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**Prognostication and monitoring of mesothelioma using biomarkers- a systematic review**

**Running title; Prognostic biomarkers in mesothelioma; systematic review**

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**Conflict of Interest**

The authors report no conflicts of interest.
Abstract

Introduction

Radiological markers of treatment response and prognostication in malignant pleural mesothelioma (MPM) 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 (QUIPS) 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.

Conclusion

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.

Keywords

mesothelioma, biomarkers, chemotherapy, monitoring, prognosis, response.
Introduction

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 and benign asbestos related plaques make radiological assessment of MPM difficult (Armato et al, 2006).

A blood or pleural fluid 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 researched is soluble mesothelin related peptide often called soluble mesothelin (SM). SM is the circulating form of a 40kDa membrane bound glycoprotein and is highly expressed by mesothelial cells in MPM and some other cancers (Robinson et al, 2003). SM levels are much higher in epithelioid MPM compared to other histological subtypes, and in larger tumours. However, its diagnostic ability in both serum and pleural fluid is limited by an inability to exclude MPM with a negative result [12]. 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 30th of 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 Appendix A. 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 & FH) screened the abstracts for study eligibility; any disagreements were resolved by mutual consensus.

Study inclusion criteria

- Involved the measurement of a serum or pleural fluid (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 & 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 Appendix B. In addition, routine data was 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 (C.I.).
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. Due to considerable heterogeneity between studies in areas such as histological sub-type, 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.

Figure 1: PRISMA flowchart

Table 1: Full table of selected studies
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 (Creaney et al, 2014; Creaney et al, 2013; Creaney et al, 2011; Cristaudo et al, 2007; Franko et al, 2012; Grigoriu et al, 2009; Grigoriu et al, 2007; Hassan et al, 2014; Hollevoet et al, 2011; Hollevoet et al, 2012; Hooper et al, 2015; Kao et al, 2012; Linch et al, 2014; Nowak et al, 2013; Pass et al, 2016; Pass et al, 2008; Robinson et al, 2003; Schneider et al, 2008; Wheatley-Price et al, 2010; Yamada et al, 2011). 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 (Cristaudo et al, 2007) and Grigoriu et al (Grigoriu et al, 2007) using the Mesomark™ ELISA found that higher baseline SM was correlated with worse OS using cut-offs of 1nmol/L (HR 1.6, C.I. 1.1-2.4) and 3.5nmol/L (HR 2.8, C.I. 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 (<3.5nmol/L) and high baseline SM levels of 17.1 months versus 8.4 months respectively (Schneider et al, 2008). This relationship was statistically significant at multivariate analysis (HR 1.9, C.I. 1.1-3.5, p=0.025) but lost statistical significance when applied to epithelioid histology alone. Two studies from Creaney et al involving 96 and 82 patients respectively found no correlation between OS and baseline serum SM (Creaney et al, 2014; Creaney et al, 2013).

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 (PR) on CT (17/55) 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 to the falling group, with HR of 2.0 (C.I. 1.1-3.1) and 23.0 (C.I. 7.5-70.9) respectively. In the small number of patients in this study who had an extrapleural pneumonectomy (EPP) (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 and colleagues 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 (Wheatley-Price et al, 2010). Changes in SM or OPN were correlated with radiological reports (using descriptive reporting, RECIST and modRECIST) 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 modRECIST criteria (p=0.004). In total, this review identified 8 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 (4 prospective, 1 retrospective), with a total of 371 patients, investigated pleural fluid (pf) SM’s ability to prognosticate from baseline (Creaney et al, 2014; Creaney et al, 2013; Creaney et al, 2007; Grigoriu et al, 2007; Yamada et al, 2011). One study by Yamada et al found patients (n=45) with higher pf SM levels survived significantly longer (dichotomy at 10nM) at univariate but not multivariate analysis (Yamada et al, 2011). This finding was not replicated in the other larger studies, which found no relationship between pf SM and OS.

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

Megakaryocyte potentiating factor (MPF) or N-ERC/mesothelin

All 5 studies (5 prospective), with a total of 232 patients, involving serum MPF were published after 2008 (Hassan et al, 2014; Hollevoet et al, 2011; Hollevoet et al, 2012; Mori et al, 2013; Tajima et al, 2008). The earliest by Tajima and colleagues (Tajima et al, 2008) included 14 patients with MPM receiving a variety of chemotherapeutic regimens and tested MPF and osteopontin (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 to those with a partial response (p<0.05). A larger study was performed by Hollevoet et al on 62 patients receiving either EPP (n=14) or pemetrexed/platinium based chemotherapy (n=48) (Hollevoet et al, 2011). 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 5 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 to 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 3 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. MPF correctly classified 15/20 patients (75% accuracy) as having progressive or stable
disease (with rising or stable/falling levels respectively) based on their CT scan, compared to 14/20 (70%) and 12/20 (60%) for SM and Ca125 respectively.

Osteopontin (OPN)

All 6 of the studies (6 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 (Abakay et al, 2014; Grigoriu et al, 2007; Hollevoet et al, 2011; Mundt et al, 2014a; Pass et al, 2016; Tajima et al, 2008; Wheatley-Price et al, 2010). Grigoriu and colleagues measured baseline serum and pf OPN (SM results above) in a cohort of 96 MPM patients (Grigoriu et al, 2007). Baseline serum OPN had a statistically significant relationship with OS at multivariate analysis in a model that included tumour histology (HR 3.46, C.I. 1.1-10.9, p=0.034). Using a cut off of 350ng/ml (selected using a maximisation of HR model), patients with a low serum OPN had a median OS of 15 months compared to 5 months in high serum OPN levels. Pf 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 and colleagues (study discussed above) (Hollevoet et al, 2011). Baseline OPN correlated with both OS and PFS (optimum cut off for their dataset was 863ng/ml) and appeared to be an independent factor with no correlation with other biomarkers or tumour stage. More recently, Pass and colleagues 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 overall survival. 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 analysed serum and pf OPN from two separate cohorts to assess its role in diagnosis and prognosis at baseline (Mundt et al, 2014a). 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 185ng/ml cut off (HR 2.5, C.I. 1.4-10.3), the median of the dataset. In the pf cohort, 40 patients had survival data available and using pf OPN cut off of 1.6µg/ml resulted in median OS times of 29m vs 13m for low to high levels respectively (HR 2.2, C.I. 1.2-4.2). However, neither of these correlations were assessed using multivariate analysis or evaluated alongside tumour histology.

Fibulin-3

Six studies (5 prospective, 1 retrospective) involving serum/plasma fibulin-3 were found by this systematic review, comprising 568 patients overall (Creaney et al, 2014; Hooper et al, 2015; Kaya et al, 2015; Kirschner et al, 2015; Pass et al, 2016; Pass et al, 2012). Of these, 3 studies (2 prospective, 1 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 and colleagues measured serum fibulin-3 before, during (after 2 cycles of Pem/Cis) and after chemotherapy (Hooper et al, 2015).
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 and colleagues (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 576ng/ml compared to 765ng/ml in stages 3&4 (p=0.040). When pf fibulin-3 levels were dichotomised at the database median of 733.4ng/ml 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 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) (Creaney et al, 2014). Patients with biphasic or sarcomatoid histology had significantly (p=0.002) higher pf fibulin-3 concentrations (median 1331ng/ml) compared to epithelioid subtypes (median 426ng/ml), 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 3 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 (2 prospective, 2 retrospective), with a total of 163 patients, assessed the utility of serum HA between 1989 and 2013 (Creaney et al, 2013; Dahl et al, 1989; Hedman et al, 2003; Thylen et al, 1999). PF HA was measured in 3 of the selected studies (1 prospective, 2 retrospective), with a total of 233 patients (Creaney et al, 2013; Dahl et al, 1989; Thylen et al, 2001). The earliest study, from Dahl and colleagues, measured serial serum HA in patients undergoing methotrexate therapy for pleural (n=34) or peritoneal (n=3) mesothelioma (Dahl et al, 1989). 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 to falling levels in responders (n=20) (median= -5, IQR -14 – 3). PF 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 to patients with benign conditions or lung cancer with no relationship with OS. PF 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 75mg/ml, there was a survival benefit for high pf HA levels (18months) compared to low levels (12.6months). The phenomena of high pf HA and improved survival is replicated by Thylen and colleagues who measured pf levels in patients receiving either chemotherapy (n=56) or BSC (n=44), although histological subtypes were not analysed separately (Thylen et al, 2001).

Vascular Endothelial Growth Factor (VEGF).
This systematic review identified 6 papers involving serum/plasma (4 prospective, 1 retrospective) or pf (1 retrospective, 1 prospective) VEGF levels, with a total of 287 and 53 patients respectively (Hirayama et al, 2011; Kao et al, 2012; Kindler et al, 2012; Nowak et al, 2013; Strizzi et al, 2001; Yasumitsu et al, 2010). 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 versus sarcomatoid histology (1071 vs 580pg/ml respectively) but due to low numbers of sarcomatoid cases (n=6) this result was not statistically significant. At multivariate analysis there was no significant correlation with OS. Kao and colleagues 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) (Kao et al, 2012). 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 to 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 enrolled, with only 1 patient with sarcomatoid MPM meeting eligibility criteria. Several serum VEGF isoforms were tested (A, C, R2, 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). VEGF 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 >2000pg/ml (a pre-defined cut-off) had lower OS at multivariate analysis (HR 961.2 C.I. 7.1 to 130446, 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 best supportive care), 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 indicator, 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 8 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. MPF is formed from the same precursor protein as SM and is a more novel biomarker in MPM (Hollevoet et al, 2010). MPF and SM correlated strongly in the 3 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 pleural fluid
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.

VEGF 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 44 studies published in the literature no serum or pleural fluid 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 report no conflicts of interest.
| 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 | 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- 22 |
| (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) | Prospective | Novel proteomes (s) | 37 | N/D | N/D |
| (Hassan et al, 2014) | Prospective | SM (s) MPF (s) Cal125 (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 BSC37 U-4 |
| (Ghanim et al, 2014) | Retrospective | Fibrinogen (p) | 176 | E- 146 S- 12 B- 18 | C- 78 Surg- 54 BSC- 44 |
| (Moni 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) CRP (s) IL-6 (s) sIL-6r (s) VEGF (s) | 63 | E- 28 S- 4 B- 30 | C- 63 |
| (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 |
| (Ghanim et al, 2012) | Retrospective | CRP (s) | 115 | E- 80 S- 27 U- 8 | C/r/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 |
| Study                  | Study Type | Biomarker | Tissue | Sensitivity | Specificity | Follow-up | Follow-up | Follow-up | Follow-up |
|-----------------------|------------|-----------|--------|-------------|-------------|-----------|-----------|-----------|-----------|
| (Hirayama et al, 2011)| Prospective| VEGF (pf) |        | 46          | E: 34        | S: 10     | B: 6      | D: 3      | N/D       |
| (Hollevoet et al, 2011)| Prospective| SM (s)    |        | 62          | E: 59        | S: 1       | B: 2      | c- 48     | surg: 14  |
| (Fasamitsu 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   |
| (Grigoroiu et al, 2009)| Retrospective| SM (s)   |        | 40          | E: 35        | S: 3       | B: 2      | im: 16    | c: 20     |
| (Wheatley-Price et al, 2010)| Prospective| SM (p)    |        | 41          | E: 41        |           |           | c: 21     | surg: 7   |
| (Schneider et al, 2008)| Prospective| SM (s)    |        | 129         | E: 81        | S: 14      | B: 17     | U: 17     | c: 68     |
| (Pass et al, 2008)    | Prospective| SM (s)    |        | 90          | E: 58        | S: 3       | B: 29     |           | surg: 90  |
| (Tajima et al, 2008)  | Prospective| MPF (s)   |        | 14          | E: 11        | S: 2       | B: 1      | c: 14     |           |
| (Grigoroiu et al, 2007)| Prospective| SM (s)    |        | 96          | E: 73        | S: 10      | B: 13     | c: 70     |
| (Cristaudo et al, 2007)| Prospective| SM (s)    |        | 107         | E: 72        | S: 10      | B: 7      | D: 3      | U: 15     |
| (Creaney et al, 2007) | Prospective| SM (s)    |        | 52          | E: 15        | S: 9       | B: 5      | U: 23     | N/D       |
| (Filiberti et al, 2005)| Prospective| PDGF-AB (s)|    | 93          | 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)    |        | 11          | N/D          |           |           |           |           |
| (Strizzi et al, 2001) | Retrospective| VEGF (s)  |        | 12          | E: 8         | S: 1       | B: 3      | N/D       |           |
| (Thylén et al, 2001)  | Retrospective| HA (pf)   |        | 100         | E: 67        | NE: 33     |           | c: 56     |
| (Thylén et al, 1999)  | Prospective| HA (s)    |        | 19          | E: 15        | S: 1       | B: 3      | N/D       |           |
| (Schouwink et al, 1999)| Retrospective| TPA (s)   |        | 52          | E: 31        | S: 9       | B: 10     | un: 2     |
| (Nakano et al, 1998)  | Prospective| IL-6 (s)  |        | 25          | N/D          |           |           |           |           |
| (Bonfrer et al, 1997) | Prospective| TPA (s)   |        | 29          | E: 21        | NE: 8      |           | N/D       |           |
| (Dah et al, 1989)     | Prospective| HA (s)    |        | 37          | E: 28        | S: 3       | B: 5      | M: 5      | C: 37     |

E- epithelioid, S- sarcomatoid, B- biphasic, D- desmoplastic, NE- non-epithelioid, U- unknown, A- anaplastic, MC- multicystic, C- chemotherapy, Surg- surgery, BSC- best supportive care, RTX- radiotherapy, Im- immunotherapy, Bio- Biological therapy, N/D- not documented, SM- soluble mesothelin, CTC- circulating tumour cells, OPN- osteopontin, MPF- megakaryocyte potentiating factor, HA- hyaluronic acid, HMGB1- high-mobility group box 1, VEGF- vascular endothelial growth factor, CRP- C reactive protein, PDGF- platelet derived growth factor, TPA- tissue polypeptide antigen, CEA- carcinoembryonic antigen, IL- interleukin, s- serum, p- plasma, pf- pleural fluid.
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 OSt | 0% | Chemotherapy group; a falling serum SM at 6-8weeks 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 & 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 | 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, Surgery- 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, Surgery- 5 | Mod RECIST CT | 15% | Partial response to chemotherapy correlated with a 34% fall in SM (p=0.010) compared to a 54% rise in progressive disease (p<0.001). |
| (Creaney et al, 2011) | Chemo- 61, BSC- 25, Surgery - 8 | Mod RECIST CT FDG-PET | 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 to static (13 months) or rising levels (15 months). (p=0.001). |
| (Wheatley-Price et al, 2010) | Chemo- 21, BSC- 13, Surgery - 8 | Mod RECIST CT RECIST CT report | 10% or 5nmol/L | 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 (>1nM/L), rising level correlated with progressive disease in 12/16 patients. OS higher in patients with stable SM compared to increasing (p=0.012). |

P- pemetrexed, C- cisplatin, G- gemcitabine, Chemo- chemotherapy (not specified), Bio- biological therapy, Im- immunotherapy, BSC- best supportive care, Surg- surgery, Mod RECIST CT- Modified Response Evaluation Criteria In Solid Tumors CT, OS- overall survival, TTP- time to progression.

Figure Legends

Figure 1: PRISMA flowchart
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