest study of SM in prognosis, correlated tumour size on CT with baseline SM, finding that levels were significantly higher in larger tumours ($P<0.01$) and those of epithelioid histology ($P<0.01$), but there was no correlation with overall survival (OS) (Robinson *et al*, 2003). Studies by Cristaudo *et al* (2007) and Grigoriu *et al* (2007) using the Mesomark ELISA found that higher baseline SM was correlated with worse OS using cut-offs of 1 nmol l^{-1} (HR: 1.6, CI: 1.1–2.4) and 3.5 nmol l^{-1} (HR: 2.8, CI: 1.4–5.6), respectively. Both papers combined patients who were treated with surgery, chemotherapy or best supportive care (BSC). The cut-offs used were selected from diagnostic studies or maximisation of HR models. In a study of 91 MPM patients who received a variety of chemotherapeutic regimens, there was a significant difference in OS between low (<3.5 nmol l^{-1}) and high baseline SM levels of 17.1 months *vs* 8.4 months, respectively (Schneider *et al*, 2008). This relationship was statistically significant at multivariate analysis (HR: 1.9, CI: 1.1–3.5, $P=0.025$) but lost statistical significance when applied to epithelioid histology alone. Two studies from Creaney *et al* (2013, 2014) involving 96 and 82 patients, respectively, found no correlation between OS and baseline serum SM.

An earlier prospective study from the same author tested the role of serum SM as a proxy for treatment response when measured serially (Creaney *et al*, 2011). They recruited 95 patients with MPM and tested serum SM at baseline, every 3 months and before every chemotherapy cycle, alongside thoracic CT scanning. Baseline SM was not correlated with OS at multivariate analysis when radiological markers of FDG-PET were included. In the chemotherapy group ($n=61$), there was a correlation between response on modified RECIST CT scans and changes in SM ($P=0.023$). They classified a rise or fall in SM as a change of greater than 25%, otherwise classifying as stable SM. In patients with partial response on CT (17 out of 55) none had a rise in SM with 5 stable levels and 12 falling. In 28 patients who had a repeat FDG-PET as part of follow-up, there was a correlation between percentage change in SM levels and percentage change in tumour TGV and volume ($P<0.01$). They also found a correlation between change in SM levels and OS with both the stable and rising groups having increased risk compared with the falling group, with HR of 2.0 (CI: 1.1–3.1) and 23.0 (CI: 7.5–70.9), respectively. In the small number of patients in this study who had an extrapleural

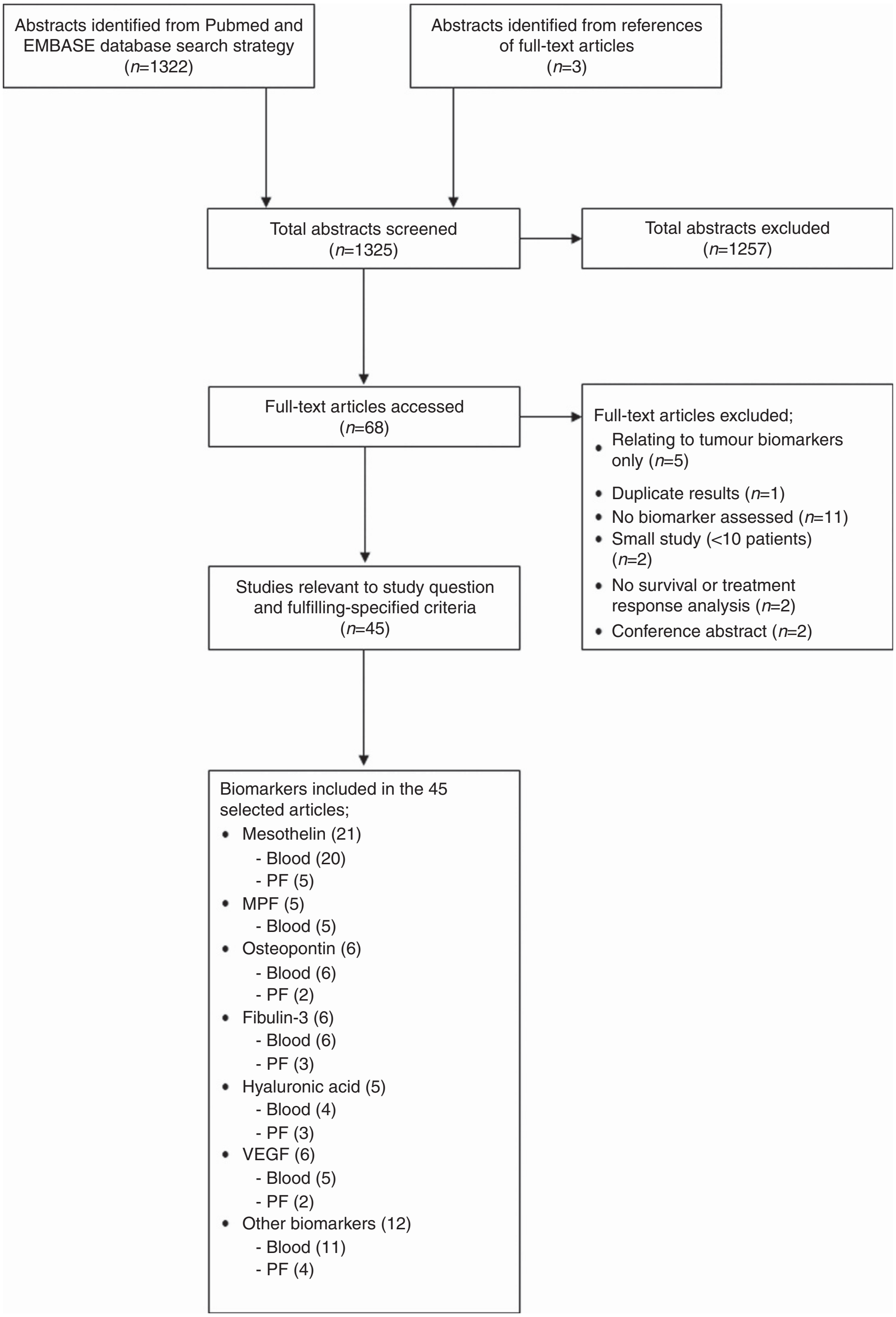

Figure 1. PRISMA flowchart.

pneumonectomy ($n = 6$), there was a mean 54% decrease in SM level pre to post surgery. There was no further testing of SM on these surgical patients to assess its role in predicting recurrent disease. Wheatley-Price *et al* (2010) published a similar study of 41 patients with non-sarcomatoid mesothelioma (39 pleural and 2 peritoneal) of whom 92% had an elevated baseline serum SM. Changes in SM or OPN were correlated with radiological reports (using descriptive reporting, RECIST and mod RECIST) during treatment. There was a significant association between relative and absolute change in SM and radiology reporting (all methods), the former having consistently better predictive value. Despite small numbers ($n = 13$), the same effect was seen in the BSC group with an average rise of SM by 26 and 60% for stable and progressed disease on mod RECIST criteria ($P = 0.004$). In total, this review identified eight studies that assessed the utility of serial serum SM testing in MPM. All found a correlation between falling SM levels and radiological response and/or improved OS (see Table 2).

Five studies (four prospective, one retrospective), with a total of 371 patients, investigated pf SM's ability to prognosticate from baseline (Creaney *et al*, 2007; Grigoriu *et al*, 2007; Yamada *et al*, 2011; Creaney *et al*, 2013, 2014). One study by Yamada *et al* (2011) found patients ($n = 45$) with higher pf SM levels survived significantly longer (dichotomy at 10 nM) at univariate but not

Table 1. Full table of selected studies

Author (year)	Study type	Biomarker	No. pts	Histology	Treatment
Pass *et al* (2016)	Prospective	SM (p) Fibulin-3 (p) OPN (p)	194	E – 135 NE – 59	Surg – 100 C – 141 RTX – 68
Kirschner *et al* (2015)	Retrospective	Fibulin-3 (p) Fibulin-3 (pf)	84 30	E – 88 S – 7 B – 17 D – 2	N/D
Hooper *et al* (2015)	Prospective	SM (s) Fibulin-3 (s)	73	E – 50 S – 19 B – 4	C – 58 BSC – 15
Kaya *et al* (2015)	Prospective	Fibulin-3 (s)	43	E – 30 NE – 13	C – 15 Surg – 7 BSC – 21
Fujimoto *et al* (2014)	Retrospective	CD26 (s) CD26 (pf)	80	E – 53 S – 9 B – 18	N/D
Raphael *et al* (2015)	Prospective	CTC (s)	27	E – 22 NE – 5	N/D
Linch *et al* (2014)	Prospective	SM (s)	53	E – 46 S – 1 B – 5 U – 1	C – 30 BSC – 23
Mundt *et al* (2014a)	Prospective	Syndecan 1 (s) OPN (s) Syndecan 1 (pf) OPN (pf)	91 89	E – 62 S – 10 B – 13 Un – 95	N/D
Mundt *et al* (2014b)	Prespective	Novel proteomes (s)	37	N/D	N/D
Hassan *et al* (2014)	Prospective	SM (s) MPF (s) Ca125 (s)	24	N/D	C/Im – 24
Creaney *et al* (2014)	Prospective	Fibulin-3 (p) SM (p) Fibulin-3 (pf) SM (pf)	82	E – 32 S – 8 B – 13 U – 29	C – 37 Surg – 4 BSC – 37 U – 4
Ghanim *et al* (2014)	Retrospective	Fibrinogen (p)	176	E – 146 S – 12 B – 18	C – 78 Surg – 54 BSC – 44
Mori *et al* (2013)	Prospective	MPF (s)	26	E – 21 S – 4 B – 1	C – 26
Creaney *et al* (2013)	Retrospective	SM (s) HA (s) SM (pf) HA (pf)	96	E – 53 S – 2 B – 9 U – 32	N/D
Tabata *et al* (2013)	Prospective	HMGB 1 (s)	61	E – 43 S – 8 B – 6 D – 3 A – 1	N/D
Nowak *et al* (2013)	Prospective	SM (s) VEGF isoforms (s) Interleukin-8 (s) S-Kit (s)	53	E – 39 S – 1 B – 10 Un – 3	Bio – 53 (second line)
Franko *et al* (2012)	Prospective	SM (s)	78	E – 64 S – 7 B – 7	C – 64 Surg – 10 BSC – 4
Kao *et al* (2012)	Prospective	SM (s) CRP (s) IL-6 (s) sIL-6r (s) VEGF (s)	63	E – 28 S – 4 B – 30	C – 63
Pass *et al* (2012)	Prospective	Fibulin-3 (p) Fibulin-3 (pf)	92 74	N/D	N/D
Kindler *et al* (2012)	Prospective	VEGF (p)	108	E – 76 NE – 32	C/Bio – 53 C – 55

Table 1. (Continued)

Author (year)	Study type	Biomarker	No. pts	Histology	Treatment
Ghanim *et al* (2012)	Retrospective	CRP (s)	115	E – 80 S – 27 U – 8	C +/– RTX – 64 Surg – 51
Hollevoet *et al* (2012)	Prospective	SM (s) MPF (s)	106	E – 91 S – 7 B – 8	C – 78 Surg – 19 BSC – 9
Yamada *et al* (2011)	Prospective	SM (pf)	45	E – 37 S – 5 B – 3	N/D
Hirayama *et al* (2011)	Prospective	VEGF (pf)	46	E – 34 S – 10 B – 2	N/D
Hollevoet *et al* (2011)	Prospective	SM (s) MPF (s) OPN (p)	62	E – 59 S – 1 B – 2	C – 48 Surg – 14
Yasumitsu *et al* (2010)	Prospective	VEGF (s)	51	E – 36 S – 6 B – 6 D – 3	N/D
Creaney *et al* (2011)	Prospective	SM (s)	95	E – 68 S – 9 B – 18	C – 61 Surg – 7 RTX – 2 BSC – 25
Grigoriu *et al* (2009)	Retrospective	SM (s)	40	E – 35 S – 3 B – 2	Im – 16 C – 20 BSC – 4
Wheatley-Price *et al* (2010)	Prospective	SM (p) OPN (p)	41	E – 41	C – 21 Surg – 7 BSC – 13
Schneider *et al* (2008)	Prospective	SM (s)	129	E – 81 S – 14 B – 17 U – 17	C – 68 Surg – 41 RTX – 4 BSC – 9 Un – 7
Pass *et al* (2008)	Prospective	SM (s)	90	E – 58 S – 3 B – 29	Surg – 90
Tajima *et al* (2008)	Prospective	MPF (s) OPN (p)	14	E – 11 S – 2 B – 1	C – 14
Grigoriu *et al* (2007)	Prospective	SM (s) OPN (s & p) SM (pf) OPN (pf)	96	E – 73 S – 10 B – 13	C – 70 Surg – 10 BSC – 16
Cristaudo *et al* (2007)	Prospective	SM (s)	107	E – 72 S – 10 B – 7 D – 3 U – 15	N/D
Creaney *et al* (2007)	Prospective	SM (s) SM (pf)	52	E – 15 S – 9 B – 5 U – 23	N/D
Filiberti *et al* (2005)	Prospective	PDGF-AB (s)	93	N/D	N/D
Robinson *et al* (2003)	Prospective	SM (s)	44	E – 25 S – 4 U – 15	N/D
Hedman *et al* (2003)	Retrospective	HA (s) Ca125 (s) TPA (s)	11	N/D	N/D
Strizzi *et al* (2001)	Retrospective	VEGF (s) VEGF (pf)	12	E – 8 S – 1 B – 3	N/D
Thylen *et al* (2001)	Retrospective	HA (pf)	100	E – 67 NE – 33	C – 56 BSC – 44

Table 1. (Continued)

Author (year)	Study type	Biomarker	No. pts	Histology	Treatment
Thylen *et al* (1999)	Prospective	HA (s)	19	E – 15 S – 1 B – 3	N/D
Schouwink *et al* (1999)	Retrospective	TPA (s) Ca125 (s) CEA (s) Cyfra21-1 (s)	52	E-31 S – 9 B – 10 Un – 2	C – 29 RTX – 5 Surg – 4 BSC – 14
Nakano *et al* (1998)	Prospective	IL-6 (s)	25	N/D	N/D
Bonfrer *et al* (1997)	Prospective	TPA (s) Cyfra21-1 (s)	29	E – 21 NE – 8	N/D
Dahl *et al* (1989)	Prospective	HA (s) HA (pf)	37	E – 28 S – 3 B – 5 MC – 1	C – 37

Abbreviations: A = Anaplastic; B = biphasic; Bio = biological therapy; BSC = best supportive care; C = chemotherapy; CEA = carcinoembryonic antigen; CRP = C reactive protein; CTC = circulating tumour cells; D = desmoplastic; E = epithelioid; HA = hyaluronic acid; HMGB1 = high-mobility group box 1; Im = immunotherapy; IL = interleukin; MC = multicystic; MPF = megakaryocyte potentiating factor; N/D = not documented; NE = non-epithelioid; OPN = osteopontin; p = plasma; pf = pleural fluid; PDGF= platelet derived growth factor; RTX = radiotherapy; S = sarcomatoid; s = serum; SM = soluble mesothelin; Surg = surgery; TPA = tissue polypeptide antigen; U = unknown; VEGF = vascular endothelial growth factor.

Table 2. Studies assessing treatment response or survival using serial serum SM during treatment

Author (year)	Treatment (no. of patients)	Outcome measure	Threshold for SM change	Results
Hooper *et al* (2015)	P/C – 58, BSC – 15	Mod RECIST CT OS, TTP	0%	Chemotherapy group; a falling serum SM at 6–8 weeks was associated with longer time to progression ($P<0.001$), and a falling SM post chemotherapy was associated with improved OS ($P=0.031$)
Hassan *et al* (2014)	P/C and Im – 20	Mod RECIST CT	15%	Fall in serum SM correlated with radiological response with 70% accuracy ($P=0.003$)
Nowak *et al* (2013)	Bio – 53	Mod RECIST CT FDG-PET OS, TTP	0%	Median change in serum SM correlated with sum change in tumour bulk on FDG-PET ($P<0.05$). % change in serum SM was associated with TTP ($P<0.001$) but not OS
Franko *et al* (2012)	G/C – 56, P/C – 8, BSC – 4, Surg – 10	Mod RECIST CT	n/a	Significantly lower mean serum SM in partial response or stable disease compared to progressive disease ($P=0.001$)
Hollevoet *et al* (2011)	P/C – 57, Surg – 5	Mod RECIST CT	15%	Partial response to chemotherapy correlated with a 34% fall in SM ($P=0.010$) compared with a 54% rise in progressive disease ($P<0.001$)
Creaney *et al* (2011)	Chemo – 61, BSC – 25, Surg – 8	Mod RECIST CT FDG-PET OS	25%	Chemotherapy group; correlation between change in serum SM and CT ($P=0.023$) and FDG-PET ($P<0.001$) Also, a falling SM was associated with better OS (19 months) compared with static (13 months) or rising levels (15 months). ($P=0.001$)
Wheatley-Price *et al* (2010)	Chemo – 21, BSC – 13, Surg – 8	Mod RECIST CT RECIST CT CT report	10% or 5 nmol l^{-1}	Chemotherapy and BSC groups; relative change in serum SM from baseline significantly associated with disease progression ($P<0.010$)
Grigoriu *et al* (2009)	Chemo – 20, Im – 16, BSC – 4	Mod RECIST CT	10%	In patients with raised SM at baseline ($>1\,\text{nM l}^{-1}$), rising level correlated with progressive disease in 12 out of 16 patients. OS higher in patients with stable SM compared with increasing ($P=0.012$)

Abbreviations: Bio = biological therapy; BSC = best supportive care; C = cisplatin; Chemo = chemotherapy (not specified); G = gemcitabine; Im = immunotherapy; Mod RECIST CT = modified response evaluation criteria in solid tumors CT; OS = overall survival; P = pemetrexed; Surg = surgery; TTP = time to progression.

multivariate analysis. This finding was not replicated in the other larger studies, which found no relationship between pf SM and OS.

Megakaryocyte potentiating factor (MPF) or N-ERC/mesothelin. All five studies (five prospective), with a total of 232 patients, involving serum MPF were published after 2008 (Tajima *et al*, 2008; Hollevoet *et al*, 2011, 2012; Mori *et al*, 2013; Hassan *et al*, 2014). The earliest by Tajima *et al* (2008) included 14 patients with MPM receiving a variety of chemotherapeutic regimens and tested MPF and OPN prior to and following treatment. Despite small numbers, the ratio between levels before and after therapy was lower in those who had progressed on RECIST criteria (i.e., levels had risen) compared with those with a partial response ($P < 0.05$). A larger study was performed by Hollevoet *et al* (2011) on 62 patients receiving either extrapleural pneumonectomy ($n = 14$) or pemetrexed/platinum-based chemotherapy ($n = 48$). Patients had modified RECIST CTs and matched MPF, SM and OPN levels (no greater than 3 weeks apart) before and after treatment. In the surgical group, only five patients had pre- and post-treatment samples and median levels of both MPF and SM fell by 76% and 78%, respectively (median OPN levels actually rose by 20%). In the chemotherapy group, the authors classified a change in biomarker level as a change of $> 15\%$ from baseline. They demonstrated that serum MPF (and SM) could predict treatment response, with a median 53% fall in partial response ($n = 14$) compared with 58% rise in progressive disease ($n = 16$). This study did not show any correlation between baseline MPF and OS. However, a study from the same author investigated the effect of age, BMI and renal function on serum MPF and SM levels, finding that only renal function altered biomarker levels in 106 MPM patients (a worsening renal function increased serum MPF and SM) (Hollevoet *et al*, 2012). Once this and other covariates were considered, baseline serum MPF (and not SM) was found to correlate with OS ($P = 0.040$). Finally, in a Phase 1 dose escalation study of an anti-mesothelin immunotherapy called SS1P (in combination with Pemetrexed and Cisplatin), the serum biomarkers MPF, SM and Ca125 were tested for correlation with treatment response (mod RECIST CT) (Hassan *et al*, 2014). Twenty patients were evaluable with biomarkers pre and post treatment. All three biomarkers showed 'strong significant correlation' with partial response, stable disease or progressive disease. The biomarkers' accuracy in predicting response on CT was assessed with 15% used as a cut-off for change in biomarker levels from baseline. Megakaryocyte potentiating factor correctly classified 15 out of 20 patients (75% accuracy) as having progressive or stable disease (with rising or stable/falling levels, respectively) based on their CT scan, compared with 14 out of 20 (70%) and 12 out of 20 (60%) for SM and Ca125, respectively.

Osteopontin (OPN). All six of the studies (six prospective) that assessed serum/plasma OPN, with a total of 498 patients, were looking at its role alongside other biomarkers. Two of these studies, total of 185 patients, also examined pf OPN (Grigoriu *et al*, 2007; Tajima *et al*, 2008; Wheatley-Price *et al*, 2010; Hollevoet *et al*, 2011; Abakay *et al*, 2014; Mundt *et al*, 2014a; Pass *et al*, 2016). Grigoriu *et al* (2007) measured baseline serum and pf OPN and SM (SM results above) in a cohort of 96 MPM patients. Baseline serum OPN had a statistically significant relationship with OS at multivariate analysis in a model that included tumour histology (HR: 3.46, CI: 1.1–10.9, $P = 0.034$). Using a cut-off of $350\,\mathrm{ng\,ml^{-1}}$ (selected using a maximisation of HR model), patients with a low-serum OPN had a median OS of 15 months compared with 5 months in high-serum OPN levels. Pleural fluid OPN was also measured and was not found to correlate with OS. Serum OPN's ability to act as a baseline prognostic marker was replicated by Hollevoet *et al* (2011) (study discussed above). Baseline OPN correlated with both OS and PFS (optimum cut-off for their data set was $863\,\mathrm{ng\,ml^{-1}}$) and appeared to be an independent factor with no correlation with other biomarkers or tumour stage. More recently, Pass *et al* investigated the benefit of adding baseline plasma biomarker levels to previously validated prognostic tools (EORTC prognostic index of mesothelioma and the CALGB index). In a discovery cohort of 83 patients, of whom two-thirds had cytoreductive surgery, baseline levels of plasma OPN, SM and fibulin-3 were measured. Both the plasma OPN and SM, but not fibulin-3, were independently correlated with OS. Interestingly, in a prognostic model including well known poor prognostic indicators such as low Hb and EORTC score (> 1.27) only high OPN remained an independently significant predictor of worse prognosis.

A study by Mundt *et al* (2014a) analysed serum and pf OPN from two separate cohorts to assess its role in diagnosis and prognosis at baseline. Although the diagnostic analysis of serum OPN involved 91 patients with MPM, full survival analysis was only available for 19 patients. Despite this data attrition, serum OPN was found to correlate with OS using a $185\,\mathrm{ng\,ml^{-1}}$ cut-off (HR: 2.5, CI: 1.4–10.3), the median of the data set. In the pf cohort, 40 patients had survival data available and using pf OPN cut-off of $1.6\,\mu\mathrm{g\,ml^{-1}}$ resulted in median OS times of 29 m *vs* 13 m for low to high levels, respectively (HR: 2.2, CI: 1.2–4.2). However, neither of these correlations were assessed using multivariate analysis or evaluated alongside tumour histology.

Fibulin-3. Six studies (five prospective, one retrospective) involving serum/plasma fibulin-3 were found by this systematic review, comprising 568 patients overall (Pass *et al*, 2012; Creaney *et al*, 2014; Hooper *et al*, 2015; Kaya *et al*, 2015; Kirschner *et al*, 2015; Pass *et al*, 2016). Of these, three studies (two prospective, one retrospective), with a total of 186 patients, also measured pf fibulin. No study found serum/plasma fibulin-3 to be a significant marker of prognostication at baseline. Hooper *et al* (2015) measured serum fibulin-3 before, during (after two cycles of Pem/Cis) and after chemotherapy. Baseline levels were higher in the epithelioid subtypes but there was no correlation with OS when histological subtypes were analysed separately. In addition, serial sampling did not predict treatment response or PFS.

The earliest study to analyse pf fibulin-3 as a prognostic marker was carried out by Pass *et al* (2012). The primary outcome was fibulin-3's diagnostic utility, so survival data was only available for a proportion of the pf cohort ($n = 54$). PF fibulin-3 correlated with pathological stage, with stages 1 and 2 ($n = 21$) having a median level of $576\,\mathrm{ng\,ml^{-1}}$ compared with $765\,\mathrm{ng\,ml^{-1}}$ in stages 3 and 4 ($P = 0.040$). When pf fibulin-3 levels were dichotomised at the database median of $733.4\,\mathrm{ng\,ml^{-1}}$ a low baseline level inferred better OS and this remained significant in a multivariate model that included gender, stage and histological subtype ($P = 0.024$). Creaney *et al* (2014) also measured both serum and pf fibulin-3 in a prospectively collected cohort of 82 patients with MPM with a focus on diagnostic utility but with follow-up data for the majority of the cohort ($n = 78$). Patients with biphasic or sarcomatoid histology had significantly ($P = 0.002$) higher pf fibulin-3 concentrations (median $1331\,\mathrm{ng\,ml^{-1}}$) compared with epithelioid subtypes (median $426\,\mathrm{ng\,ml^{-1}}$), but no relationship to tumour stage. A linear negative relation was found between OS and pf fibulin-3 and remained significant at multivariate analysis ($P = 0.017$). Lastly, a retrospective analysis of three cohorts of MPM patients (serum $n = 37$ and $n = 47$, pf $n = 30$) found that lower pf fibulin-3, but not serum, was associated with improved OS at multivariate analysis (Kirschner *et al*, 2015).

Hyaluronic acid (HA). Four studies (two prospective, two retrospective), with a total of 163 patients, assessed the utility of serum HA between 1989 and 2013 (Dahl *et al*, 1989; Thylen *et al*, 1999; Hedman *et al*, 2003; Creaney *et al*, 2013). Pleural fluid HA was measured in three of the selected studies (one prospective, two retrospective), with a total of 233 patients (Dahl *et al*, 1989; Thylen *et al*, 2001; Creaney *et al*, 2013). The earliest study, from Dahl *et al* (1989), measured serial serum HA in patients undergoing methotrexate therapy for pleural ($n = 34$) or peritoneal ($n = 3$) mesothelioma. They showed that serum HA were higher in later disease stage but presented no data regarding histological subtypes. Additionally, in patients who were deemed to have progressed, based on subjective CT reporting ($n = 13$), levels of HA rose (median $= 25$, IQR 6–37) compared with falling levels in responders ($n = 20$) (median $= -5$, IQR: -14–3). Pleural fluid HA did not correlate with serum levels and there was no relationship between tumour stage or disease response. In contrast, a more recent case series retrospectively analysed serum and pf HA in 96 MPM cases (Creaney *et al*, 2013). In this study, serum HA was not significantly raised in MPM patients compared with patients with benign conditions or lung cancer with no relationship with OS. Pleural fluid HA was significantly higher in MPM patients and demonstrated a biphasic distribution that was independent of tumour histology. Although no treatment data was presented, using a cut-off of 75 mg ml^{-1}, there was a survival benefit for high pf HA levels (18 months) compared with low levels (12.6 months). The phenomena of high pf HA and improved survival is replicated by Thylen *et al* (2001) who measured pf levels in patients receiving either chemotherapy ($n=56$) or BSC ($n=44$), although histological subtypes were not analysed separately.

Vascular endothelial growth factor (VEGF). This systematic review identified six papers involving serum/plasma (four prospective, one retrospective) or pf (one retrospective, one prospective) vascular endothelial growth factor (VEGF) levels, with a total of 287 and 53 patients, respectively, (Strizzi *et al*, 2001; Yasumitsu *et al*, 2010; Hirayama *et al*, 2011; Kao *et al*, 2012; Kindler *et al*, 2012; Nowak *et al*, 2013). A 7-year prospective single centre case series of 51 patients with MPM analysed serum pan-VEGF levels and compared them to a non-MPM asbestos-exposed population ($n=42$) (Yasumitsu *et al*, 2010). Serum VEGF levels were significantly higher in the MPM population and increased with tumour stage. Median levels were higher in epithelioid *vs* sarcomatoid histology (1071 pg ml^{-1} *vs* 580 pg ml^{-1}, respectively) but because of low numbers of sarcomatoid cases ($n=6$), this result was not statistically significant. At multivariate analysis, there was no significant correlation with OS. Kao *et al* (2012) analysed a variety of novel biomarkers (pan-VEGF, CRP, IL-6, sIL-6R and SM) in a non-randomised trial of thalidomide as a chemotherapy adjunct ($n=34$) or single agent ($n=29$). At multivariate analysis, baseline serum VEGF was the only significant biomarker in predicting OS ($P=0.025$), with higher median survival in lower VEGF levels. In addition, VEGF levels were tested post chemotherapy (at 8 weeks). Patients with high baseline levels that subsequently fell had median OS of 79 weeks compared with 39 weeks ($P=0.050$).

A phase II trial of second-line therapy (Sunitinib Malate, a multitargeted tyrosine kinase inhibitor) robustly assessed the role of several serum VEGF isoforms in prognostication (Nowak *et al*, 2013). Fifty-three patients with progression following conventional chemotherapy were enroled, with only one patient with sarcomatoid MPM meeting eligibility criteria. Several serum VEGF isoforms were tested (A, C, R2 and R3) as well as SM, c-kit and IL-8 at baseline, 6 weeks and every 12 weeks thereafter. Baseline VEGF-A and VEGF-R2 were predictive of radiological response at multivariate analysis, with only percentage change in SM being associated with time to progression (HR: 3.84, $P<0.001$). Another trial of biological therapy compared bevacizumab (a monoclonal antibody to VEGF) to placebo when added to chemotherapy with gemcitabine and cisplatin in 108 patients with MPM (Kindler *et al*, 2012). Vascular endothelial growth factor levels were measured pre-treatment in 56 patients. The trial found no difference between the two treatment arms with partial response rates of 24.5% and 21.8% for the bevacizumab and placebo arms, respectively, ($P=0.74$). There was no significant difference in baseline VEGF levels between responders and non-responders, although higher baseline levels were associated with worse PFS ($P=0.049$) and OS ($P=0.014$).

A diagnostic study measured pf pan-VEGF levels in 46 MPM patients (Hirayama *et al*, 2011). In the 28 patients followed up to 600 days, those with a pf VEGF >2000 pg ml^{-1} (a pre-defined cut-off) had lower OS at multivariate analysis (HR; 961.2, CI: 7.1–130 446, $P=0.006$).

DISCUSSION

This systematic review identified 45 studies from the current literature that assessed the prognostic or treatment monitoring ability of biomarkers in MPM. There was significant variation in the quality of the selected studies with many having a moderate to high risk of bias due to study attrition or lack of reporting of confounding factors (as evidenced by the variation in QUIPS scores). In addition, there was considerable heterogeneity within studies regarding patient treatment (variation in numbers undergoing standard chemotherapy, trial drugs, surgery or BSC), which is a major confounder in prognostic studies that can only be partly adjusted for using multivariate analysis. Several papers combined these groups when assessing a biomarker's baseline prognostic ability, making many of their conclusions invalid.

Robust methodology was used to capture all available literature, including an evidence-based search strategy, multiple independent reviewers, PRISMA reporting methodology and the use of the QUIPS tool for study assessment. However, given the inter-study variability in biomarker cut-offs, histological subtypes, treatment modalities and outcome measures (OS, PFS or radiological treatment response) no attempt to meta-analyse the studies was made.

The majority of selected studies examined the utility of serum SM as a baseline prognostic indictor, often as a secondary outcome to its diagnostic utility. Earlier case series suggested higher baseline levels inferred a worse prognosis, but this finding was inconsistently replicated by more recent studies. Soluble mesothelin is only expressed by tumours with full or partial epithelioid histology, so variation between studies in histological subtypes has a significant impact on the interpretation of these results. Several studies have demonstrated no correlation with OS when tumour histology and renal function (renal function is inversely correlated with serum SM) are included in multivariate analyses. When serum SM is measured serially it has been consistently shown to correlate with changes in modified RECIST CT findings or survival (OS and PFS). The eight studies examining this relationship focused on patients receiving chemotherapy, with only 34 patients having had surgery. Between studies, there was variation in the thresholds used to define a significant change in serum SM as well as the appropriate sampling intervals during or after treatment. Before serial SM testing can be recommended in routine clinical practice a large prospective study is required to address these uncertainties and assess its use in surgical and BSC cohorts. Megakaryocyte potentiating factor is formed from the same precursor protein as SM and is a more novel biomarker in MPM (Hollevoet *et al*, 2010). Megakaryocyte potentiating factor and SM correlated strongly in the three studies that measured both concurrently but the strength of evidence for serum MPF is far smaller than for SM.

This systematic review identified several studies that correlated high OPN levels with poor prognosis, including within prognostic tools. Also plasma OPN showed no correlation with other biomarkers, indicating that it may offer independent prognostic information. There was significant variability in the cut-offs used between studies, which is likely reflective of the variation in treatment modality between cohorts as well as the ELISA platforms used (Anborgh *et al*, 2009). In addition, because OPN is cleaved by thrombin following blood coagulation, plasma sampling is superior to serum (Pass *et al*, 2016). The majority of studies found by this review analysed plasma OPN, but in order for this biomarker to be validated in the future a consensus approach is required for sampling and analysis.

Serum fibulin-3 has shown promise as a diagnostic biomarker (Pass *et al*, 2012) but was not a marker of prognosis on the basis of this systematic review. However, higher levels of fibulin-3 in pf did inversely correlate with survival, although this is likely in part due to the much higher levels found in effusions of the more aggressive sarcomatoid MPM.

Vascular endothelial growth factor is a well-documented marker of tumour angiogenesis and is raised in the serum of patients with MPM (Strizzi *et al*, 2001). It is of particular importance in MPM given the emergence of antiangiogenic VEGF-targeted treatments that have been shown to improve survival when given in combination with pemetrexed and cisplatin (Zalcman *et al*, 2016). The studies involving serum VEGF were heterogeneous in terms of design but showed positive results for pan-VEGF and its isoforms as prognostic or monitoring biomarkers. No studies demonstrated any ability of serum VEGF to select responders from non-responders for biologic therapy, but this area demands further study given the development of promising but expensive biologicals (Kindler *et al*, 2012; Nowak *et al*, 2013).

In conclusion, from the 45 studies published in the literature no serum or pf biomarker was identified that could be recommended currently for use in clinical practice. There was considerable heterogeneity within studies for patient treatment, tumour histology and follow-up, as well as inter-study variability in terms of biomarker cut-offs. Serum SM when measured before and after treatment has been shown to track treatment response but further studies are required to ascertain its place in the chemotherapy or surgical management pathway. Serum OPN showed an ability to prognosticate from baseline, but whether this has clinical utility is uncertain. With considerable variation in response rates to chemotherapy and the emergence of promising biological therapies, biomarkers that could select responders from non-responders at baseline or during treatment would aid clinical decision making, prevent patients getting ineffective therapy and improve cost effectiveness.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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