# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: meta-analyses

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# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: meta-analyses

Short running head: SMRPs in mesothelioma

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ABSTRACT

**Objective** Although the values of soluble mesothelin-related peptides (SMRPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma have been extensively studied, the exact diagnostic accuracy of these SMRPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMRPs in serum, and further to establish that of SMRPs in pleural fluid for mesothelioma.

**Design** Systematic review and meta-analysis.

**Methods** In total, 27 articles from 30 diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMRPs in serum and pleural fluid for the diagnosis of malignant pleural mesothelioma were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.63, 0.87, 5.68, 0.42, and 14.95, respectively; pleural fluid: 0.80, 0.83, 4.00, 0.30, and 15.31, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMRPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, and the positive test results indicated that further

invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of MPM is always a challenging endeavor because.
- To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.
- The concentrations of soluble mesothelin-related peptides, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin-related peptides in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- The negative test results are not sufficiently to exclude non-mesothelioma, while the positive test results would indicate that further invasive diagnostic steps might be necessary.

**Strengths and limitations of this study**

- The studies included this meta-analysis were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural

mesothelioma and the other diseases.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in malignant mesothelioma, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble megakaryocyte potentiating factor (MPF).[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin-related peptides (SMRPs), including both soluble mesothelin and MPF, have been found in human serum and pleural fluid (PF).[9,10] Actually, the diagnostic accuracy of SMRP detections for MPM has been extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we

performed and published first meta-analysis reporting the overall diagnostic accuracy of serum SMRPs for diagnosing MPM, and our results showed that serum SMRP determinations played a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMRP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMRPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMRPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMRPs, and further to establish that of PF SMRPs for diagnosing MPM.

## METHODS

### Search strategy and study selection

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until July, 2013; no lower date limit was applied. Search keywords included: "soluble mesothelin-related peptides/SMRP", "mesothelin", "megakaryocyte potentiating factor/MPF", "mesothelioma". Articles were also identified by use of the related-articles function in PubMed. References of articles identified were also searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

A study was included in the meta-analyses when it provided SMRP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected in the study, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

### Data extraction and quality assessment

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, fifty-six publications determining concentrations of human SMRPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, twenty-nine were excluded (Appendix excluded references, available online). Subsequently, twenty-seven publications [21-47] were available for analysis of diagnostic accuracy of SMRPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 16 publications from 18 studies [32-47] were added in the current meta-analyses.

The methods of determining SMRPs in all studies included were enzyme-linked immunosorbent assay. Serum mesothelin concentrations were determined in 20 studies (19 articles), [21,23-28,30,31,33,34,36-39,41,43,45,47] and serum MPF concentrations were determined in 5 studies [22,29,30,32,36] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMRP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. In addtion, another 2 articles [30,36] were also treated as independent studies, since both mesothelin and MPF in serum were investigated in these 2 articles. PF mesothelin concentrations were determined in 9 studies.[23,35,40-44,46,47]

The clinical characteristics of the studies, along with STARD and QUADAS scores, are outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMRPs (Appdendix Table 1). For serum SMRP studies, the average samples size was 270 (range from 40 – 1,086), the subjects included 1,046 patients with MPM and 5,356 non-MPM. For PF SMRP studies, the average samples size was 127 (range from 40 – 275), the subjects included 352 patients with MPM and 794 non-MPM.

Except for 2 studies, all samples were collected from the consecutive patients in the remaining 28 studies. Nine studies reported blinded interpretation of SMRP assays independent of the reference standard. Eight studies reported the study design was prospective. In 21 studies, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 studies, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 23 of 27 publications had higher STARD scores (≥ 13) and 18 studies had higher QUADAS scores (≥ 10).

**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMRP studies (Figure 1A), evaluation of publication bias showed that Egger tests were significant for serum SMRPs (p = 0.044). Although the funnel plots for publication bias showed somehow asymmetry due to the

limited number of PF SMRP studies (Figure 1B), Egger tests showed that this was not significant for PF SMRPs (p = 0.149). These results indicated a potential for publication bias for serum SMRP, but not for PF SMRP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 25 serum SMRP assays in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.63, 95% CI 0.60 – 0.65), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.68 (95% CI 4.15 – 7.76), NLR was 0.42 (95% CI 0.36 – 0.49), and DOR was 14.95 (95% CI 9.93 – 22.50). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 121.53, 443.37, 261.05, 107.18, and 137.67, respectively, with all $p < 0.001$, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity 9 PF SMRP assays in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.80, 95% CI 0.76 – 0.84), while specificity ranged from 0.65 – 0.90 (pooled 0.83, 95% CI 0.80 – 0.85). We also noted that PLR was 4.00 (95% CI 2.98 – 5.36), NLR was 0.30 (95% CI 0.23 – 0.39), and DOR was 15.31 (95% CI 9.32 – 25.16). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 37.07 ($p < 0.001$), 30.45 ($p < 0.001$), 23.85 ($p = 0.002$), 11.30 ( $p = 0.185$) , and 15.14 ($p = 0.056$), respectively, indicating somehow a heterogeneity between studies.

The graphs of SROC curves for SMRP determinations showing sensitivity versus 1 – specificity from individual studies are shown in Figure 3. SROC curve of serum SMRPs was not positioned near the desirable upper left corner of SROC curve, and that the maximum

joint sensitivity and specificity was 0.737 (SEM, 0.031) (Figure 3A); while area under curve (AUC) was 0.802 (SEM, 0.035). The maximum joint sensitivity and specificity of PF SMRP was 0.805 (SEM, 0.022); while AUC was 0.875 (SEM, 0.023) (Figure 3B).

Totally, the diagnostic performance of SMRPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMRPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,44,45] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,39] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMRPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMRPs, it was the most difficult to identify MPM from other cancers, compared with from healthy controls or asbestos-exposed people.

Five studies [23,35,40,41,46] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [35,40,41,46] for

differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was very similar to that of differentiating MPM from benign pleural effusions.

## DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[48,49]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMRPs was 0.63 and 0.80, respectively; and their specificity was 0.87 and 0.83 respectively. These data indicated that sensitivity and specificity of SMRPs in serum and PF were not as high as expected. The positive SMRPs results might be somehow helpful in confirming (ruling in) MPM, suggesting that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. However, the relative low sensitivity, especially serum SMRPs, that was not sufficiently low to exclude non-MPM when a patient's SMRP results were lower than the cut-off values. Therefore, the associated poor sensitivity of SMRPs clearly limits their added value to diagnosis of MPM.

Unlike a traditional ROC plot that explores the effect of varying cut-off values on sensitivity and specificity in a single study, each data point in the SROC plot represents a separate study. The SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMRPs were 0.737 and 0.805, respectively; while their AUCs were 0.802 and 0.875, respectively, indicating level

of overall accuracy were also not as high as expected.

The DOR is a single indicator of test accuracy [50] that combines the data from sensitivity and specificity into a single number. The DOR of a test is the ratio of the odds of positive test results in the diseased relative to the odds of positive test results in the non-diseased. The value of a DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance. A DOR of 1.0 indicates that a test does not discriminate between patients with the disorder and those without it. In the current meta-analyses, we found that the pooled DORs of serum and PF SMRPs were 14.95, and 15.31, respectively, indicating that SMRPs seemed to be helpful in the diagnosis of MPM.

Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[51,52] we further presented both PLR and NLR as our measures of diagnostic accuracy. It a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[53] A PLR value of 5.68 with serum SMRPs suggests that patients with MPM have a near 6-fold higher chance of being SMRP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. This might lead to an inordinate number of individuals undergoing unnecessary diagnostic work-ups or biopsies. On the other hand, NLR of serum SMRPs was found to be 0.42. If serum SMRP results were negative, the probability that this patient has MPM is 42%, which is not low enough to rule out MPM. The very similar results were found with PF SMRPs.

Although both mesothelin and MPF belong to SMRPs, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR,

NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMRP for discriminating MPM from healthy control subjects was the best (although not as good as expexted), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

Our meta-analyses had several limitations. First, exclusion of conference abstracts, letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for serum SMRP studies, but not for PF SMRP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 2 studies,[34,41] epithelioid subtype of MPM was the most common pathological type in all remaining studies, but not one.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMRP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMRPs in MPM diagnosis. Third, control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. These issues regarding accuracy of diagnosis could also lead to biased results

In conclusion, current evidence supported that SMRPs in both serum and PF were

helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMRPs in serum and PF was similar, and serum MPF had superior diagnostic accury compared to serum mesothelin. The negative results of SMRP determinations were not sufficiently to exclude non-MPM; on the other hand, the positive test results would indicate that further invasive diagnostic steps might be necessary and could possibly lead to an earlier diagnosis.

**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

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**Data sharing statement:** There is no additional data available.

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## Figure legends

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of SMPRs in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | MPF | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[34] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[36] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[36] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[37] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[38] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[39] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[41] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[43] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Ferro et al[45] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[47] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 2.** Study summary of SMPRs in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Fujimoto et al[35] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[40] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[41] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[42] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[43] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[44] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Filiberti et al[46] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[47] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23, 24, 25, 26, 27, 28, 30, 31, 33, 34, 36, 37, 38, 39, 41, 43, 45, 47 | 22, 29, 30, 32, 36 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.66 (0.60 – 0.71) |
| Heterogeneity* (p) | 70.03 (< 0.001) | 50.26 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.95 (0.93 – 0.96) |
| Heterogeneity (p) | 345.53 (< 0.001) | 19.42 (0.001) |
| PLR (95% CI) | 4.75 (3.51 – 6.44) | 12.31 (6.21 – 24.42) |
| Heterogeneity (p) | 179.04 (< 0.001) | 15.48 (0.004) |
| NLR (95% CI) | 0.45 (0.39 – 0.51) | 0.30 (0.14 – 0.64) |
| Heterogeneity (p) | 50.33 (< 0.001) | 74.23 (< 0.001) |
| DOR (95% CI) | 11.84 (7.91 – 17.70) | 40.15 (16.55 – 97.39) |
| Heterogeneity (p) | 92.67 (< 0.001) | 12.07 (0.017) |
| AUC (SEM) | 0.791 (0.035) | 0.933 (0.083) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 44, 45 | 21, 23, 24, 25, 28, 30, 33, 39 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 35, 40, 41, 46 | 35, 40, 41, 46 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.81) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.14 (0.887) | 1.08 (0.783) |
| Specificity (95% CI) | 0.75 (0.68 –0.81) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 3.93 (0.416) | 6.74 (0.081) |
| PLR (95% CI) | 2.81 (2.13 –3.70) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.22 (0.337) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.26 – 0.44) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.90 (0.755) | 1.83 (0.608) |
| DOR (95% CI) | 8.75 (5.41 –14.15) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.28 (0.512) | 5.26 (0.154) |
| AUC (SEM) | 0.812 (0.026) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

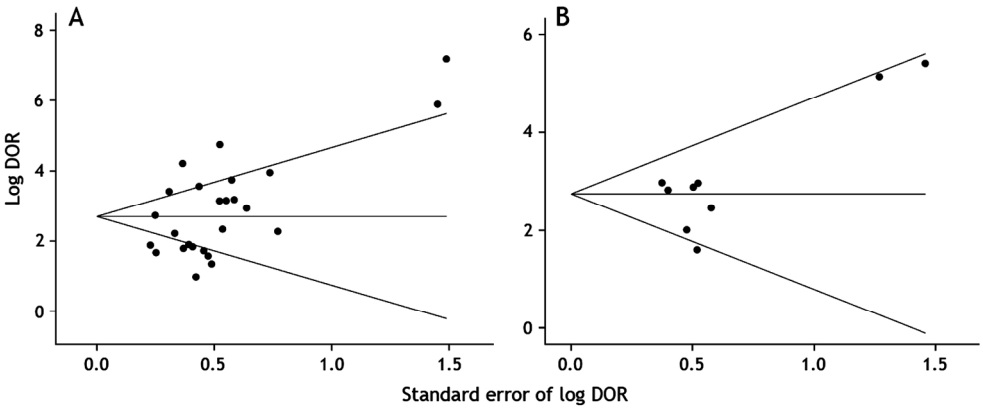


Figure 1. Funnel graphs for the assessment of potential publication bias in soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.
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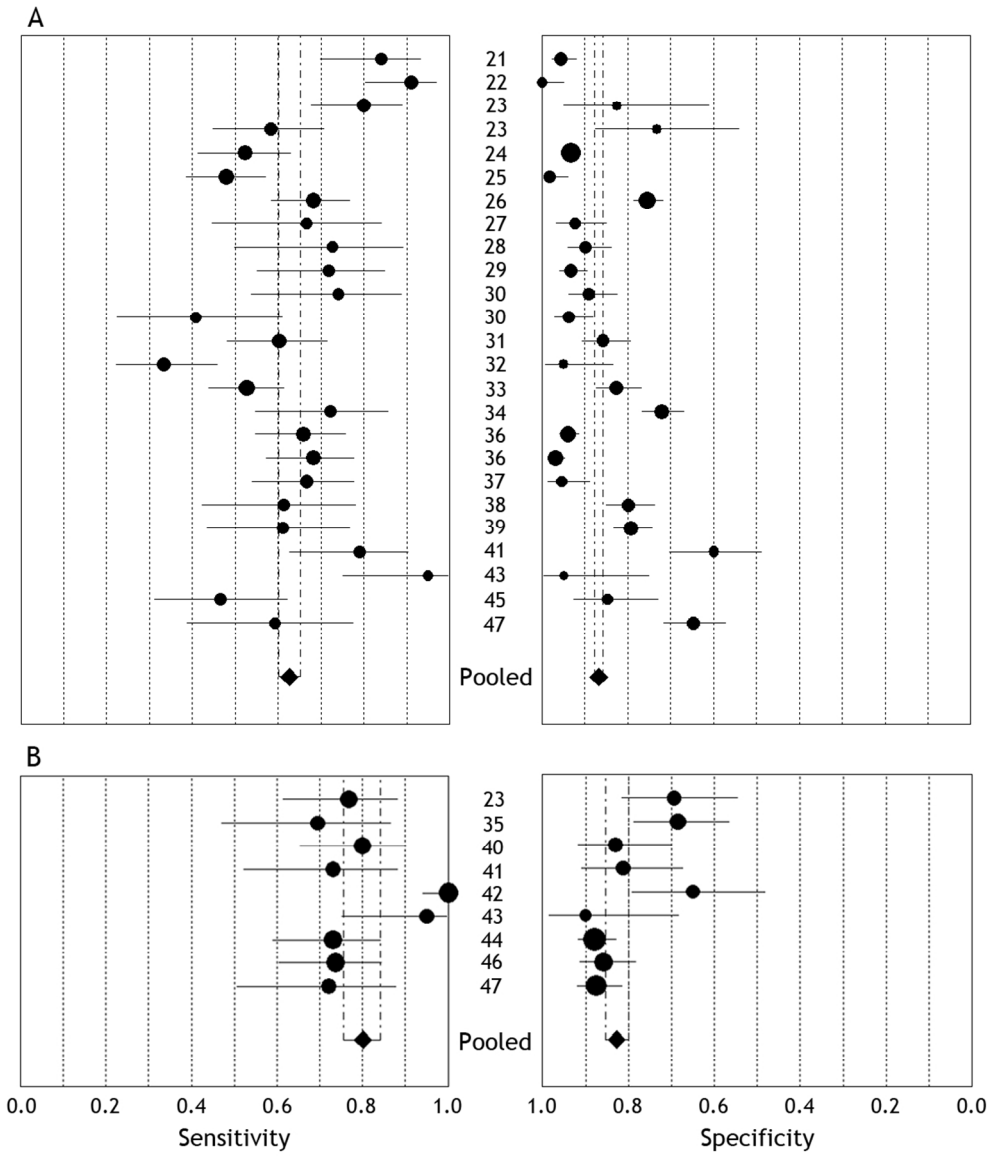


Figure 2. Forest plots of estimates of sensitivity and specificity for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.
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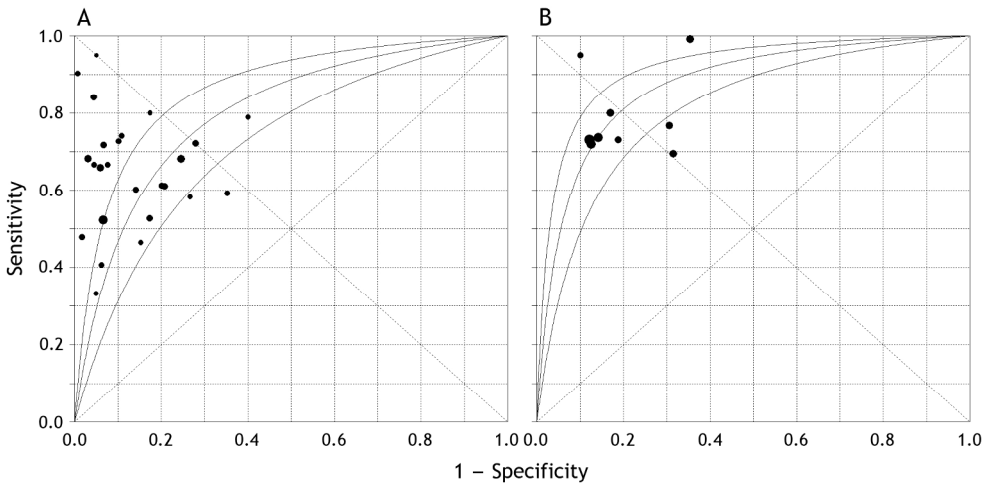


Figure 3. Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.
205x101mm (300 x 300 DPI)

**Supplemental Data**

# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: meta-analyses

Short running head: SMRPs in mesothelioma

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## Appendix Excluded References

One were excluded because it recruited less than 10 patients in one of study groups,[1] seven were excluded because the same authors published several reports on the same patients, and only the best-quality study was considered,[2-8] twenty-one were excluded because they did not allow the calculation of sensitivity or specificity.[9-29]

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Appendix Table 1 The characteristics of subjects studied

| Study | MPM Patients | Non-MPM Subjects |
|---|---|---|
| Robinson et al[21] | Epithelioid type (n = 25)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 15) | Healthy controls without asbestos exposure (n = 28)<br>Healthy controls with asbestos exposure (n = 40)<br>Patients with inflammatory non-pleural lung disease (n = 92)<br>Patients with non-MPM pleural diseases (n = 38)<br>Patients with non-pleural malignant lung disease (n = 30) |
| Onda et al[22] | Epithelioid type (n = 56) | Healthy controls (n = 70) |
| Scherpereel et al[23] | Epithelioid type (n = 55)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 13) | Patients with benign asbestos-related pleural diseases (n = 28)<br>Patients with pleural metastasis of carcinomas (n = 35) |
| Beyer et al[24] | Epithelioid type (n = 59)<br>Sarcomatoid type (n = 8)<br>Other or not specified (n = 21) | Healthy controls (n = 409)<br>Patients with non-MPM malignancy (n = 412)<br>Patients with nonmalignant conditions (n = 116)<br>Subjects with asbestos exposure (n = 61) |
| Creaney et al[25] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 15) | Healthy controls with asbestos exposure (n = 33)<br>Patients with benign asbestos-related diseases (n = 53) |

| | | |
|---|---|---|
| | Other or not specified (n = 67) | Patients with benign pleural effusions (n = 30) |
| Cristaudo et al[26] | Epithelioid type (n = 72)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 25) | Healthy controls (n = 262)<br>Patients with benign respiratory diseases (n = 130)<br>Patients with lung cancer (n = 215) |
| Di Serio et al[27] | Epithelioid type (n = 20)<br>Sarcomatoid type (n = 2)<br>Other or not specified (n = 2) | Healthy controls with asbestos exposure (n = 26)<br>Patients with asbestos-related diseases (n = 66) |
| Amati et al[28] | Epithelioid type (n =11)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 5) | Healthy controls without asbestos exposure (n = 54)<br>Subjects with asbestos exposure (n = 94) |
| Shiomi et al[29] | Epithelioid type (n = 21)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 9) | Patients with benign asbestos-related diseases and healthy controls with asbestos exposure (n = 201)<br>Patients with lung cancer (n = 45)<br>Others (n = 8) |
| Iwahori et al[30] | Epithelioid type (n = 13)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 11) | Healthy controls without asbestos exposure (n = 38)<br>Healthy controls with asbestos exposure (n = 9)<br>Patients with lung cancer (n = 47)<br>Patients with other cancers (n = 55) |

| | | |
|---|---|---|
| van den Heuvel[31] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 20) | Healthy controls (n = 50)<br>Patients with lung cancer (n = 106) |
| Creaney et al[32] | Epithelioid type (n = 57)<br>Sarcomatoid type (n = 9) | Healthy controls without asbestos exposure (n = 10)<br>Healthy controls with asbestos exposure (n = 10)<br>Patients with benign asbestos-related diseases (n = 21)<br>Patients with lung cancer (n = 103) |
| Schneider et al[33] | Epithelioid type (n = 81)<br>Sarcomatoid type (n = 14)<br>Other or not specified (n = 34) | Patients with lung cancer (n = 139)<br>Patients with benign asbestos-related diseases (n = 75) |
| Portal et al[34] | Not specified (n = 36) | Healthy controls (n = 48)<br>Patients with asbestos exposure and no pleural disease (n = 177)<br>Patients with benign asbestos-related diseases (n = 101) |
| Fujimoto et al[35] | Epithelioid type (n = 15)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 4) | Patients with lung cancer (n = 38)<br>Patients with benign asbestos pleurisy (n = 26)<br>Patients with tuberculosis pleurisy (n = 5)<br>Patients with no pleural diseases (n = 4) |
| Hollevoet et al[36] | Epithelioid type (n = 73) | Healthy controls (n = 101) |

| | | |
|---|---|---|
| | Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Healthy controls with asbestos exposure (n = 89)<br>Patients with benign asbestos-related diseases (n = 123)<br>Patients with benign respiratory diseases (n = 46)<br>Patients with lung cancer (n = 63) |
| Creaney et al[37] | Epithelioid type (n = 59)<br>Other or not specified (n = 7) | Patients with benign asbestos-related diseases (n = 47)<br>Patients with benign respiratory diseases (n = 42) |
| Cristaudo et al[38] | Epithelioid type (n = 31) | Healthy controls (n = 93)<br>Patients with benign respiratory diseases (n = 111) |
| Dipalma et al[39] | Epithelioid type (n = 29)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 3) | Healthy controls without asbestos exposure (n = 109)<br>Healthy controls with asbestos exposure (n = 26)<br>Patients with benign asbestos-related diseases (n = 48)<br>Patients with benign respiratory diseases (n = 110)<br>Patients with lung cancer (n = 252) |
| Yamada et al[40] | Epithelioid type (n = 37)<br>Sarcomatoid type (n = 5)<br>Other or not specified (n = 3) | Patients with non-malignant pleural effusions (n = 24)<br>Patients with lung cancer involving malignant pleural effusion (n = 29) |
| Ashour et al[41] | Not specified (n = 38) | Healthy controls with asbestos exposure (n = 32)<br>Patients with benign pleural diseases (n = 29) |

| | | |
|---|---|---|
| | | Patients with pleural carcinomas (n = 24) |
| Blanquart et al[42] | Epithelioid type (n = 49)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Patients with adenocarcinoma effusions (n = 25)<br>Patients with benign pleural effusions (n = 15) |
| Amany et al[43] | Epithelioid type (n = 14)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 2) | Patients with benign asbestos pleural effusions (n = 10)<br>Patients with tuberculosis pleural effusions (n = 10) |
| Canessa et al[44] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignant effusions (n = 94)<br>Patients with benign pleural effusions (n = 129) |
| Ferro et al[45] | Epithelioid type (n = 26)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignancy (n = 23)<br>Patients with benign diseases (n = 36) |
| Filiberti et al[46] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 2) | Patients with malignant effusions (n = 64)<br>Patients with benign effusions (n = 56) |
| Hooper et al[47] | Epithelioid type (n = 23)<br>Sarcomatoid type (n = 3) | Patients with non-MPM malignant effusions (n = 74)<br>Patients with benign asbestos-related effusions (n = 13) |

| | | |
|---|---|---|
| | Other or not specified (n = 2) | Patients with benign pleural diseases (n = 100) |

MPM = malignant pleural mesothelioma.

# STARD checklist for reporting of studies of diagnostic accuracy

*(version January 2003)*

| Section and Topic | Item # | | On page # |
|---|---|---|---|
| TITLE/ABSTRACT/ KEYWORDS | 1 | Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity'). | 0-2 |
| INTRODUCTION | 2 | State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups. | 7,8 |
| METHODS | | | |
| *Participants* | 3 | The study population: The inclusion and exclusion criteria, setting and locations where data were collected. | 11 |
| | 4 | Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard? | 12 |
| | 5 | Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected. | 12 |
| | 6 | Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)? | 12 |
| *Test methods* | 7 | The reference standard and its rationale. | 12 |
| | 8 | Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard. | 12 |
| | 9 | Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard. | 11,12 |
| | 10 | The number, training and expertise of the persons executing and reading the index tests and the reference standard. | A/N |
| | 11 | Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers. | 12 |
| *Statistical methods* | 12 | Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals). | A/N |
| | 13 | Methods for calculating test reproducibility, if done. | A/N |
| RESULTS | | | |
| *Participants* | 14 | When study was performed, including beginning and end dates of recruitment. | A/N |
| | 15 | Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms). | 11 |
| | 16 | The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended). | A/N |
| *Test results* | 17 | Time-interval between the index tests and the reference standard, and any treatment administered in between. | A/N |
| | 18 | Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition. | 12 |
| | 19 | A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard. | 12-15 |
| | 20 | Any adverse events from performing the index tests or the reference standard. | A/N |
| *Estimates* | 21 | Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals). | 14,15 |
| | 22 | How indeterminate results, missing data and outliers of the index tests were handled. | A/N |
| | 23 | Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done. | 14,15 |
| | 24 | Estimates of test reproducibility, if done. | A/N |
| DISCUSSION | 25 | Discuss the clinical applicability of the study findings. | 18,19 |

# Diagnostic values of soluble mesothelin family proteins for malignant pleural mesothelioma: updated meta-analyses

| | |
|---|---|
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# Diagnostic values of soluble mesothelin family proteins for malignant pleural mesothelioma: updated meta-analyses

Short running head: Mesothelin family proteins in mesothelioma

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**Keywords:** Diagnosis; malignant pleural mesothelioma; soluble mesothelin family proteins

**Word counts:** 2,729

ABSTRACT

**Objective** Although the values of soluble mesothelin family proteins (SMFPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma have been extensively studied, the exact diagnostic accuracy of these SMFPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMFPs in serum, and further to establish that of SMFPs in pleural fluid for mesothelioma.

**Design** Systematic review and meta-analysis.

**Methods** In total, 27 articles from 30 diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMFPs in serum and pleural fluid for the diagnosis of malignant pleural mesothelioma were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.63, 0.87, 5.68, 0.42, and 14.95, respectively; pleural fluid: 0.80, 0.83, 4.00, 0.30, and 15.31, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMFPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMFP determinations were not sufficiently to exclude non-MPM, and the positive test results indicated that further invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of MPM is always a challenging endeavor because.
- To date, no single marker or panel of soluble biomarkers is available for a clear diagnosis of MPM.
- The concentrations of soluble mesothelin family proteins, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin family proteins in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- Determination of soluble mesothelin family proteins might be helpful in confirming pleural mesothelioma if the results were higher than the cut-off values, while the negative results were not sufficiently to exclude non-mesothelioma.

**Strengths and limitations of this study**

- The studies included this meta-analysis were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural mesothelioma and the other diseases.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in malignant mesothelioma, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble megakaryocyte potentiating factor (MPF).[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin family proteins (SMFPs), including both soluble mesothelin and MPF, have been found in human serum and pleural fluid (PF).[9,10] Actually, the diagnostic accuracy of SMFP detections for MPM has been extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we performed and published first meta-analysis reporting the overall diagnostic accuracy of serum SMFPs for

diagnosing MPM, and our results showed that serum SMFP determinations played a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMFP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMFPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMFPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMFPs, and further to establish that of PF SMFPs for diagnosing MPM.

## METHODS

### Search strategy and study selection

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until July, 2013; no lower date limit was applied. Search keywords included: "soluble mesothelin family proteins", "soluble mesothelin-related peptides", "mesothelin", "megakaryocyte potentiating factor", "mesothelioma". Articles were also identified by use of the related-articles function in PubMed. References of articles identified were also searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

A study was included in the meta-analyses when it provided SMFP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected to be included in the meta-analyses, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

### Data extraction and quality assessment

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, fifty-six publications determining concentrations of human SMFPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, twenty-nine were excluded (Online supplementary appendix 1). Subsequently, twenty-seven publications [21-47] were available for analysis of diagnostic accuracy of SMFPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 16 publications from 18 studies [32-47] were added in the current meta-analyses.

The methods of determining SMFPs in all studies included were enzyme-linked immunosorbent assay. Serum mesothelin concentrations were determined in 20 studies (19 articles), [21,23-28,30,31,33,34,36-39,41,43,45,47] and serum MPF concentrations were determined in 5 studies [22,29,30,32,36] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMFP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. In addition, another 2 articles [30,36] were also treated as independent studies, since both mesothelin and MPF in serum were investigated in these 2 articles. PF mesothelin concentrations were determined in 9 studies.[23,35,40-44,46,47]

The clinical characteristics of the studies, along with STARD and QUADAS scores, are outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMFPs (Online supplementary appendix 2). For serum SMFP studies, the average samples size was 270 (range from 40 – 1,086), the subjects included 1,046 patients with MPM and 5,356 non-MPM. For PF SMFP studies, the average samples size was 127 (range from 40 – 275), the subjects included 352 patients with MPM and 794 non-MPM.

The samples were collected from the consecutive patients in all studies but not 2 studies. Nine studies reported blinded interpretation of SMFP assays independent of the reference standard. Eight studies reported the study design was prospective. In 21 studies, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 studies, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 23 of 27 publications had higher STARD scores (≥ 13) and 18 studies had higher QUADAS scores (≥ 10).

**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMFP studies (Figure 1A), evaluation of publication bias showed that Egger tests were significant for serum SMFPs (p = 0.044). Although the funnel plots for publication bias showed somehow asymmetry due to the limited number of PF SMFP studies (Figure 1B), Egger tests showed that this was not

significant for PF SMFPs (p = 0.149). These results indicated a potential for publication bias for serum SMFP, but not for PF SMFP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 25 serum SMFP studies in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.63, 95% CI 0.60 – 0.65), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.68 (95% CI 4.15 – 7.76), NLR was 0.42 (95% CI 0.36 – 0.49), and DOR was 14.95 (95% CI 9.93 – 22.50). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 121.53, 443.37, 261.05, 107.18, and 137.67, respectively, with all p < 0.001, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity 9 PF SMFP studies in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.80, 95% CI 0.76 – 0.84), while specificity ranged from 0.65 – 0.90 (pooled 0.83, 95% CI 0.80 – 0.85). We also noted that PLR was 4.00 (95% CI 2.98 – 5.36), NLR was 0.30 (95% CI 0.23 – 0.39), and DOR was 15.31 (95% CI 9.32 – 25.16). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 37.07 (p < 0.001), 30.45 (p < 0.001), 23.85 (p = 0.002), 11.30 ( p = 0.185) , and 15.14 (p = 0.056), respectively, indicating some a heterogeneity between studies.

The graphs of SROC curves for SMFP determinations showing sensitivity versus 1 – specificity from individual studies are shown in Figure 3. SROC curve of serum SMFPs was not positioned near the desirable upper left corner of SROC curve, and the maximum joint sensitivity and specificity was 0.737 (SEM, 0.031) (Figure 3A); while area under curve (AUC)

was 0.802 (SEM, 0.035). The maximum joint sensitivity and specificity of PF SMFP was 0.805 (SEM, 0.022); while AUC was 0.875 (SEM, 0.023) (Figure 3B).

Totally, the diagnostic performance of SMFPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMFPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,44,45] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,39] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMFPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMFPs, it was the most difficult to identify MPM from other cancers, compared with healthy controls or asbestos-exposed people.

Five studies [23,35,40,41,46] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [35,40,41,46] for differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of

PF SMFP differentiating MPM from the other cancers was very similar to that of differentiating MPM from benign pleural effusions.

## DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[48,49]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMFPs was 0.63 and 0.80, respectively; and their specificity was 0.87 and 0.83 respectively. These data indicated that sensitivity and specificity of SMFPs in serum and PF were not as high as expected. SMFPs might be helpful in confirming (ruling in) MPM if the results were higher than the cut-off values. Thus, positive SMFP test results suggested that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. However, the relative low sensitivity, especially serum SMFPs, that was not sufficiently low to exclude non-MPM when a patient's SMFP results were lower than the cut-off values. Therefore, the associated poor sensitivity of SMFPs clearly limits their added value to diagnosis of MPM.

As previously described,[11] SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity, while DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMFPs were 0.737 and 0.805, respectively; while their AUCs were 0.802 and 0.875, respectively, indicating level of overall accuracy were also not as high

as expected. We also found that the pooled DORs of serum and PF SMFPs were 14.95, and 15.31, respectively, indicating that SMFPs seemed to be helpful in the diagnosis of MPM.

Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[50,51] we further presented both PLR and NLR as our measures of diagnostic accuracy. If a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[52] A PLR value of 5.68 with serum SMFPs suggests that patients with MPM have a near 6-fold higher chance of being SMFP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. On the other hand, NLR of serum SMFPs was found to be 0.42. If serum SMFP results were negative, the probability that this patient has MPM is 42%, which is not low enough to rule out MPM. The very similar results were found with PF SMFPs.

Although both mesothelin and MPF belong to SMFPs, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR, NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMFP for discriminating MPM from healthy control subjects was the best (although not as good as expected), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMFP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

Our meta-analyses had several limitations. First, exclusion of conference abstracts,

letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for serum SMFP studies, but not for PF SMFP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 2 studies,[34,41] epithelioid subtype of MPM was the most common pathological type in all studies, excluding the one reported by Creaney et al.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMFP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMFPs in MPM diagnosis. Third, control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. Fourth, multiple assays were available for determining mesothelin concentrations, and Mesomark, which has been approved by the US Food and Drug Administration, was used in most studies. The other mesothelin ELISA kits were used in 4 studies.[21, 30, 31, 35] These issues regarding accuracy of diagnosis could also lead to biased results

In conclusion, current evidence supported that SMFPs in both serum and PF were helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMFPs in serum and PF was similar, and serum MPF had superior diagnostic accuracy compared to serum mesothelin. The negative results of SMFP determinations were not sufficiently to exclude non-MPM, whereas the positive test results might be helpful in confirming MPM.

**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

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**Competing interests:** The authors have declared that no conflict of interest exists.

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**Data sharing statement:** There is no additional data available.

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**Figure legends**

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of SMPRs in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | MPF | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[34] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[36] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[36] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[37] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[38] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[39] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[41] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[43] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Ferro et al[45] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[47] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 2.** Study summary of SMPRs in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Fujimoto et al[35] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[40] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[41] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[42] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[43] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[44] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Filiberti et al[46] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[47] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23, 24, 25, 26, 27, 28, 30, 31, 33, 34, 36, 37, 38, 39, 41, 43, 45, 47 | 22, 29, 30, 32, 36 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.66 (0.60 – 0.71) |
| Heterogeneity* (p) | 70.03 (< 0.001) | 50.26 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.95 (0.93 – 0.96) |
| Heterogeneity (p) | 345.53 (< 0.001) | 19.42 (0.001) |
| PLR (95% CI) | 4.75 (3.51 – 6.44) | 12.31 (6.21 – 24.42) |
| Heterogeneity (p) | 179.04 (< 0.001) | 15.48 (0.004) |
| NLR (95% CI) | 0.45 (0.39 – 0.51) | 0.30 (0.14 – 0.64) |
| Heterogeneity (p) | 50.33 (< 0.001) | 74.23 (< 0.001) |
| DOR (95% CI) | 11.84 (7.91 – 17.70) | 40.15 (16.55 – 97.39) |
| Heterogeneity (p) | 92.67 (< 0.001) | 12.07 (0.017) |
| AUC (SEM) | 0.791 (0.035) | 0.933 (0.083) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 44, 45 | 21, 23, 24, 25, 28, 30, 33, 39 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 35, 40, 41, 46 | 35, 40, 41, 46 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.81) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.14 (0.887) | 1.08 (0.783) |
| Specificity (95% CI) | 0.75 (0.68 –0.81) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 3.93 (0.416) | 6.74 (0.081) |
| PLR (95% CI) | 2.81 (2.13 –3.70) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.22 (0.337) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.26 – 0.44) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.90 (0.755) | 1.83 (0.608) |
| DOR (95% CI) | 8.75 (5.41 –14.15) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.28 (0.512) | 5.26 (0.154) |
| AUC (SEM) | 0.812 (0.026) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analyses

Comment [U1]: mesothelin family proteins (throughout)

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Short running head: SMRPs in mesothelioma

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**Keywords:** Diagnosis; malignant pleural mesothelioma; soluble mesothelin-related peptides

**Word counts:** 2,822

**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

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**Data sharing statement:** There is no additional data available.

ABSTRACT

**Objective** Although the values of soluble mesothelin-related peptides (SMRPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma have been extensively studied, the exact diagnostic accuracy of these SMRPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMRPs in serum, and further to establish that of SMRPs in pleural fluid for mesothelioma.

**Design** Systematic review and meta-analysis.

**Methods** In total, 27 articles from 30 diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMRPs in serum and pleural fluid for the diagnosis of malignant pleural mesothelioma were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.63, 0.87, 5.68, 0.42, and 14.95, respectively; pleural fluid: 0.80, 0.83, 4.00, 0.30, and 15.31, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMRPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, and the positive test results indicated that further

invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of MPM is always a challenging endeavor because.
- To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.
- The concentrations of soluble mesothelin-related peptides, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin-related peptides in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- The negative test results are not sufficiently to exclude non-mesothelioma, while the positive test results would indicate that further invasive diagnostic steps might be necessary.

**Strengths and limitations of this study**

- The studies included this meta-analysis were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural

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mesothelioma and the other diseases.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in malignant mesothelioma, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble megakaryocyte potentiating factor (MPF).[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin-related peptides (SMRPs), including both soluble mesothelin and MPF, have been found in human serum and pleural fluid (PF).[9,10] Actually, the diagnostic accuracy of SMRP detections for MPM has been extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we

performed and published first meta-analysis reporting the overall diagnostic accuracy of serum SMRPs for diagnosing MPM, and our results showed that serum SMRP determinations played a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMRP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMRPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMRPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMRPs, and further to establish that of PF SMRPs for diagnosing MPM.

METHODS

**Search strategy and study selection**

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until July, 2013; no lower date limit was applied. Search keywords included: "soluble mesothelin-related peptides/SMRP", "mesothelin", "megakaryocyte potentiating factor/MPF", "mesothelioma". Articles were also identified by use of the related-articles function in PubMed. References of articles identified were also searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

A study was included in the meta-analyses when it provided SMRP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected in the study, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

Comment [U6]: revised

**Data extraction and quality assessment**

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, fifty-six publications determining concentrations of human SMRPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, twenty-nine were excluded (Appendix excluded references, available online). Subsequently, twenty-seven publications [21-47] were available for analysis of diagnostic accuracy of SMRPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 16 publications from 18 studies [32-47] were added in the current meta-analyses.

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The methods of determining SMRPs in all studies included were enzyme-linked immunosorbent assay. Serum mesothelin concentrations were determined in 20 studies (19 articles), [21,23-28,30,31,33,34,36-39,41,43,45,47] and serum MPF concentrations were determined in 5 studies [22,29,30,32,36] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMRP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. In addition, another 2 articles [30,36] were also treated as independent studies, since both mesothelin and MPF in serum were investigated in these 2 articles. PF mesothelin concentrations were determined in 9 studies.[23,35,40-44,46,47]

The clinical characteristics of the studies, along with STARD and QUADAS scores, are outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMRPs (Appdendix Table 1). For serum SMRP studies, the average samples size was 270 (range from 40 – 1,086), the subjects included 1,046 patients with MPM and 5,356 non-MPM. For PF SMRP studies, the average samples size was 127 (range from 40 – 275), the subjects included 352 patients with MPM and 794 non-MPM.

Except for 2 studies, all samples were collected from the consecutive patients in the remaining 28 studies. Nine studies reported blinded interpretation of SMRP assays independent of the reference standard. Eight studies reported the study design was prospective. In 21 studies, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 studies, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 23 of 27 publications had higher STARD scores (≥ 13) and 18 studies had higher QUADAS scores (≥ 10).

**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMRP studies (Figure 1A), evaluation of publication bias showed that Egger tests were significant for serum SMRPs (p = 0.044). Although the funnel plots for publication bias showed somehow asymmetry due to the

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limited number of PF SMRP studies (Figure 1B), Egger tests showed that this was not significant for PF SMRPs (p = 0.149). These results indicated a potential for publication bias for serum SMRP, but not for PF SMRP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 25 serum SMRP assays in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.63, 95% CI 0.60 – 0.65), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.68 (95% CI 4.15 – 7.76), NLR was 0.42 (95% CI 0.36 – 0.49), and DOR was 14.95 (95% CI 9.93 – 22.50). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 121.53, 443.37, 261.05, 107.18, and 137.67, respectively, with all p < 0.001, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity 9 PF SMRP assays in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.80, 95% CI 0.76 – 0.84), while specificity ranged from 0.65 – 0.90 (pooled 0.83, 95% CI 0.80 – 0.85). We also noted that PLR was 4.00 (95% CI 2.98 – 5.36), NLR was 0.30 (95% CI 0.23 – 0.39), and DOR was 15.31 (95% CI 9.32 – 25.16). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 37.07 (p < 0.001), 30.45 (p < 0.001), 23.85 (p = 0.002), 11.30 ( p = 0.185) , and 15.14 (p = 0.056), respectively, indicating somehow a heterogeneity between studies.

The graphs of SROC curves for SMRP determinations showing sensitivity versus 1 – specificity from individual studies are shown in Figure 3. SROC curve of serum SMRPs was not positioned near the desirable upper left corner of SROC curve, and that the maximum

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joint sensitivity and specificity was 0.737 (SEM, 0.031) (Figure 3A); while area under curve (AUC) was 0.802 (SEM, 0.035). The maximum joint sensitivity and specificity of PF SMRP was 0.805 (SEM, 0.022); while AUC was 0.875 (SEM, 0.023) (Figure 3B).

Totally, the diagnostic performance of SMRPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMRPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,44,45] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,39] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMRPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMRPs, it was the most difficult to identify MPM from other cancers, compared with from healthy controls or asbestos-exposed people.

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Five studies [23,35,40,41,46] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [35,40,41,46] for

differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was very similar to that of differentiating MPM from benign pleural effusions.

DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[48,49]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMRPs was 0.63 and 0.80, respectively; and their specificity was 0.87 and 0.83 respectively. These data indicated that sensitivity and specificity of SMRPs in serum and PF were not as high as expected. The positive SMRPs results might be somehow helpful in confirming (ruling in) MPM, suggesting that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. However, the relative low sensitivity, especially serum SMRPs, that was not sufficiently low to exclude non-MPM when a patient's SMRP results were lower than the cut-off values. Therefore, the associated poor sensitivity of SMRPs clearly limits their added value to diagnosis of MPM.

As previously described,[11] Unlike a traditional ROC plot that explores the effect of varying cut-off values on sensitivity and specificity in a single study, each data point in the SROC plot represents a separate study. The SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMRPs were 0.737 and 0.805, respectively; while their AUCs were 0.802 and 0.875,

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respectively, indicating level of overall accuracy were also not as high as expected.

The DOR is a single indicator of test accuracy [50] that combines the data from sensitivity and specificity into a single number. The DOR of a test is the ratio of the odds of positive test results in the diseased relative to the odds of positive test results in the non-diseased. The value of a DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance. A DOR of 1.0 indicates that a test does not discriminate between patients with the disorder and those without it. In the current meta-analyses, we found that the pooled DORs of serum and PF SMRPs were 14.95, and 15.31, respectively, indicating that SMRPs seemed to be helpful in the diagnosis of MPM.

Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[51,52] we further presented both PLR and NLR as our measures of diagnostic accuracy. It a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[53] A PLR value of 5.68 with serum SMRPs suggests that patients with MPM have a near 6-fold higher chance of being SMRP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. This might lead to an inordinate number of individuals undergoing unnecessary diagnostic work-ups or biopsies. On the other hand, NLR of serum SMRPs was found to be 0.42. If serum SMRP results were negative, the probability that this patient has MPM is 42%, which is not low enough to rule out MPM. The very similar results were found with PF SMRPs.

Although both mesothelin and MPF belong to SMRPs, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR,

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NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMRP for discriminating MPM from healthy control subjects was the best (although not as good as expected), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

Our meta-analyses had several limitations. First, exclusion of conference abstracts, letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for serum SMRP studies, but not for PF SMRP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 2 studies,[34,41] epithelioid subtype of MPM was the most common pathological type in all remaining studies, but not one.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMRP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMRPs in MPM diagnosis. Third, control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. Fourth, multiple assays were available for determining mesothelin concentrations, and Mesomark, which has been approved by the US Food and Drug

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Administration, was used in most studies. The other mesothelin ELISA kits were used in 4 studies.[21, 30, 31, 35] These issues regarding accuracy of diagnosis could also lead to biased results

In conclusion, current evidence supported that SMRPs in both serum and PF were helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMRPs in serum and PF was similar, and serum MPF had superior diagnostic accuracy compared to serum mesothelin. The negative results of SMRP determinations were not sufficiently to exclude non-MPM; on the other hand, the positive test results would indicate that further invasive diagnostic steps might be necessary and could possibly lead to an earlier diagnosis.

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**Figure legends**

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of SMPRs in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | MPF | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[34] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[36] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[36] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[37] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[38] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[39] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[41] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[43] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Ferro et al[45] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[47] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 2.** Study summary of SMPRs in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Fujimoto et al[35] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[40] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[41] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[42] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[43] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[44] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Filiberti et al[46] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[47] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23, 24, 25, 26, 27, 28, 30, 31, 33, 34, 36, 37, 38, 39, 41, 43, 45, 47 | 22, 29, 30, 32, 36 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.66 (0.60 – 0.71) |
| Heterogeneity* (p) | 70.03 (< 0.001) | 50.26 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.95 (0.93 – 0.96) |
| Heterogeneity (p) | 345.53 (< 0.001) | 19.42 (0.001) |
| PLR (95% CI) | 4.75 (3.51 – 6.44) | 12.31 (6.21 – 24.42) |
| Heterogeneity (p) | 179.04 (< 0.001) | 15.48 (0.004) |
| NLR (95% CI) | 0.45 (0.39 – 0.51) | 0.30 (0.14 – 0.64) |
| Heterogeneity (p) | 50.33 (< 0.001) | 74.23 (< 0.001) |
| DOR (95% CI) | 11.84 (7.91 – 17.70) | 40.15 (16.55 – 97.39) |
| Heterogeneity (p) | 92.67 (< 0.001) | 12.07 (0.017) |
| AUC (SEM) | 0.791 (0.035) | 0.933 (0.083) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 44, 45 | 21, 23, 24, 25, 28, 30, 33, 39 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 35, 40, 41, 46 | 35, 40, 41, 46 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.81) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.14 (0.887) | 1.08 (0.783) |
| Specificity (95% CI) | 0.75 (0.68 –0.81) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 3.93 (0.416) | 6.74 (0.081) |
| PLR (95% CI) | 2.81 (2.13 –3.70) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.22 (0.337) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.26 – 0.44) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.90 (0.755) | 1.83 (0.608) |
| DOR (95% CI) | 8.75 (5.41 –14.15) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.28 (0.512) | 5.26 (0.154) |
| AUC (SEM) | 0.812 (0.026) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio;

NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

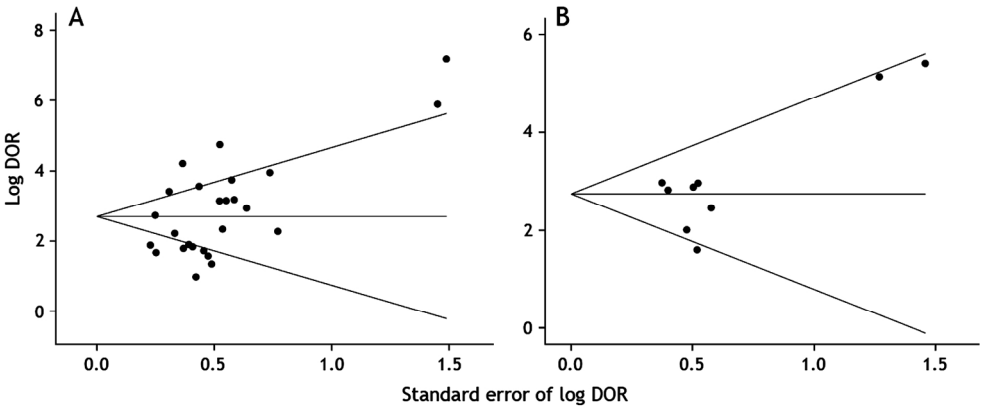


Figure 1. Funnel graphs for the assessment of potential publication bias in soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.
162x69mm (300 x 300 DPI)

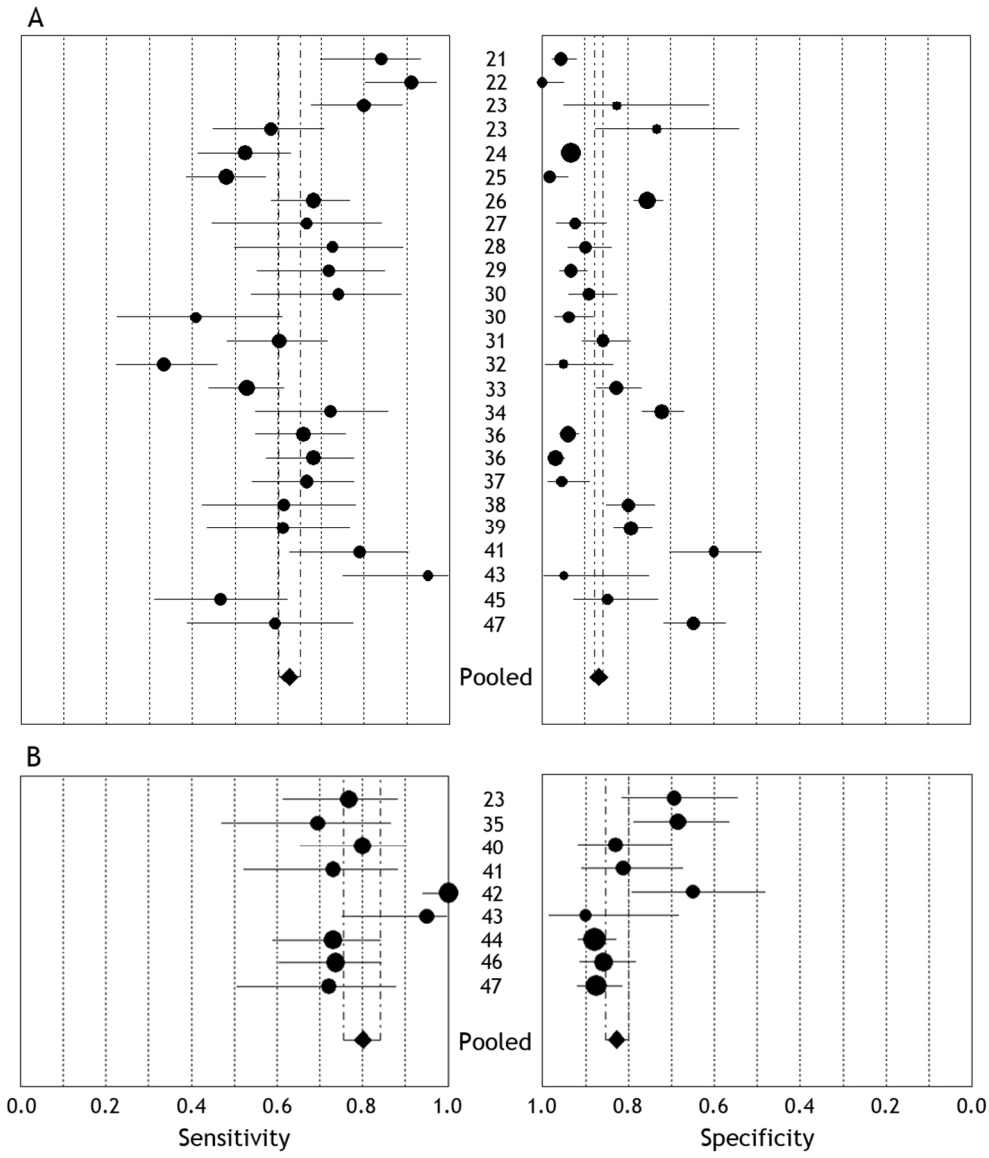


Figure 2. Forest plots of estimates of sensitivity and specificity for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.
101x118mm (300 x 300 DPI)

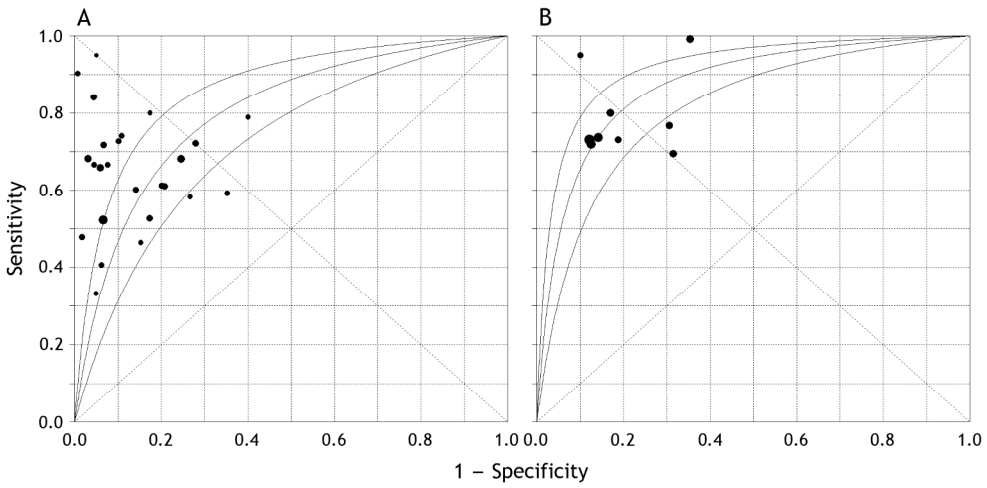


Figure 3. Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin-related peptides in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.
205x101mm (300 x 300 DPI)

**Online supplementary appendix 1**

**Excluded References**

One were excluded because it recruited less than 10 patients in one of study groups [1], seven were excluded because the same authors published several reports on the same patients, and only the best-quality study was considered [2-8], twenty-one were excluded because they did not allow the calculation of sensitivity or specificity [9-29].

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**Online supplementary appendix 2**

**The characteristics of subjects studied**

| Study | MPM Patients | Non-MPM Subjects |
|---|---|---|
| Robinson et al[21] | Epithelioid type (n = 25)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 15) | Healthy controls without asbestos exposure (n = 28)<br>Healthy controls with asbestos exposure (n = 40)<br>Patients with inflammatory non-pleural lung disease (n = 92)<br>Patients with non-MPM pleural diseases (n = 38)<br>Patients with non-pleural malignant lung disease (n = 30) |
| Onda et al[22] | Epithelioid type (n = 56) | Healthy controls (n = 70) |
| Scherpereel et al[23] | Epithelioid type (n = 55)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 13) | Patients with benign asbestos-related pleural diseases (n = 28)<br>Patients with pleural metastasis of carcinomas (n = 35) |
| Beyer et al[24] | Epithelioid type (n = 59)<br>Sarcomatoid type (n = 8)<br>Other or not specified (n = 21) | Healthy controls (n = 409)<br>Patients with non-MPM malignancy (n = 412)<br>Patients with nonmalignant conditions (n = 116) |

| | | |
|---|---|---|
| | | Subjects with asbestos exposure (n = 61) |
| Creaney et al[25] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 15)<br>Other or not specified (n = 67) | Healthy controls with asbestos exposure (n = 33)<br>Patients with benign asbestos-related diseases (n = 53)<br>Patients with benign pleural effusions (n = 30) |
| Cristaudo et al[26] | Epithelioid type (n = 72)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 25) | Healthy controls (n = 262)<br>Patients with benign respiratory diseases (n = 130)<br>Patients with lung cancer (n = 212) |
| Di Serio et al[27] | Epithelioid type (n = 20)<br>Sarcomatoid type (n = 2)<br>Other or not specified (n = 2) | Healthy controls with asbestos exposure (n = 26)<br>Patients with asbestos-related diseases (n = 66) |
| Amati et al[28] | Epithelioid type (n =11)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 5) | Healthy controls without asbestos exposure (n = 54)<br>Subjects with asbestos exposure (n = 94) |
| Shiomi et al[29] | Epithelioid type (n = 21)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 9) | Patients with benign asbestos-related diseases and healthy controls with asbestos exposure (n = 201)<br>Patients with lung cancer (n = 45)<br>Others (n = 8) |
| Iwahori et al[30] | Epithelioid type (n = 13) | Healthy controls without asbestos exposure (n = 38) |

| | | |
|---|---|---|
| | Sarcomatoid type (n = 3)<br>Other or not specified (n = 11) | Healthy controls with asbestos exposure (n = 9)<br>Patients with lung cancer (n = 47)<br>Patients with other cancers (n = 35) |
| van den Heuvel[31] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 20) | Healthy controls (n = 50)<br>Patients with lung cancer (n = 106) |
| Creaney et al[32] | Epithelioid type (n = 57)<br>Sarcomatoid type (n = 9) | Healthy controls without asbestos exposure (n = 10)<br>Healthy controls with asbestos exposure (n = 10)<br>Patients with benign asbestos-related diseases (n = 21)<br>Patients with lung cancer (n = 16) |
| Schneider et al[33] | Epithelioid type (n = 81)<br>Sarcomatoid type (n = 14)<br>Other or not specified (n = 34) | Patients with lung cancer (n = 139)<br>Patients with benign asbestos-related diseases (n = 75) |
| Portal et al[34] | Not specified (n = 36) | Healthy controls (n = 48)<br>Patients with asbestos exposure and no pleural disease (n = 177)<br>Patients with benign asbestos-related diseases (n = 101) |
| Fujimoto et al[35] | Epithelioid type (n = 15)<br>Sarcomatoid type (n = 4) | Patients with lung cancer (n = 38)<br>Patients with benign asbestos pleurisy (n = 26) |

| | | |
|---|---|---|
| | Other or not specified (n = 4) | Patients with tuberculosis pleurisy (n = 5)<br>Patients with no pleural diseases (n = 4) |
| Hollevoet et al[36] | Epithelioid type (n = 73)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Healthy controls (n = 101)<br>Healthy controls with asbestos exposure (n = 89)<br>Patients with benign asbestos-related diseases (n = 123)<br>Patients with benign respiratory diseases (n = 46)<br>Patients with lung cancer (n = 63) |
| Creaney et al[37] | Epithelioid type (n = 59)<br>Other or not specified (n = 7) | Patients with benign asbestos-related diseases (n = 47)<br>Patients with benign respiratory diseases (n = 42) |
| Cristaudo et al[38] | Epithelioid type (n = 31) | Healthy controls (n = 93)<br>Patients with benign respiratory diseases (n = 111) |
| Dipalma et al[39] | Epithelioid type (n = 29)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 3) | Healthy controls without asbestos exposure (n = 109)<br>Healthy controls with asbestos exposure (n = 26)<br>Patients with benign asbestos-related diseases (n = 48)<br>Patients with benign respiratory diseases (n = 110)<br>Patients with lung cancer (n = 25) |
| Yamada et al[40] | Epithelioid type (n = 37)<br>Sarcomatoid type (n = 5) | Patients with non-malignant pleural effusions (n = 24)<br>Patients with lung cancer involving malignant pleural effusion (n = |

| | | |
|---|---|---|
| | Other or not specified (n = 3) | 29) |
| Ashour et al[41] | Not specified (n = 38) | Healthy controls with asbestos exposure (n = 32)<br>Patients with benign pleural diseases (n = 29)<br>Patients with pleural carcinomas (n = 24) |
| Blanquart et al[42] | Epithelioid type (n = 49)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Patients with adenocarcinoma effusions (n = 25)<br>Patients with benign pleural effusions (n = 15) |
| Amany et al[43] | Epithelioid type (n = 14)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 2) | Patients with benign asbestos pleural effusions (n = 10)<br>Patients with tuberculosis pleural effusions (n = 10) |
| Canessa et al[44] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignant effusions (n = 94)<br>Patients with benign pleural effusions (n = 129) |
| Ferro et al[45] | Epithelioid type (n = 26)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignancy (n = 23)<br>Patients with benign diseases (n = 36) |
| Filiberti et al[46] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 3) | Patients with malignant effusions (n = 64)<br>Patients with benign effusions (n = 56) |

| | | |
|---|---|---|
| | Other or not specified (n = 2) | |
| Hooper et al[47] | Epithelioid type (n = 23)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 2) | Patients with non-MPM malignant effusions (n = 74)<br>Patients with benign asbestos-related effusions (n = 13)<br>Patients with benign pleural diseases (n = 100) |

MPM = malignant pleural mesothelioma.

## STARD checklist for reporting of studies of diagnostic accuracy

*(version January 2003)*

| Section and Topic | Item # | | On page # |
|---|---|---|---|
| TITLE/ABSTRACT/ KEYWORDS | 1 | Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity'). | 0-2 |
| INTRODUCTION | 2 | State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups. | 7,8 |
| METHODS | | | |
| *Participants* | 3 | The study population: The inclusion and exclusion criteria, setting and locations where data were collected. | 11 |
| | 4 | Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard? | 12 |
| | 5 | Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected. | 12 |
| | 6 | Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)? | 12 |
| *Test methods* | 7 | The reference standard and its rationale. | 12 |
| | 8 | Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard. | 12 |
| | 9 | Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard. | 11,12 |
| | 10 | The number, training and expertise of the persons executing and reading the index tests and the reference standard. | N/A |
| | 11 | Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers. | 12 |
| *Statistical methods* | 12 | Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals). | N/A |
| | 13 | Methods for calculating test reproducibility, if done. | N/A |
| RESULTS | | | |
| *Participants* | 14 | When study was performed, including beginning and end dates of recruitment. | N/A |
| | 15 | Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms). | 11 |
| | 16 | The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended). | N/A |
| *Test results* | 17 | Time-interval between the index tests and the reference standard, and any treatment administered in between. | N/A |
| | 18 | Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition. | 12 |
| | 19 | A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard. | 12-15 |
| | 20 | Any adverse events from performing the index tests or the reference standard. | N/A |
| *Estimates* | 21 | Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals). | 14,15 |
| | 22 | How indeterminate results, missing data and outliers of the index tests were handled. | N/A |
| | 23 | Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done. | 14,15 |
| | 24 | Estimates of test reproducibility, if done. | N/A |
| DISCUSSION | 25 | Discuss the clinical applicability of the study findings. | 18,19 |

# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analyses

| | |
|---|---|
| Journal: | *BMJ Open* |
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# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analyses

Short running head: SMRPs in mesothelioma

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ABSTRACT

**Objective** Although the values of soluble mesothelin-related peptides (SMRPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma (MPM) have been extensively studied, the exact diagnostic accuracy of these SMRPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMRPs in serum, and further to establish that of SMRPs in pleural fluid for MPM.

**Design** Systematic review and meta-analysis.

**Methods** In total, 30 articles of diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMRPs in serum and pleural fluid for the diagnosis of MPM were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.61, 0.87, 5.71, 0.43, and 14.43, respectively; pleural fluid: 0.79, 0.85, 4.78, 0.30, and 19.50, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMRPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMRP determinations were

not sufficiently to exclude non-MPM, and the positive test results indicated that further invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of malignant pleural mesothelioma is always a challenging endeavor.
- To date, no single marker or panel of soluble biomarkers is available for a clear diagnosis of malignant pleural mesothelioma.
- The concentrations of soluble mesothelin-related peptides, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin-related peptides in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- Determination of soluble mesothelin-related peptides might be helpful in confirming pleural mesothelioma if the results were higher than the cut-off values, while the negative results were not sufficiently to exclude non-mesothelioma.

**Strengths and limitations of this study**

- The studies included this meta-analyses were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural

mesothelioma and the other diseases.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in MPM, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble 31-kD N-terminal fraction, megakaryocyte potentiating factor (MPF), also denominated "N-ERC/mesothelin".[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin-related peptides (SMRPs), including both soluble mesothelin and MPF, have been found in human serum and pleural fluid (PF).[9,10] Actually, the diagnostic accuracy of SMRP detections for MPM has been

extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we performed and published first meta-analysis reporting the overall diagnostic accuracy of serum SMRPs for diagnosing MPM, and our results showed that serum SMRP determinations played a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMRP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMRPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMRPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMRPs, and further to establish that of PF SMRPs for diagnosing MPM.

## METHODS

### Search strategy and study selection

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until November 28, 2013; no lower date limit was applied. Search keywords included: "soluble mesothelin-related peptides/SMRP", "mesothelin", "megakaryocyte potentiating factor/MPF", "mesothelioma". Articles were also identified by use of the related-articles function in PubMed. References of articles identified were further searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

A study was included in the meta-analyses when it provided SMRP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected to be included in the meta-analyses, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

### Data extraction and quality assessment

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, sixty-two publications determining concentrations of human SMRPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, thirty-two were excluded (Online supplementary appendix 1). Subsequently, thirty publications [21-50] were available for analysis of diagnostic accuracy of SMRPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 19 publications from 28 studies [32-50] were added in the current meta-analyses.

Multiple ELISA kits were available for determining SMRP concentrations. Mesomark, which has been approved by the US Food and Drug Administration, was used to determine mesothelin in most studies, and the other mesothelin ELISA kits were used in the other 4 studies.[21,30,31,34] Serum mesothelin concentrations were determined in 23 studies (22 articles), [21,23-31,33,35,37-40,42,44,46,47,49,50] and serum MPF concentrations were determined in 5 studies [22,30,32,37,46] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMRP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. SMRP concentrations in PF were determined in 11 articles from 12 studies (mesothelin in 11 and MPF in 1) (Table 2). [23,34,36,41-47,49]

The clinical characteristics of the studies, along with STARD and QUADAS scores, are

outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMRPs (Online supplementary appendix 2). For serum SMRP studies, the average samples size was 265 (range from 40 – 1,086), the subjects included 1,562 patients with MPM and 5,988 non-MPM. For PF SMRP studies, the average samples size was 126 (range from 40 – 275), the subjects included 460 patients with MPM and 1,046 non-MPM.

In 21 publications, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 publications, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 26 of 30 publications had higher STARD scores (≥ 13) and 21 studies had higher QUADAS scores (≥ 10).

**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMRP studies (Figure 1A), evaluation of publication bias showed that Egger tests were significant for serum SMRPs (p = 0.038). Similarly, the funnel plots for publication bias also showed asymmetry for PF SMRP studies (Figure 1B), Egger tests showed that this was significant for PF SMRPs (p = 0.035).

These results indicated a potential for publication bias for both serum and PF SMRP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 28 serum SMRP studies in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.61, 95% CI 0.58 – 0.63), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.71 (95% CI 4.28 – 7.62), NLR was 0.43 (95% CI 0.38 – 0.50), and DOR was 14.43 (95% CI 9.98 – 20.87). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 153.68, 460.32, 272.50, 143.64, and 142.07, respectively, with all $p < 0.001$, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity 12 PF SMRP studies in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.79, 95% CI 0.75 – 0.83), while specificity ranged from 0.65 – 0.95 (pooled 0.85, 95% CI 0.83 – 0.87). We also noted that PLR was 4.78 (95% CI 3.52 – 6.50), NLR was 0.30 (95% CI 0.24 – 0.36), and DOR was 19.50 (95% CI 12.14 – 31.33). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 41.33 ($p < 0.001$), 46.78 ($p < 0.001$), 38.64 ($p < 0.001$), 14.53 ( $p = 0.205$) , and 23.49 ($p = 0.015$), respectively, indicating some a heterogeneity between studies.

The graphs of SROC curves for SMRP determinations showing sensitivity versus 1 – specificity from individual studies are shown in Figure 3. SROC curve of serum SMRPs was not positioned near the desirable upper left corner of SROC curve, and the maximum joint sensitivity and specificity was 0.741 (SEM, 0.029) (Figure 3A); while area under curve (AUC) was 0.806 (SEM, 0.032). The maximum joint sensitivity and specificity of PF SMRP was

0.820 (SEM, 0.022); while AUC was 0.890 (SEM, 0.021) (Figure 3B).

Totally, the diagnostic performance of SMRPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMRPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,45,47] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,40] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMRPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMRPs, it was the most difficult to identify MPM from other cancers, compared with healthy controls or asbestos-exposed people.

Five studies [23,35,41,42,48] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [36,41,42,48] for differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was very similar to that of

differentiating MPM from benign pleural effusions.

## DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[51,52]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMRPs was 0.61 and 0.79, respectively; and their specificity was 0.87 and 0.85 respectively. These data indicated that sensitivity and specificity of SMRPs in serum and PF were not as high as expected. SMRPs might be helpful in confirming (ruling in) MPM if the results were higher than the cut-off values. Thus, positive SMRP test results suggested that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. However, the relative low sensitivity, especially serum SMRPs, that was not sufficiently low to exclude non-MPM when a patient's SMRP results were lower than the cut-off values. Therefore, the associated poor sensitivity of SMRPs clearly limits their added value to diagnosis of MPM.

As previously described,[11] SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity, while DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMRPs were 0.741 and 0.820, respectively; while their AUCs were 0.806 and 0.890, respectively, indicating level of overall accuracy were also not as high

as expected. We also found that the pooled DORs of serum and PF SMRPs were 14.43, and 19.50, respectively, indicating that SMRPs seemed to be helpful in the diagnosis of MPM, although they were not perfect.

Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[53,54] we further presented both PLR and NLR as our measures of diagnostic accuracy. If a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[55] A PLR value of 5.71 with serum SMRPs suggests that patients with MPM have a near 6-fold higher chance of being SMRP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. On the other hand, NLR of serum SMRPs was found to be 0.43. If serum SMRP results were negative, the probability that this patient has MPM is 43%, which is not low enough to rule out MPM. The very similar results were found with PF SMRPs.

Although both mesothelin and MPF belong to SMRPs, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR, NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMRP for discriminating MPM from healthy control subjects was the best (although not as good as expected), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

Our meta-analyses had several limitations. First, exclusion of conference abstracts, letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for both serum and PF SMRP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 5 studies,[35,42,50] epithelioid subtype of MPM was the most common pathological type in all studies, excluding the one reported by Creaney et al.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMRP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMRPs in MPM diagnosis. Third, control populations were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. These issues regarding accuracy of diagnosis could also lead to biased results.

In conclusion, current evidence supported that SMRPs in both serum and PF were helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMRPs in serum and PF was similar, and serum MPF had superior diagnostic accuracy compared to serum mesothelin. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, whereas the positive test results might be helpful in confirming MPM.

Finally, it should be mentioned that since our previous meta-analysis [11] had been published, the field concerning the use of SMRPs in clinical practice moved forward

significantly.[10] It has been recognized that SMRPs are not only diagnostic markers, but also serve as markers of disease course and response to treatment.[56,57] Therefore, the application of SMRPs in the near future clinical practice may probably be in monitoring response to therapy, rather than in guiding diagnostic decisions and risk assessment of asbestos-exposed populations.

**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

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**Competing interests:** The authors have declared that no conflict of interest exists.

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## Figure legends

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of soluble mesothelin-related peptides in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | MPF | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[35] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[37] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[37] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[38] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[39] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[40] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[42] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Creaney et al[46] | 121 | Mesothelin | 2.4 nmol/L | 40 | 3 | 26 | 52 | 13 | 11 |
| Creaney et al[46] | 121 | MPF | 33.2 ng/mL | 34 | 3 | 32 | 52 | 13 | 11 |
| Ferro et al[47] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[49] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |
| Bayram et al[50] | 546 | Mesothelin | 1.63 nmol/L | 14 | 89 | 10 | 433 | 13 | 10 |

SMRP = soluble mesothelin-related peptide; MPF = megakaryocyte potentiating factor, OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 2.** Study summary of soluble mesothelin-related peptides in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Davies et al[34] | 166 | Mesothelin | 20.0 nmol/L | 17 | 14 | 7 | 128 | 14 | 11 |
| Fujimoto et al[36] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[41] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[42] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[43] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[45] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Creaney et al[46] | 98 | Mesothelin | 20.0 nmol/L | 30 | 3 | 13 | 52 | 13 | 11 |
| Creaney et al[46] | 98 | MPF | 600.0 ng/mL | 35 | 3 | 6 | 52 | 13 | 11 |
| Filiberti et al[48] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[49] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23-28, 30, 31, 33, 35, 37-40, 42, 44, 46, 47, 49, 50 | 22, 29, 30, 32, 37, 46 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.62 (0.56 – 0.67) |
| Heterogeneity* (p) | 70.20 (< 0.001) | 53.08 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.96 (0.94 – 0.97) |
| Heterogeneity (p) | 352.24 (< 0.001) | 17.60 (0.001) |
| PLR (95% CI) | 4.78 (3.59 – 6.36) | 12.39 (5.53 – 27.74) |
| Heterogeneity (p) | 185.80 (< 0.001) | 14.42 (0.006) |
| NLR (95% CI) | 0.45 (0.40 – 0.51) | 0.34 (0.19 – 0.63) |
| Heterogeneity (p) | 50.65 (< 0.001) | 67.07 (< 0.001) |
| DOR (95% CI) | 11.84 (8.12 – 17.27) | 36.08 (12.91 – 100.85) |
| Heterogeneity (p) | 95.80 (< 0.001) | 13.40 (0.009) |
| AUC (SEM) | 0.785 (0.033) | 0.941 (0.094) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 45, 47 | 21, 23, 24, 25, 28, 30, 33, 40 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 34, 36, 41, 42, 48 | 36, 41, 42, 48 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.80) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.36 (0.929) | 1.08 (0.783) |
| Specificity (95% CI) | 0.76 (0.71 –0.82) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 4.88 (0.430) | 6.74 (0.081) |
| PLR (95% CI) | 2.95 (2.32 – 3.75) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.83 (0.437) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.27 – 0.43) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.94 (0.857) | 1.83 (0.608) |
| DOR (95% CI) | 8.96 (5.78 – 13.89) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.34 (0.648) | 5.26 (0.154) |
| AUC (SEM) | 0.809 (0.025) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

# Diagnostic values of soluble mesothelin family proteins for malignant pleural mesothelioma: updated meta-analyses

Short running head: Mesothelin family proteins in mesothelioma

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**Keywords:** Diagnosis; malignant pleural mesothelioma; soluble mesothelin family proteins

**Word counts:** 2,729

**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

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**Data sharing statement:** There is no additional data available.

ABSTRACT

**Objective** Although the values of soluble mesothelin family proteins (SMFPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma (MPM) have been extensively studied, the exact diagnostic accuracy of these SMFPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMFPs in serum, and further to establish that of SMFPs in pleural fluid for mesothelioma.

**Design** Systematic review and meta-analysis.

**Methods** In total, 30 articles with diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMFPs in serum and pleural fluid for the diagnosis of malignant pleural mesothelioma were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.63, 0.87, 5.68, 0.42, and 14.95, respectively; pleural fluid: 0.80, 0.83, 4.00, 0.30, and 15.31, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMFPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMFP determinations were not sufficiently to exclude non-MPM, and the positive test results indicated that further

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invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of MPM is always a challenging endeavor.
- To date, no single marker or panel of soluble biomarkers is available for a clear diagnosis of MPM.
- The concentrations of soluble mesothelin family proteins, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin family proteins in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- Determination of soluble mesothelin family proteins might be helpful in confirming pleural mesothelioma if the results were higher than the cut-off values, while the negative results were not sufficiently to exclude non-mesothelioma.

**Strengths and limitations of this study**

- The studies included this meta-analyses were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural

mesothelioma and the other diseases.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in malignant mesothelioma, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble megakaryocyte potentiating factor (MPF).[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin family proteins (SMFPs), including both soluble mesothelin and MPF, have been found in human serum and pleural fluid (PF).[9,10] Actually, the diagnostic accuracy of SMFP detections for MPM has been extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we performed and

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published first meta-analysis reporting the overall diagnostic accuracy of serum SMFPs for diagnosing MPM, and our results showed that serum SMFP determinations played a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMFP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMFPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMFPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMFPs, and further to establish that of PF SMFPs for diagnosing MPM.

METHODS

**Search strategy and study selection**

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until July, 2013; no lower date limit was applied. Search keywords included: “soluble mesothelin family proteins”, “soluble mesothelin-related peptides”, “mesothelin”, “megakaryocyte potentiating factor”, “mesothelioma”. Articles were also identified by use of the related-articles function in PubMed. References of articles identified were also searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

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A study was included in the meta-analyses when it provided SMFP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected to be included in the meta-analyses, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

**Data extraction and quality assessment**

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, fifty-six publications determining concentrations of human SMFPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, thirty-two were excluded (Online supplementary appendix 1). Subsequently, twenty-seven publications [21-47] were available for analysis of diagnostic accuracy of SMFPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 16 publications from 18 studies [32-47] were added in the current meta-analyses.

The methods of determining SMFPs in all studies included were enzyme-linked immunosorbent assay. Multiple enzyme-linked immunosorbent assays were available for determining SMRP concentrations. Mesomark, which has been approved by the US Food and Drug Administration, was used to determine mesothelin in most studies, and the other mesothelin ELISA kits were used in the other 4 studies.[21,30,31,34] Serum mesothelin concentrations were determined in 20 studies (19 articles), [21,23-28,30,31,33,34,36-39,41,43,45,47] and serum MPF concentrations were determined in 5 studies [22,29,30,32,36] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMFP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. In addition, another 2 articles [30,36] were also treated as independent studies, since both mesothelin and MPF in serum were investigated in these 2 articles. PF mesothelin concentrations were determined in 9 studies.[23,35,40-44,46,47]

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The clinical characteristics of the studies, along with STARD and QUADAS scores, are outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMFPs (Online supplementary appendix 2). For serum SMFP studies, the average samples size was 270 (range from 40 – 1,086), the subjects included 1,046 patients with MPM and 5,356 non-MPM. For PF SMFP studies, the average samples size was 127 (range from 40 – 275), the subjects included 352 patients with MPM and 794 non-MPM.

The samples were collected from the consecutive patients in all studies but not 2 studies. Nine studies reported blinded interpretation of SMFP assays independent of the reference standard. Eight studies reported the study design was prospective. In 21 studies, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 studies, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 23 of 27 publications had higher STARD scores (≥ 13) and 18 studies had higher QUADAS scores (≥ 10).

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**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMFP studies (Figure 1A),

evaluation of publication bias showed that Egger tests were significant for serum SMFPs (p = 0.044). Although the funnel plots for publication bias showed somehow asymmetry due to the limited number of PF SMFP studies (Figure 1B), Egger tests showed that this was not significant for PF SMFPs (p = 0.149). These results indicated a potential for publication bias for serum SMFP, but not for PF SMFP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 25 serum SMFP studies in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.63, 95% CI 0.60 – 0.65), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.68 (95% CI 4.15 – 7.76), NLR was 0.42 (95% CI 0.36 – 0.49), and DOR was 14.95 (95% CI 9.93 – 22.50). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 121.53, 443.37, 261.05, 107.18, and 137.67, respectively, with all p < 0.001, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity 9 PF SMFP studies in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.80, 95% CI 0.76 – 0.84), while specificity ranged from 0.65 – 0.90 (pooled 0.83, 95% CI 0.80 – 0.85). We also noted that PLR was 4.00 (95% CI 2.98 – 5.36), NLR was 0.30 (95% CI 0.23 – 0.39), and DOR was 15.31 (95% CI 9.32 – 25.16). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 37.07 (p < 0.001), 30.45 (p < 0.001), 23.85 (p = 0.002), 11.30 ( p = 0.185) , and 15.14 (p = 0.056), respectively, indicating some a heterogeneity between studies.

The graphs of SROC curves for SMFP determinations showing sensitivity versus 1 –

specificity from individual studies are shown in Figure 3. SROC curve of serum SMFPs was not positioned near the desirable upper left corner of SROC curve, and the maximum joint sensitivity and specificity was 0.737 (SEM, 0.031) (Figure 3A); while area under curve (AUC) was 0.802 (SEM, 0.035). The maximum joint sensitivity and specificity of PF SMFP was 0.805 (SEM, 0.022); while AUC was 0.875 (SEM, 0.023) (Figure 3B).

Totally, the diagnostic performance of SMFPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMFPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,44,45] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,39] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMFPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMFPs, it was the most difficult to identify MPM from other cancers, compared with healthy controls or asbestos-exposed people.

Five studies [23,35,40,41,46] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [35,40,41,46] for differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of PF SMFP differentiating MPM from the other cancers was very similar to that of differentiating MPM from benign pleural effusions.

## DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[48,49]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMFPs was 0.63 and 0.80, respectively; and their specificity was 0.87 and 0.83 respectively. These data indicated that sensitivity and specificity of SMFPs in serum and PF were not as high as expected. SMFPs might be helpful in confirming (ruling in) MPM if the results were higher than the cut-off values. Thus, positive SMFP test results suggested that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. However, the relative low sensitivity, especially serum SMFPs, that was not sufficiently low to exclude non-MPM when a patient's SMFP results were lower than the cut-off values. Therefore, the associated poor sensitivity of SMFPs clearly limits their added value to diagnosis of MPM.

As previously described,[11] SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity, while DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMFPs were 0.737 and 0.805, respectively; while their AUCs were 0.802 and 0.875, respectively, indicating level of overall accuracy were also not as high

as expected. We also found that the pooled DORs of serum and PF SMFPs were 14.95, and 15.31, respectively, indicating that SMFPs seemed to be helpful in the diagnosis of MPM, although they were not perfect.

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Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[50,51] we further presented both PLR and NLR as our measures of diagnostic accuracy. If a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[52] A PLR value of 5.68 with serum SMFPs suggests that patients with MPM have a near 6-fold higher chance of being SMFP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. On the other hand, NLR of serum SMFPs was found to be 0.42. If serum SMFP results were negative, the probability that this patient has MPM is 42%, which is not low enough to rule out MPM. The very similar results were found with PF SMFPs.

Although both mesothelin and MPF belong to SMFPs, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR, NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMFP for discriminating MPM from healthy control subjects was the best (although not as good as expected), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMFP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

Our meta-analyses had several limitations. First, exclusion of conference abstracts, letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for serum SMFP studies, but not for PF SMFP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 2 studies,[34,41] epithelioid subtype of MPM was the most common pathological type in all studies, excluding the one reported by Creaney et al.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMFP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMFPs in MPM diagnosis. Third, control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. Fourth, multiple assays were available for determining mesothelin concentrations, and Mesomark, which has been approved by the US Food and Drug Administration, was used in most studies. The other mesothelin ELISA kits were used in 4 studies.[21, 30, 31, 35] These issues regarding accuracy of diagnosis could also lead to biased results

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In conclusion, current evidence supported that SMFPs in both serum and PF were helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMFPs in serum and PF was similar, and serum MPF had superior diagnostic accuracy compared to serum mesothelin. The negative results of SMFP determinations were not sufficiently to exclude

non-MPM, whereas the positive test results might be helpful in confirming MPM.

Finally, it should be mentioned that since our previous meta-analysis [11] had been published, the field concerning the use of SMRPs in clinical practice moved forward significantly.[10] It has been recognized that SMRPs are not only diagnostic markers, but also serve as markers of disease course and response to treatment.[56,57] Therefore, the application of SMRPs in the near future clinical practice may probably be in monitoring response to therapy, rather than in guiding diagnostic decisions and risk assessment of asbestos-exposed populations.

Comment [U13]: added

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**Figure legends**

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of SMPRs in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | N-ERC/ Mesothelin | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[35] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[37] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[37] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[38] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[39] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[40] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[42] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Creaney et al[46] | 121 | Mesothelin | 2.4 nmol/L | 40 | 3 | 26 | 52 | 13 | 11 |
| Creaney et al[46] | 121 | MPF | 33.2 ng/mL | 34 | 3 | 32 | 52 | 13 | 11 |
| Ferro et al[47] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[49] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |
| Bayram et al[50] | 546 | Mesothelin | 1.63 nmol/L | 14 | 89 | 10 | 433 | 13 | 10 |

SMRP = soluble mesothelin-related peptide; MPF = megakaryocyte potentiating factor, OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

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**Table 2.** Study summary of SMPRs in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Davies et al[34] | 166 | Mesothelin | 20.0 nmol/L | 17 | 14 | 7 | 128 | 14 | 11 |
| Fujimoto et al[36] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[41] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[42] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[43] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[45] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Creaney et al[46] | 98 | Mesothelin | 20.0 nmol/L | 30 | 3 | 13 | 52 | 13 | 11 |
| Creaney et al[46] | 98 | MPF | 600.0 ng/mL | 35 | 3 | 6 | 52 | 13 | 11 |
| Filiberti et al[47] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[49] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23, 24, 25, 26, 27, 28, 30, 31, 33, 34, 36, 37, 38, 39, 41, 43, 45, 47 | 22, 29, 30, 32, 36 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.66 (0.60 – 0.71) |
| Heterogeneity* (p) | 70.03 (< 0.001) | 50.26 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.95 (0.93 – 0.96) |
| Heterogeneity (p) | 345.53 (< 0.001) | 19.42 (0.001) |
| PLR (95% CI) | 4.75 (3.51 – 6.44) | 12.31 (6.21 – 24.42) |
| Heterogeneity (p) | 179.04 (< 0.001) | 15.48 (0.004) |
| NLR (95% CI) | 0.45 (0.39 – 0.51) | 0.30 (0.14 – 0.64) |
| Heterogeneity (p) | 50.33 (< 0.001) | 74.23 (< 0.001) |
| DOR (95% CI) | 11.84 (7.91 – 17.70) | 40.15 (16.55 – 97.39) |
| Heterogeneity (p) | 92.67 (< 0.001) | 12.07 (0.017) |
| AUC (SEM) | 0.791 (0.035) | 0.933 (0.083) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 44, 45 | 21, 23, 24, 25, 28, 30, 33, 39 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 35, 40, 41, 46 | 35, 40, 41, 46 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.81) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.14 (0.887) | 1.08 (0.783) |
| Specificity (95% CI) | 0.75 (0.68 –0.81) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 3.93 (0.416) | 6.74 (0.081) |
| PLR (95% CI) | 2.81 (2.13 –3.70) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.22 (0.337) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.26 – 0.44) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.90 (0.755) | 1.83 (0.608) |
| DOR (95% CI) | 8.75 (5.41 –14.15) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.28 (0.512) | 5.26 (0.154) |
| AUC (SEM) | 0.812 (0.026) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio;

NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

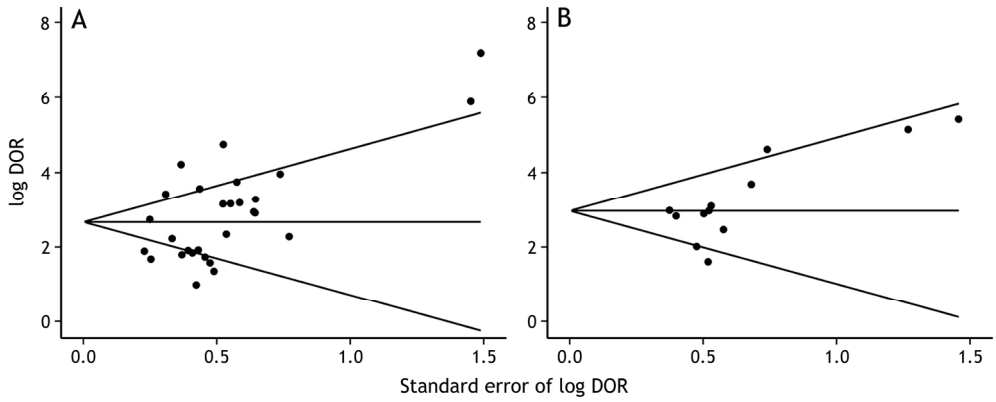


Figure 1. Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.
160x66mm (300 x 300 DPI)

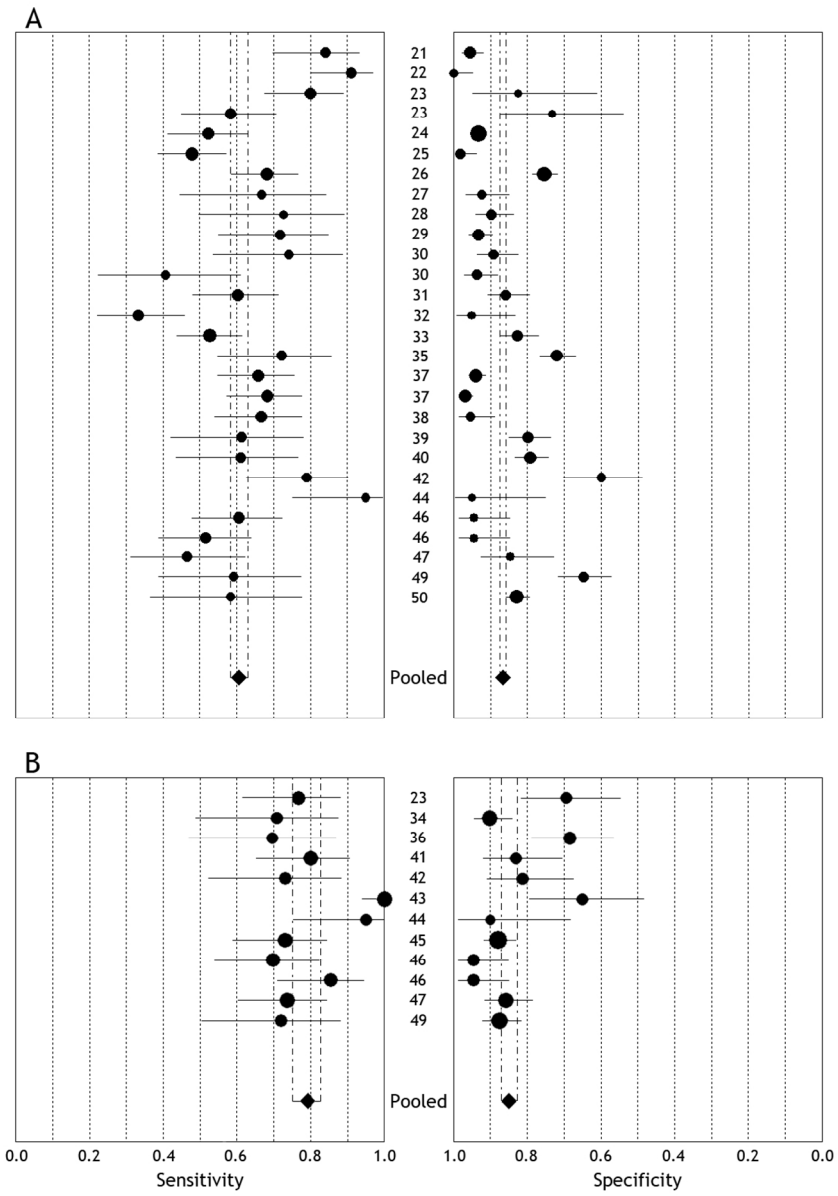


Figure 2. Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.
94x134mm (300 x 300 DPI)

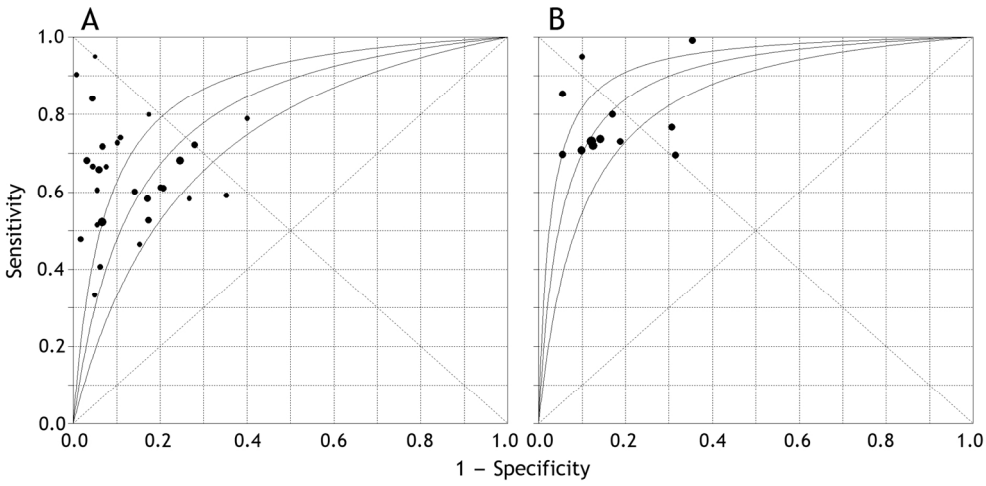


Figure 3. Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.
160x78mm (300 x 300 DPI)

**Online supplementary appendix 1**

**Excluded References**

One were excluded because it recruited less than 10 patients in one of study groups [1], nine were excluded because the same authors published several reports on the same patients, and only the best-quality study was considered [2-10], twenty-two were excluded because they did not allow the calculation of sensitivity or specificity [11-32].

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**Online supplementary appendix 2**

**The characteristics of subjects studied**

| Study | MPM Patients | Non-MPM Subjects |
|---|---|---|
| Robinson et al[21] | Epithelioid type (n = 25)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 15) | Healthy controls without asbestos exposure (n = 28)<br>Healthy controls with asbestos exposure (n = 40)<br>Patients with inflammatory non-pleural lung disease (n = 92)<br>Patients with non-MPM pleural diseases (n = 38)<br>Patients with non-pleural malignant lung disease (n = 30) |
| Onda et al[22] | Epithelioid type (n = 56) | Healthy controls (n = 70) |
| Scherpereel et al[23] | Epithelioid type (n = 55)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 13) | Patients with benign asbestos-related pleural diseases (n = 28)<br>Patients with pleural metastasis of carcinomas (n = 35) |
| Beyer et al[24] | Epithelioid type (n = 59)<br>Sarcomatoid type (n = 8)<br>Other or not specified (n = 21) | Healthy controls (n = 409)<br>Patients with non-MPM malignancy (n = 412)<br>Patients with nonmalignant conditions (n = 116) |

| | | |
|---|---|---|
| | | Subjects with asbestos exposure (n = 61) |
| Creaney et al[25] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 15)<br>Other or not specified (n = 67) | Healthy controls with asbestos exposure (n = 33)<br>Patients with benign asbestos-related diseases (n = 53)<br>Patients with benign pleural effusions (n = 30) |
| Cristaudo et al[26] | Epithelioid type (n = 72)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 25) | Healthy controls (n = 262)<br>Patients with benign respiratory diseases (n = 130)<br>Patients with lung cancer (n = 215) |
| Di Serio et al[27] | Epithelioid type (n = 20)<br>Sarcomatoid type (n = 2)<br>Other or not specified (n = 2) | Healthy controls with asbestos exposure (n = 26)<br>Patients with asbestos-related diseases (n = 66) |
| Amati et al[28] | Epithelioid type (n =11)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 5) | Healthy controls without asbestos exposure (n = 54)<br>Subjects with asbestos exposure (n = 94) |
| Shiomi et al[29] | Epithelioid type (n = 21)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 9) | Patients with benign asbestos-related diseases and healthy controls with asbestos exposure (n = 201)<br>Patients with lung cancer (n = 45)<br>Others (n = 8) |
| Iwahori et al[30] | Epithelioid type (n = 13) | Healthy controls without asbestos exposure (n = 38) |

| | | |
|---|---|---|
| | Sarcomatoid type (n = 3)<br>Other or not specified (n = 11) | Healthy controls with asbestos exposure (n = 9)<br>Patients with lung cancer (n = 47)<br>Patients with other cancers (n = 35) |
| van den Heuvel[31] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 20) | Healthy controls (n = 50)<br>Patients with lung cancer (n = 106) |
| Creaney et al[32] | Epithelioid type (n = 57)<br>Sarcomatoid type (n = 9) | Healthy controls without asbestos exposure (n = 10)<br>Healthy controls with asbestos exposure (n = 10)<br>Patients with benign asbestos-related diseases (n = 21)<br>Patients with lung cancer (n = 10) |
| Schneider et al[33] | Epithelioid type (n = 81)<br>Sarcomatoid type (n = 14)<br>Other or not specified (n = 34) | Patients with lung cancer (n = 139)<br>Patients with benign asbestos-related diseases (n = 75) |
| Davies et al[34] | Epithelioid type (n = 11)<br>Sarcomatoid type (n = 5)<br>Other or not specified (n = 8) | Patients with nonmesothelioma malignancy (n = 67)<br>Patients with benign pleural effusion (n = 75) |
| Rodriguex Portal et al[35] | Not specified (n = 36) | Healthy controls (n = 48)<br>Patients with asbestos exposure and no pleural disease (n = 177) |

| | | |
|---|---|---|
| | | Patients with benign asbestos-related diseases (n = 101) |
| Fujimoto et al[36] | Epithelioid type (n = 15)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 4) | Patients with lung cancer (n = 38)<br>Patients with benign asbestos pleurisy (n = 26)<br>Patients with tuberculosis pleurisy (n = 5)<br>Patients with no pleural diseases (n = 4) |
| Hollevoet et al[36] | Epithelioid type (n = 73)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Healthy controls (n = 101)<br>Healthy controls with asbestos exposure (n = 89)<br>Patients with benign asbestos-related diseases (n = 123)<br>Patients with benign respiratory diseases (n = 46)<br>Patients with lung cancer (n = 63) |
| Creaney et al[38] | Epithelioid type (n = 59)<br>Other or not specified (n = 7) | Patients with benign asbestos-related diseases (n = 47)<br>Patients with benign respiratory diseases (n = 42) |
| Cristaudo et al[39] | Epithelioid type (n = 31) | Healthy controls (n = 93)<br>Patients with benign respiratory diseases (n = 111) |
| Dipalma et al[40] | Epithelioid type (n = 29)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 3) | Healthy controls without asbestos exposure (n = 109)<br>Healthy controls with asbestos exposure (n = 26)<br>Patients with benign asbestos-related diseases (n = 48)<br>Patients with benign respiratory diseases (n = 110) |

| | | |
|---|---|---|
| | | Patients with lung cancer (n = 25) |
| Yamada et al[41] | Epithelioid type (n = 37)<br>Sarcomatoid type (n = 5)<br>Other or not specified (n = 3) | Patients with non-malignant pleural effusions (n = 24)<br>Patients with lung cancer (n = 29) |
| Ashour et al[42] | Not specified (n = 38) | Healthy controls with asbestos exposure (n = 32)<br>Patients with benign pleural diseases (n = 29)<br>Patients with pleural carcinomas (n = 24) |
| Blanquart et al[43] | Epithelioid type (n = 49)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Patients with adenocarcinoma effusions (n = 25)<br>Patients with benign pleural effusions (n = 15) |
| Amany et al[44] | Epithelioid type (n = 14)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 2) | Patients with benign asbestos pleural effusions (n = 10)<br>Patients with tuberculosis pleural effusions (n = 10) |
| Canessa et al[45] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignant effusions (n = 94)<br>Patients with benign pleural effusions (n = 129) |
| Creaney et al[46] | Epithelioid type (n = 32)<br>Sarcomatoid type (n = 9) | Patients with non-MPM malignant effusions (n = 39)<br>Patients with benign disease (n = 37) |

| | | |
|---|---|---|
| | Other or not specified (n = 25) | Patients with chronic kidney disease (n = 53)<br>Healthy controls without asbestos exposure (n = 18) |
| Ferro et al[47] | Epithelioid type (n = 26)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignancy (n = 23)<br>Patients with benign diseases (n = 36) |
| Filiberti et al[48] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 2) | Patients with malignant effusions (n = 64)<br>Patients with benign effusions (n = 56) |
| Hooper et al[49] | Epithelioid type (n = 23)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 2) | Patients with non-MPM malignant effusions (n = 74)<br>Patients with benign asbestos-related effusions (n = 13)<br>Patients with benign pleural diseases (n = 100) |
| Bayram et al[50] | Not specified (n = 24) | Patients with pleural plaques (n = 279)<br>Healthy controls with asbestos exposure (n = 123)<br>Healthy controls without asbestos exposure (n = 120) |

MPM = malignant pleural mesothelioma.

## STARD checklist for reporting of studies of diagnostic accuracy

*(version January 2003)*

| Section and Topic | Item # | | On page # |
|---|---|---|---|
| TITLE/ABSTRACT/ KEYWORDS | 1 | Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity'). | 0-2 |
| INTRODUCTION | 2 | State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups. | 7,8 |
| METHODS | | | |
| *Participants* | 3 | The study population: The inclusion and exclusion criteria, setting and locations where data were collected. | 11 |
| | 4 | Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard? | 12 |
| | 5 | Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected. | 12 |
| | 6 | Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)? | 12 |
| *Test methods* | 7 | The reference standard and its rationale. | 12 |
| | 8 | Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard. | 12 |
| | 9 | Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard. | 11,12 |
| | 10 | The number, training and expertise of the persons executing and reading the index tests and the reference standard. | N/A |
| | 11 | Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers. | 12 |
| *Statistical methods* | 12 | Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals). | N/A |
| | 13 | Methods for calculating test reproducibility, if done. | N/A |
| RESULTS | | | |
| *Participants* | 14 | When study was performed, including beginning and end dates of recruitment. | N/A |
| | 15 | Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms). | 11 |
| | 16 | The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended). | N/A |
| *Test results* | 17 | Time-interval between the index tests and the reference standard, and any treatment administered in between. | N/A |
| | 18 | Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition. | 12 |
| | 19 | A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard. | 12-15 |
| | 20 | Any adverse events from performing the index tests or the reference standard. | N/A |
| *Estimates* | 21 | Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals). | 14,15 |
| | 22 | How indeterminate results, missing data and outliers of the index tests were handled. | N/A |
| | 23 | Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done. | 14,15 |
| | 24 | Estimates of test reproducibility, if done. | N/A |
| DISCUSSION | 25 | Discuss the clinical applicability of the study findings. | 18,19 |

# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analyses

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID: | bmjopen-2013-004145.R3 |
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| Complete List of Authors: | Cui, Ai; Beijing Chaoyang Hospital, Capital Medical University, Department of Respiratory and Critical Care Medicine<br>Jin, Xiao-Guang; Beijing Chaoyang Hospital, Capital Medical University, Department of Respiratory and Critical Care Medicine<br>Zhai, Kan; Beijing Institute of Respiratory Diseases, Center of Medical Research<br>Tong, Zhao-Hui; Beijing Chaoyang Hospital, Capital Medical University, Department of Respiratory and Critical Care Medicine<br>Shi, Huan-Zhong; Beijing Chaoyang Hospital, Capital Medical University, Department of Respiratory and Critical Care Medicine |
| Primary Subject Heading: | Diagnostics |
| Secondary Subject Heading: | Diagnostics, Respiratory medicine |
| Keywords: | Adult thoracic medicine < THORACIC MEDICINE, Immunology < THORACIC MEDICINE, RESPIRATORY MEDICINE (see Thoracic Medicine) |
| | |

# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analyses

Short running head: SMRPs in mesothelioma

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ABSTRACT

**Objective** Although the values of soluble mesothelin-related peptides (SMRPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma (MPM) have been extensively studied, the exact diagnostic accuracy of these SMRPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMRPs in serum, and further to establish that of SMRPs in pleural fluid for MPM.

**Design** Systematic review and meta-analysis.

**Methods** In total, 30 articles of diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMRPs in serum and pleural fluid for the diagnosis of MPM were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.61, 0.87, 5.71, 0.43, and 14.43, respectively; pleural fluid: 0.79, 0.85, 4.78, 0.30, and 19.50, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMRPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, and the positive test results indicated that further invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of malignant pleural mesothelioma is always a challenging endeavor.
- To date, no single marker or panel of soluble biomarkers is available for a clear diagnosis of malignant pleural mesothelioma.
- The concentrations of soluble mesothelin family proteins, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin family proteins in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- Determination of soluble mesothelin family proteins might be helpful in confirming pleural mesothelioma if the results were higher than the cut-off values, while the negative results were not sufficiently to exclude non-mesothelioma.

**Strengths and limitations of this study**

- The studies included this meta-analyses were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural mesothelioma and the other diseases.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers, such as mesothelin family proteins, in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in MPM, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble 31-kD N-terminal fraction, megakaryocyte potentiating factor (MPF), also denominated "N-ERC/mesothelin".[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin-related peptides (SMRPs), including both soluble mesothelin and MPF, have been found in human serum and pleural

fluid (PF).[9,10] Actually, the diagnostic accuracy of SMRP detections for MPM has been extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we performed and published first meta-analysis reporting the overall diagnostic accuracy of serum SMRPs for diagnosing MPM, and our results showed that serum SMRP determinations could play a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMRP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMRPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMRPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMRPs, and further to establish that of PF SMRPs for diagnosing MPM.

## METHODS

### Search strategy and study selection

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until November 28, 2013; no lower date limit was applied. Search keywords included: "soluble mesothelin-related peptides/SMRP", "mesothelin", "megakaryocyte potentiating factor/MPF", "mesothelioma". Articles were also identified by use of the related-articles function in PubMed. References of articles identified were further searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

A study was included in the meta-analyses when it provided SMRP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected to be included in the meta-analyses, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

### Data extraction and quality assessment

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, sixty-two publications determining concentrations of human SMRPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, thirty-two were excluded (Online supplementary appendix 1). Subsequently, thirty publications [21-50] were available for analysis of diagnostic accuracy of SMRPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 19 publications from 28 studies [32-50] were added in the current meta-analyses.

Multiple ELISA kits were available for determining SMRP concentrations. Mesomark, which has been approved by the US Food and Drug Administration, was used to determine mesothelin in most studies, and the other mesothelin ELISA kits were used in the other 4 studies.[21,30,31,34] Serum mesothelin concentrations were determined in 23 studies (22 articles), [21,23-31,33,35,37-40,42,44,46,47,49,50] and serum MPF concentrations were determined in 5 studies [22,30,32,37,46] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMRP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. SMRP concentrations in PF were determined in 11 articles from 12 studies (mesothelin in 11 and MPF in 1) (Table 2). [23,34,36,41-47,49]

The clinical characteristics of the studies, along with STARD and QUADAS scores, are

outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMRPs (Online supplementary appendix 2). For serum SMRP studies, the average samples size was 265 (range from 40 – 1,086), the subjects included 1,562 patients with MPM and 5,988 non-MPM. For PF SMRP studies, the average samples size was 126 (range from 40 – 275), the subjects included 460 patients with MPM and 1,046 non-MPM.

In 21 publications, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 publications, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 26 of 30 publications had higher STARD scores (≥ 13) and 21 studies had higher QUADAS scores (≥ 10).

**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMRP studies (Figure 1A), evaluation of publication bias showed that Egger tests were significant for serum SMRPs (p = 0.038). Similarly, the funnel plots for publication bias also showed asymmetry for PF SMRP studies (Figure 1B), Egger tests showed that this was significant for PF SMRPs (p = 0.035).

These results indicated a potential for publication bias for both serum and PF SMRP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 28 serum SMRP studies in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.61, 95% CI 0.58 – 0.63), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.71 (95% CI 4.28 – 7.62), NLR was 0.43 (95% CI 0.38 – 0.50), and DOR was 14.43 (95% CI 9.98 – 20.87). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 153.68, 460.32, 272.50, 143.64, and 142.07, respectively, with all $p < 0.001$, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity for 12 PF SMRP studies in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.79, 95% CI 0.75 – 0.83), while specificity ranged from 0.65 – 0.95 (pooled 0.85, 95% CI 0.83 – 0.87). We also noted that PLR was 4.78 (95% CI 3.52 – 6.50), NLR was 0.30 (95% CI 0.24 – 0.36), and DOR was 19.50 (95% CI 12.14 – 31.33). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 41.33 ($p < 0.001$), 46.78 ($p < 0.001$), 38.64 ($p < 0.001$), 14.53 ( $p = 0.205$) , and 23.49 ($p = 0.015$), respectively, indicating some a heterogeneity between studies.

The graphs of SROC curves for SMRP determinations showing sensitivity versus 1 – specificity from individual studies are shown in Figure 3. SROC curve of serum SMRPs was not positioned near the desirable upper left corner of SROC curve, and the maximum joint sensitivity and specificity was 0.741 (SEM, 0.029) (Figure 3A); while area under curve (AUC) was 0.806 (SEM, 0.032). The maximum joint sensitivity and specificity of PF SMRP was

0.820 (SEM, 0.022); while AUC was 0.890 (SEM, 0.021) (Figure 3B).

Totally, the diagnostic performance of SMRPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMRPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,45,47] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,40] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMRPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMRPs, it was the most difficult to identify MPM from other cancers, compared with healthy controls or asbestos-exposed people.

Five studies [23,35,41,42,48] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [36,41,42,48] for differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was very similar to that of

differentiating MPM from benign pleural effusions.

## DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[51,52]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMRPs was 0.61 and 0.79, respectively; and their specificity was 0.87 and 0.85 respectively. These data indicated that sensitivity and specificity of SMRPs in serum and PF were not as high as expected. SMRPs might be helpful in confirming (ruling in) MPM if the results were higher than the cut-off values. Thus, positive SMRP test results suggested that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. On the other hand, the low sensitivity will not allow exclusion of non-MM patients even if patients have mesothelin concentrations lower than the cutoff value. Therefore, the associated poor sensitivity of SMRPs clearly limits their added value to diagnosis of MPM.

As previously described,[11] SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity, while DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMRPs were 0.741 and 0.820, respectively; while their AUCs were 0.806 and 0.890, respectively, indicating level of overall accuracy were also not as high

as expected. We also found that the pooled DORs of serum and PF SMRPs were 14.43, and 19.50, respectively, indicating that SMRPs seemed to be helpful in the diagnosis of MPM, although they were not perfect.

Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[53,54] we further presented both PLR and NLR as our measures of diagnostic accuracy. If a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[55] A PLR value of 5.71 with serum SMRPs suggests that patients with MPM have a near 6-fold higher chance of being SMRP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. On the other hand, NLR of serum SMRPs was found to be 0.43. If serum SMRP results were negative, the probability that this patient has MPM is 43%, which is not low enough to rule out MPM. The very similar results were found with PF SMRPs.

Although both mesothelin and MPF belong to mesothelin family proteins, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR, NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMRP for discriminating MPM from healthy control subjects was the best (although not as good as expected), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

Our meta-analyses had several limitations. First, exclusion of conference abstracts, letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for both serum and PF SMRP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 5 studies,[35,42,50] epithelioid subtype of MPM was the most common pathological type in all studies, excluding the one reported by Creaney et al.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMRP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMRPs in MPM diagnosis. Third, control populations were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. These issues regarding accuracy of diagnosis could also lead to biased results.

It should be mentioned that since our previous meta-analysis [11] had been published, the field concerning the use of SMRPs in clinical practice moved forward significantly.[10] It has been recognized that SMRPs are not only diagnostic markers, but also serve as markers of disease course and response to treatment.[56,57] Therefore, the application of SMRPs in the near future clinical practice may probably be in monitoring response to therapy, rather than in guiding diagnostic decisions and risk assessment of asbestos-exposed populations.

In conclusion, current evidence supported that SMRPs in both serum and PF were

helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMRPs in serum and PF was similar, and serum MPF had superior diagnostic accuracy compared to serum mesothelin. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, whereas the positive test results might be helpful in confirming MPM.

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**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

**Competing interests:** The authors have declared that no conflict of interest exists.

**Data sharing statement:** No additional data available.

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## Figure legends

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of soluble mesothelin-related peptides in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | MPF | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[35] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[37] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[37] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[38] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[39] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[40] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[42] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Creaney et al[46] | 121 | Mesothelin | 2.4 nmol/L | 40 | 3 | 26 | 52 | 13 | 11 |
| Creaney et al[46] | 121 | MPF | 33.2 ng/mL | 34 | 3 | 32 | 52 | 13 | 11 |
| Ferro et al[47] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[49] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |
| Bayram et al[50] | 546 | Mesothelin | 1.63 nmol/L | 14 | 89 | 10 | 433 | 13 | 10 |

SMRP = soluble mesothelin-related peptide; MPF = megakaryocyte potentiating factor, OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 2.** Study summary of soluble mesothelin-related peptides in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Davies et al[34] | 166 | Mesothelin | 20.0 nmol/L | 17 | 14 | 7 | 128 | 14 | 11 |
| Fujimoto et al[36] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[41] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[42] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[43] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[45] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Creaney et al[46] | 98 | Mesothelin | 20.0 nmol/L | 30 | 3 | 13 | 52 | 13 | 11 |
| Creaney et al[46] | 98 | MPF | 600.0 ng/mL | 35 | 3 | 6 | 52 | 13 | 11 |
| Filiberti et al[48] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[49] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23-28, 30, 31, 33, 35, 37-40, 42, 44, 46, 47, 49, 50 | 22, 29, 30, 32, 37, 46 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.62 (0.56 – 0.67) |
| Heterogeneity* (p) | 70.20 (< 0.001) | 53.08 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.96 (0.94 – 0.97) |
| Heterogeneity (p) | 352.24 (< 0.001) | 17.60 (0.001) |
| PLR (95% CI) | 4.78 (3.59 – 6.36) | 12.39 (5.53 – 27.74) |
| Heterogeneity (p) | 185.80 (< 0.001) | 14.42 (0.006) |
| NLR (95% CI) | 0.45 (0.40 – 0.51) | 0.34 (0.19 – 0.63) |
| Heterogeneity (p) | 50.65 (< 0.001) | 67.07 (< 0.001) |
| DOR (95% CI) | 11.84 (8.12 – 17.27) | 36.08 (12.91 – 100.85) |
| Heterogeneity (p) | 95.80 (< 0.001) | 13.40 (0.009) |
| AUC (SEM) | 0.785 (0.033) | 0.941 (0.094) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 45, 47 | 21, 23, 24, 25, 28, 30, 33, 40 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 34, 36, 41, 42, 48 | 36, 41, 42, 48 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.80) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.36 (0.929) | 1.08 (0.783) |
| Specificity (95% CI) | 0.76 (0.71 –0.82) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 4.88 (0.430) | 6.74 (0.081) |
| PLR (95% CI) | 2.95 (2.32 – 3.75) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.83 (0.437) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.27 – 0.43) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.94 (0.857) | 1.83 (0.608) |
| DOR (95% CI) | 8.96 (5.78 – 13.89) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.34 (0.648) | 5.26 (0.154) |
| AUC (SEM) | 0.809 (0.025) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

# Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analyses

Short running head: SMRPs in mesothelioma

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**Word counts:** 2,767

**Contributors**

HZS was responsible for the study conception and protocol design, and wrote the first draft of the paper. AC and XGJ were responsible for the overall study co-ordination under the supervision of HZS with contributions from KZ and ZHT. All literature searching, abstract screening, study selection and data extraction was undertaken independently by AC and XGJ with referral to KZ as a third reviewer as necessary. Assessment of methodological quality was also undertaken by ZHT and HZS. All authors have read and approved the final version of the manuscript.

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**Data sharing statement:** There is no additional data available.

ABSTRACT

**Objective** Although the values of soluble mesothelin-related peptides (SMRPs), including mesothelin and megakaryocyte potentiating factor, in serum or/and pleural fluid for diagnosing malignant pleural mesothelioma (MPM) have been extensively studied, the exact diagnostic accuracy of these SMRPs remains controversial. The purpose of the present meta-analyses was to update the overall diagnostic accuracy of SMRPs in serum, and further to establish that of SMRPs in pleural fluid for MPM.

**Design** Systematic review and meta-analysis.

**Methods** In total, 30 articles of diagnostic studies were included in the current meta-analyses. Sensitivity, specificity, and other measures of accuracy of SMRPs in serum and pleural fluid for the diagnosis of MPM were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

**Results** The summary estimates of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were as follows: serum: 0.61, 0.87, 5.71, 0.43, and 14.43, respectively; pleural fluid: 0.79, 0.85, 4.78, 0.30, and 19.50, respectively. It was also found that megakaryocyte potentiating factor in serum had a superior diagnostic accuracy compared to mesothelin for MPM.

**Conclusions** SMRPs in both serum and pleural fluid were helpful markers for diagnosing MPM with similar diagnostic accuracy. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, and the positive test results indicated that further

invasive diagnostic steps might be necessary for the diagnosis of MPM.

ARTICLE SUMMARY

**Article focus**

- The diagnosis of malignant pleural mesothelioma is always a challenging endeavor.
- To date, no single marker or panel of soluble biomarkers is available for a clear diagnosis of malignant pleural mesothelioma.
- The concentrations of soluble mesothelin-related peptides, including mesothelin and megakaryocyte potentiating factor, have been found to be increased in serum and pleural fluid of patients with malignant pleural mesothelioma.

**Key messages**

- Soluble mesothelin-related peptides in both serum and pleural fluid are helpful markers for the diagnosis of malignant pleural mesothelioma.
- Determination of soluble mesothelin-related peptides might be helpful in confirming pleural mesothelioma if the results were higher than the cut-off values, while the negative results were not sufficiently to exclude non-mesothelioma.

**Strengths and limitations of this study**

- The studies included this meta-analyses were methodologically satisfactory and their results were consistent and close.
- The subjects in control groups were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between malignant pleural

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mesothelioma and the other diseases.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a highly aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos.[1] Current therapeutic options for MPM are limited, and the prognosis is poor.[2] When the patients are treated with standard of care chemotherapy, cisplatin and an antifolate, median survival is approximately one year.[3,4] Early diagnosis offers the best hope for a favorable prognosis, however, the early and reliable diagnosis of MPM is extremely difficult, only 5% of patients with MPM present with stage IA disease.[5,6] Therefore, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Many soluble markers, such as mesothelin family proteins, in serum or pleural fluid have been evaluated to facilitate the non-invasive diagnostic work-up for MPM.[6] Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in MPM, pancreatic cancers, ovarian cancers, and some other cancers. Mesothelin is synthesized as a precursor 69-kDa protein and forms two proteins, the membrane-bound mesothelin and a soluble 31-kD N-terminal fraction, megakaryocyte potentiating factor (MPF), also denominated "N-ERC/mesothelin".[7] Although mesothelin is bound to cell membrane, a circulating form termed soluble mesothelin has been reported to be related to abnormal splicing events leading to synthesis of a secreted protein and to an enzymatic cleavage from membrane-bound mesothelin.[8]

It has been well documented that soluble mesothelin-related peptides (SMRPs), including both soluble mesothelin and MPF, have been found in human serum and pleural fluid (PF).[9,10] Actually, the diagnostic accuracy of SMRP detections for MPM has been

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extensively studied, but the exact role of these detections needs to be elucidated. In 2010, we performed and published first meta-analysis reporting the overall diagnostic accuracy of serum SMRPs for diagnosing MPM, and our results showed that serum SMRP determinations played a role in the diagnosis of MPM.[11] More recently, Hollevoet et al performed an individual patient data meta-analysis to evaluate serum SMRP for diagnosing MPM, and found that a positive test result at a high-specificity threshold is a strong incentive to urge further diagnostic steps; however, the poor sensitivity of SMRPs limits its added value to early diagnosis of MPM.[12] Since that time, many additional clinical studies determining the concentrations of SMRPs in serum and PF have been reported. We thus performed the present meta-analyses to update the overall diagnostic accuracy of serum SMRPs, and further to establish that of PF SMRPs for diagnosing MPM.

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METHODS

**Search strategy and study selection**

MEDLINE (PubMed database) and EMBASE were searched for suitable studies until November 28, 2013; no lower date limit was applied. Search keywords included: "soluble mesothelin-related peptides/SMRP", "mesothelin", "megakaryocyte potentiating factor/MPF", "mesothelioma". Articles were also identified by use of the related-articles function in PubMed. References of articles identified were further searched manually. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles. Conference abstracts and letters to the journal editors were excluded because of the limited data presented in them.

A study was included in the meta-analyses when it provided SMRP values in serum or/and PF for both sensitivity and specificity of the diagnosis of MPM. The studies including at least 10 specimens were selected to be included in the meta-analyses, since very small studies may be vulnerable to selection bias. Publications with evidence of possible overlap of patients with other studies were discussed by AC, XGJ, and KZ and only the best-quality study was used. Two reviewers (ZHT and HZS) independently judged study eligibility while screening the citations. Disagreements were resolved by consensus.

**Data extraction and quality assessment**

The final set of English articles was assessed independently by two reviewers (AC and XGJ). Data retrieved from the reports included author, publication year, study characteristics,

participant characteristics, diagnostic methods, sensitivity and specificity data, cut-off value and methodological quality. All eligible studies were assessed for methodologic quality using guidelines published by the STARD (standards for reporting diagnostic accuracy, maximum score 25) initiative [13] (ie, guidelines that aim to improve the quality of reporting in diagnostic studies) and the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14) tool [14] (ie, appraisal by use of empirical evidence, expert opinion, and formal consensus to assess the quality of primary studies of diagnostic accuracy).

**Statistical analyses**

Standard methods recommended for meta-analyses of diagnostic test evaluations [15] were used. Analyses were performed using two statistical software programs (Stata, version 9; Stata Corporation; College Station, TX; and Meta-DiSc for Windows; XI Cochrane Colloquium; Barcelona, Spain). We computed the following measures of test accuracy for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR).

The analyses were based on a summary receiver operating characteristic (SROC) curves.[15,16] The sensitivity and specificity for the single test threshold identified for each study were used to plot an SROC curve.[16,17] A random-effects model was used to calculate the average sensitivity, specificity and the other measures across studies.[18,19] The Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. Since publication bias is of concern for meta-analyses of diagnostic studies, we tested for the potential presence of this bias using funnel plots and the Egger test.[20]

RESULTS

**Studies included**

After independent review, sixty-two publications determining concentrations of human SMRPs in serum or/and PF were considered to be eligible for inclusion in the meta-analyses. Of these publications, thirty-two were excluded (Online supplementary appendix 1). Subsequently, thirty publications [21-50] were available for analysis of diagnostic accuracy of SMRPs. Eleven publications from 12 studies [21-31] were included in our previous meta-analysis.[11] After that, additional 19 publications from 28 studies [32-50] were added in the current meta-analyses.

Multiple ELISA kits were available for determining SMRP concentrations. Mesomark, which has been approved by the US Food and Drug Administration, was used to determine mesothelin in most studies, and the other mesothelin ELISA kits were used in the other 4 studies.[21,30,31,34] Serum mesothelin concentrations were determined in 23 studies (22 articles), [21,23-31,33,35,37-40,42,44,46,47,49,50] and serum MPF concentrations were determined in 5 studies [22,30,32,37,46] (Table 1). In the study by Scherpereel et al, [23] the authors compared serum SMRP concentrations in MPM patients with those in asbestos exposed-patients with benign pleural lesions and with patients with pleural metastasis of carcinomas separately, using two different cut-off values, we thus treated these research data as two independent studies. SMRP concentrations in PF were determined in 11 articles from 12 studies (mesothelin in 11 and MPF in 1) (Table 2). [23,34,36,41-47,49]

The clinical characteristics of the studies, along with STARD and QUADAS scores, are

outlined in Table 1 and Table 2.

**Study characteristics**

On review, the studies showed large differences in number of participants, clinical characteristics (especially histologic subtypes of MPM, and type of control groups), and reported diagnostic cut-off values of SMRPs (Online supplementary appendix 2). For serum SMRP studies, the average samples size was 265 (range from 40 – 1,086), the subjects included 1,562 patients with MPM and 5,988 non-MPM. For PF SMRP studies, the average samples size was 126 (range from 40 – 275), the subjects included 460 patients with MPM and 1,046 non-MPM.

In 21 publications, MPM diagnosis was completely based on pathological findings in pleural biopsies, with or without positive cytological results; while in the remaining 9 publications, some MPM patients were diagnosed just based on the cytological findings. Totally, the quality of study design and reporting diagnostic accuracy of most studies were good, since 26 of 30 publications had higher STARD scores (≥ 13) and 21 studies had higher QUADAS scores (≥ 10).

**Publication bias**

The funnel plots for publication bias showed asymmetry for serum SMRP studies (Figure 1A), evaluation of publication bias showed that Egger tests were significant for serum SMRPs ($p = 0.038$). Similarly, the funnel plots for publication bias also showed asymmetry for PF SMRP studies (Figure 1B), Egger tests showed that this was significant for PF SMRPs ($p = 0.035$).

These results indicated a potential for publication bias for both serum and PF SMRP studies.

**Diagnostic accuracy**

Figure 2A shows forest plot of sensitivity and specificity for 28 serum SMRP studies in the diagnosis of MPM. The sensitivity ranged from 0.33 to 0.95 (pooled 0.61, 95% CI 0.58 – 0.63), while specificity ranged from 0.60 – 1.00 (pooled 0.87, 95% CI 0.86 – 0.88). It was also noted that PLR was 5.71 (95% CI 4.28 – 7.62), NLR was 0.43 (95% CI 0.38 – 0.50), and DOR was 14.43 (95% CI 9.98 – 20.87). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 153.68, 460.32, 272.50, 143.64, and 142.07, respectively, with all $p < 0.001$, indicating a significant heterogeneity between studies.

Figure 2B shows forest plot of sensitivity and specificity for 12 PF SMRP studies in the diagnosis of MPM. The sensitivity ranged from 0.70 to 1.00 (pooled 0.79, 95% CI 0.75 – 0.83), while specificity ranged from 0.65 – 0.95 (pooled 0.85, 95% CI 0.83 – 0.87). We also noted that PLR was 4.78 (95% CI 3.52 – 6.50), NLR was 0.30 (95% CI 0.24 – 0.36), and DOR was 19.50 (95% CI 12.14 – 31.33). $X^2$ values of sensitivity, specificity, PLR, NLR, and DOR were 41.33 ($p < 0.001$), 46.78 ($p < 0.001$), 38.64 ($p < 0.001$), 14.53 ( $p = 0.205$) , and 23.49 ($p = 0.015$), respectively, indicating some a heterogeneity between studies.

The graphs of SROC curves for SMRP determinations showing sensitivity versus 1 – specificity from individual studies are shown in Figure 3. SROC curve of serum SMRPs was not positioned near the desirable upper left corner of SROC curve, and the maximum joint sensitivity and specificity was 0.741 (SEM, 0.029) (Figure 3A); while area under curve (AUC) was 0.806 (SEM, 0.032). The maximum joint sensitivity and specificity of PF SMRP was

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0.820 (SEM, 0.022); while AUC was 0.890 (SEM, 0.021) (Figure 3B).

Totally, the diagnostic performance of SMRPs in serum and PF was similar.

**Subgroup analysis**

We first analyzed the diagnostic values of serum mesothelin and MPF separately, and the results are presented in Table 3. Based on the comparisons of sensitivity, specificity, PLR, NLR, DOR, and AUC, the diagnostic performance of serum MPF was superior to that of serum mesothelin.

Data from 6 studies [21,22,24,28,30,31] were available for comparing diagnostic accuracy of serum SMRPs differentiating MPM from healthy control subjects, 9 studies [21,23,24,26,30,31,33,45,47] were available for comparing that of differentiating MPM from other malignancies, 8 studies [21,23,24,25,28,30,33,40] were available for comparing that of differentiating MPM from asbestos-exposed subjects. As shown in Table 4, the values of sensitivity, specificity, PLR, NLR, DOR, and AUC of SMRPs for discriminating MPM from healthy control subjects were quite acceptable, which were better than those for discriminating MPM from patients with the other cancers or from asbestos-exposed people. By using serum SMRPs, it was the most difficult to identify MPM from other cancers, compared with healthy controls or asbestos-exposed people.

Five studies [23,35,41,42,48] provided required data for comparing diagnostic accuracy of PF mesothelin differentiating MPM from the other cancers, and 4 studies [36,41,42,48] for differentiating MPM from benign pleural effusions (Table 5). Totally, diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was very similar to that of

differentiating MPM from benign pleural effusions.

DISCUSSION

The diagnosis of MPM is always a challenging endeavor because (1) MPM may appear in patients up to 30 – 40 years after exposure to asbestos, (2) the clinical and imaging signs of MPM are unspecific, and (3) a definitive diagnosis, which relies on histology, can sometimes be very difficult to achieve, even with the use of immunohistochemistry.[5] To date, no single marker or panel of soluble biomarkers are available for a clear diagnosis of MPM.[51,52]

In the present meta-analyses, our results indicated that the pooled sensitivity of serum and PF SMRPs was 0.61 and 0.79, respectively; and their specificity was 0.87 and 0.85 respectively. These data indicated that sensitivity and specificity of SMRPs in serum and PF were not as high as expected. SMRPs might be helpful in confirming (ruling in) MPM if the results were higher than the cut-off values. Thus, positive SMRP test results suggested that invasive diagnostic steps, such as medical thoracoscopy, might be necessary. However, the relative low sensitivity, especially serum SMRPs, that was not sufficiently low to exclude non-MPM when a patient's SMRP results were lower than the cut-off values. Therefore, the associated poor sensitivity of SMRPs clearly limits their added value to diagnosis of MPM.

Comment [U7]: revised

As previously described,[11] SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity, while DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number. The results of our analyses based SROC curves showed the maximum joint sensitivity and specificity of serum and PF SMRPs were 0.741 and 0.820, respectively; while their AUCs were 0.806 and 0.890, respectively, indicating level of overall accuracy were also not as high

as expected. We also found that the pooled DORs of serum and PF SMRPs were 14.43, and 19.50, respectively, indicating that SMRPs seemed to be helpful in the diagnosis of MPM, although they were not perfect.

Since SROC curve and DOR are not easy to interpret and use in clinical practice, and since PLR and NLR are considered more clinically meaningful,[53,54] we further presented both PLR and NLR as our measures of diagnostic accuracy. If a value is greater than 10 or less than 0.1, PLR or NLR generates large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[55] A PLR value of 5.71 with serum SMRPs suggests that patients with MPM have a near 6-fold higher chance of being SMRP-positive compared with patients without MPM, and this was not high enough for the clinical purpose. On the other hand, NLR of serum SMRPs was found to be 0.43. If serum SMRP results were negative, the probability that this patient has MPM is 43%, which is not low enough to rule out MPM. The very similar results were found with PF SMRPs.

Although both mesothelin and MPF belong to SMRPs, we noted in the current meta-analyses that the overall diagnostic measures, including sensitivity, specificity, PLR, NLR, DOR, and AUC, of serum MPF, were better than those of serum mesothelin. Therefore, MPF had a superior diagnostic accuracy compared to mesothelin for MPM. We also noted that diagnostic performance of serum SMRP for discriminating MPM from healthy control subjects was the best (although not as good as expected), followed by that for discriminating MPM from patients with other cancers or from asbestos-exposed people. In addition, the overall diagnostic accuracy of PF SMRP differentiating MPM from the other cancers was similar to that of differentiating MPM from benign pleural effusions.

**Comment [U8]:** mesothelin family proteins

Our meta-analyses had several limitations. First, exclusion of conference abstracts, letters to the editors, and non-English language studies may have led to publication bias. Indeed, we observed a publication bias for both serum and PF SMRP studies. Publication bias may also be introduced by inflation of diagnostic accuracy estimates since studies that report positive results are more likely to be accepted for publication. Second, pathological types of MPM were not specified in 5 studies,[35,42,50] epithelioid subtype of MPM was the most common pathological type in all studies, excluding the one reported by Creaney et al.[25] Totally, 69.9% (982/1404) MPM were epithelioid subtype (ranged from 29.9% to 100%). Analysis in terms of histologic type has shown that SMRP levels were significantly elevated in epithelioid subtype MPM than other types.[21,23,29] This could explain partly the quite low sensitivity of SMRPs in MPM diagnosis. Third, control populations were very heterogeneous from one study to another; and various cutoff points were used for distinguishing between MPM and controls, other cancers or benign respiratory diseases, according to the best combination of sensitivity and specificity. These issues regarding accuracy of diagnosis could also lead to biased results.

In conclusion, current evidence supported that SMRPs in both serum and PF were helpful markers for the diagnosis of MPM. The overall diagnostic performance of SMRPs in serum and PF was similar, and serum MPF had superior diagnostic accuracy compared to serum mesothelin. The negative results of SMRP determinations were not sufficiently to exclude non-MPM, whereas the positive test results might be helpful in confirming MPM.

Finally, it should be mentioned that since our previous meta-analysis [11] had been published, the field concerning the use of SMRPs in clinical practice moved forward

Comment [U9]: deleted

significantly.[10] It has been recognized that SMRPs are not only diagnostic markers, but also serve as markers of disease course and response to treatment.[56,57] Therefore, the application of SMRPs in the near future clinical practice may probably be in monitoring response to therapy, rather than in guiding diagnostic decisions and risk assessment of asbestos-exposed populations.

**Comment [U10]:** moved forward.

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**Figure legends**

**Figure 1.** Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.

**Figure 2.** Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.

**Figure 3.** Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.

**Table 1**. Study summary of soluble mesothelin-related peptides in sera

| Study | Subjects, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Robinson et al[21] | 272 | Mesothelin | 0.218 OD | 37 | 10 | 7 | 218 | 16 | 11 |
| Onda et al[22] | 126 | MPF | 0.034 OD | 51 | 0 | 5 | 70 | 11 | 9 |
| Scherpereel et al[23] | 83 | Mesothelin | 0.93 nmol/L | 48 | 4 | 12 | 19 | 14 | 10 |
| Scherpereel et al[23] | 90 | Mesothelin | 1.85 nmol/L | 35 | 8 | 25 | 22 | 14 | 10 |
| Beyer et al[24] | 1,086 | Mesothelin | 1.5 nmol/L | 46 | 66 | 42 | 932 | 16 | 12 |
| Creaney et al[25] | 233 | Mesothelin | 2.5 nmol/L | 56 | 2 | 61 | 114 | 13 | 11 |
| Cristaudo et al[26] | 714 | Mesothelin | 1.0 nmol/L | 73 | 149 | 34 | 458 | 14 | 9 |
| Di Serio et al[27] | 116 | Mesothelin | 1.5 nmol/L | 16 | 7 | 8 | 85 | 14 | 12 |
| Amati et al[28] | 170 | Mesothelin | 1.9 nmol/L | 16 | 15 | 6 | 133 | 12 | 9 |
| Shiomi et al[29] | 293 | MPF | 5.6 ng/ml | 28 | 17 | 11 | 237 | 20 | 13 |
| Iwahori et al[30] | 156 | MPF | 19.1 ng/ml | 20 | 14 | 7 | 115 | 14 | 11 |
| Iwahori et al[30] | 156 | Mesothelin | 123.7 ng/ml | 11 | 8 | 16 | 121 | 14 | 11 |
| van den Heuvel et al[31] | 229 | Mesothelin | 1.3 nmol/L | 44 | 22 | 29 | 134 | 17 | 12 |
| Creaney et al[32] | 107 | MPF | 1.0 ng/ml | 22 | 2 | 44 | 39 | 13 | 11 |
| Schneider et al[33] | 343 | Mesothelin | 1.35 nmol/L | 68 | 37 | 61 | 177 | 15 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Portal et al[35] | 362 | Mesothelin | 0.55 nmol/L | 26 | 91 | 10 | 235 | 15 | 11 |
| Hollevoet et al[37] | 507 | Mesothelin | 1.89 nmol/L | 56 | 25 | 29 | 397 | 20 | 11 |
| Hollevoet et al[37] | 507 | MPF | 13.46 ng/ml | 58 | 13 | 27 | 409 | 20 | 11 |
| Creaney et al[38] | 155 | Mesothelin | 1.6 nmol/L | 44 | 4 | 22 | 85 | 13 | 11 |
| Cristaudo et al[39] | 235 | Mesothelin | 1.0 nmol/L | 19 | 41 | 12 | 163 | 16 | 10 |
| Dipalma et al[40] | 354 | Mesothelin | 1.2 nmol/L | 22 | 66 | 14 | 252 | 15 | 10 |
| Ashour et al[42] | 123 | Mesothelin | 0.55 nmol/L | 30 | 34 | 8 | 51 | 13 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.3 nmol/L | 19 | 1 | 1 | 19 | 12 | 9 |
| Creaney et al[46] | 121 | Mesothelin | 2.4 nmol/L | 40 | 3 | 26 | 52 | 13 | 11 |
| Creaney et al[46] | 121 | MPF | 33.2 ng/mL | 34 | 3 | 32 | 52 | 13 | 11 |
| Ferro et al[47] | 102 | Mesothelin | 1.08 nmol/L | 20 | 9 | 23 | 50 | 14 | 9 |
| Hooper et al[49] | 203 | Mesothelin | 1.5 nmol/L | 16 | 62 | 11 | 114 | 15 | 12 |
| Bayram et al[50] | 546 | Mesothelin | 1.63 nmol/L | 14 | 89 | 10 | 433 | 13 | 10 |

SMRP = soluble mesothelin-related peptide; MPF = megakaryocyte potentiating factor, OD = optical density; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 2.** Study summary of soluble mesothelin-related peptides in pleural fluids *

| Study | Patients, n | SMRPs | Cut-off | Test Results | | | | Quality Scores | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TP | FP | FN | TN | STARD | QUADAS |
| Scherperel et al[23] | 92 | Mesothelin | 10.4 nmol/L | 33 | 15 | 10 | 34 | 14 | 10 |
| Davies et al[34] | 166 | Mesothelin | 20.0 nmol/L | 17 | 14 | 7 | 128 | 14 | 11 |
| Fujimoto et al[36] | 96 | Mesothelin | 8.0 nmol/L | 16 | 23 | 7 | 50 | 16 | 9 |
| Yamada et al[41] | 98 | Mesothelin | 10.0 nmol/L | 36 | 9 | 9 | 44 | 16 | 9 |
| Ashour et al[42] | 74 | Mesothelin | 3.0 nmol/L | 19 | 9 | 7 | 39 | 13 | 9 |
| Blanquart et al[43] | 101 | Mesothelin | 24.05 nmol/L | 61 | 14 | 0 | 26 | 12 | 9 |
| Amany et al[44] | 40 | Mesothelin | 3.5 nmol/L | 19 | 2 | 1 | 18 | 12 | 9 |
| Canessa et al[45] | 275 | Mesothelin | 9.3 nmol/L | 38 | 27 | 14 | 196 | 16 | 10 |
| Creaney et al[46] | 98 | Mesothelin | 20.0 nmol/L | 30 | 3 | 13 | 52 | 13 | 11 |
| Creaney et al[46] | 98 | MPF | 600.0 ng/mL | 35 | 3 | 6 | 52 | 13 | 11 |
| Filiberti et al[48] | 177 | Mesothelin | 12.0 nmol/L | 42 | 17 | 15 | 103 | 14 | 12 |
| Hooper et al[49] | 193 | Mesothelin | 20.0 nmol/L | 18 | 21 | 7 | 147 | 15 | 12 |

SMRP = soluble mesothelin-related peptide; TP = true positive; FP = false positive; FN= false negative; TN = true negative; STARD = standards for reporting diagnostic accuracy; QUADAS = quality assessment for studies of diagnostic accuracy.

**Table 3.** Comparison of diagnostic accuracy of mesothelin and megakaryocyte potentiating factor in sera

| | Mesothelin | Megakaryocyte potentiating factor |
|---|---|---|
| Study, Ref. No. | 21, 23-28, 30, 31, 33, 35, 37-40, 42, 44, 46, 47, 49, 50 | 22, 29, 30, 32, 37, 46 |
| Sensitivity (95% CI) | 0.62 (0.59 – 0.65) | 0.62 (0.56 – 0.67) |
| Heterogeneity* (p) | 70.20 (< 0.001) | 53.08 (< 0.001) |
| Specificity (95% CI) | 0.85 (0.84 – 0.86) | 0.96 (0.94 – 0.97) |
| Heterogeneity (p) | 352.24 (< 0.001) | 17.60 (0.001) |
| PLR (95% CI) | 4.78 (3.59 – 6.36) | 12.39 (5.53 – 27.74) |
| Heterogeneity (p) | 185.80 (< 0.001) | 14.42 (0.006) |
| NLR (95% CI) | 0.45 (0.40 – 0.51) | 0.34 (0.19 – 0.63) |
| Heterogeneity (p) | 50.65 (< 0.001) | 67.07 (< 0.001) |
| DOR (95% CI) | 11.84 (8.12 – 17.27) | 36.08 (12.91 – 100.85) |
| Heterogeneity (p) | 95.80 (< 0.001) | 13.40 (0.009) |
| AUC (SEM) | 0.785 (0.033) | 0.941 (0.094) |

* Q value

CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio;

AUC = area under curve; SEM = standard error of mean.

**Table 4.** Comparisons of diagnostic accuracy of SMRPs in sera for differentiating MPM from different control subpopulations

| | MPM *vs* healthy controls | MPM *vs* other cancers | MPM *vs* benign asbestos-related diseases |
|---|---|---|---|
| Study, Ref. No. | 21, 22, 24, 28, 30, 31 | 21, 23, 24, 26, 30, 31, 33, 45, 47 | 21, 23, 24, 25, 28, 30, 33, 40 |
| Sensitivity (95% CI) | 0.66 (.61 – 0.71) | 0.60 (0.56 – 0.64) | 0.58 (0.54 – 0.62) |
| Heterogeneity* (p) | 42.46 (< 0.001) | 31.33 (< 0.001) | 39.91 (< 0.001) |
| Specificity (95% CI) | 0.97 (0.96 – 0.98) | 0.81 (0.78 – 0.83) | 0.89 (0.86 – 0.91) |
| Heterogeneity (p) | 46.70 (< 0.001) | 52.59 (< 0.001) | 39.48 (< 0.001) |
| PLR (95% CI) | 24.07 (4.03 –143.68) | 2.81 (2.11 – 3.73) | 6.65 (3.69 –12.00) |
| Heterogeneity (p) | 48.35 (< 0.001) | 26.73 (0.001) | 25.91 (0.001) |
| NLR (95% CI) | 0.33 (0.22 – 0.50) | 0.52 (0.43 – 0.63) | 0.44 (0.36 – 0.55) |
| Heterogeneity (p) | 32.11 (< 0.001) | 25.45 (0.001) | 24.89 (0.001) |
| DOR (95% CI) | 69.27 (15.67 – 306.21) | 5.62 (3.67 – 8.59) | 18.03 (8.90 – 36.52) |
| Heterogeneity (p) | 20.80 (0.001) | 22.70 (0.004) | 19.16 (0.008) |
| AUC (SEM) | 0.870 (0.120) | 0.734 (0.055) | 0.845 (0.057) |

* Q value

SMRP = soluble mesothelin-related peptide; MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

**Table 5.** Comparisons of diagnostic accuracy of mesothelin in pleural fluid for differentiation of MPM from different control subpopulations

| | MPM *vs* other cancers | MPM *vs* benign diseases |
|---|---|---|
| Study, Ref. No. | 23, 34, 36, 41, 42, 48 | 36, 41, 42, 48 |
| Sensitivity (95% CI) | 0.75 (0.69 – 0.80) | 0.75 (0.67 – 0.82) |
| Heterogeneity* (p) | 1.36 (0.929) | 1.08 (0.783) |
| Specificity (95% CI) | 0.76 (0.71 –0.82) | 0.87 (0.80 – 0.93) |
| Heterogeneity (p) | 4.88 (0.430) | 6.74 (0.081) |
| PLR (95% CI) | 2.95 (2.32 – 3.75) | 4.74 (2.30 – 9.76) |
| Heterogeneity (p) | 4.83 (0.437) | 7.01 (0.071) |
| NLR (95% CI) | 0.34 (0.27 – 0.43) | 0.30 (0.22 – 0.40) |
| Heterogeneity (p) | 1.94 (0.857) | 1.83 (0.608) |
| DOR (95% CI) | 8.96 (5.78 – 13.89) | 16.87 (6.79 – 41.92) |
| Heterogeneity (p) | 3.34 (0.648) | 5.26 (0.154) |
| AUC (SEM) | 0.809 (0.025) | 0.818 (0.050) |

* Q value

MPM = malignant pleural mesothelioma; CI = confidence interval; PLR = positive likelihood ratio;

NLR = negative likelihood ratio; DOR = diagnostic odds ratio; AUC = area under curve; SEM = standard error of mean.

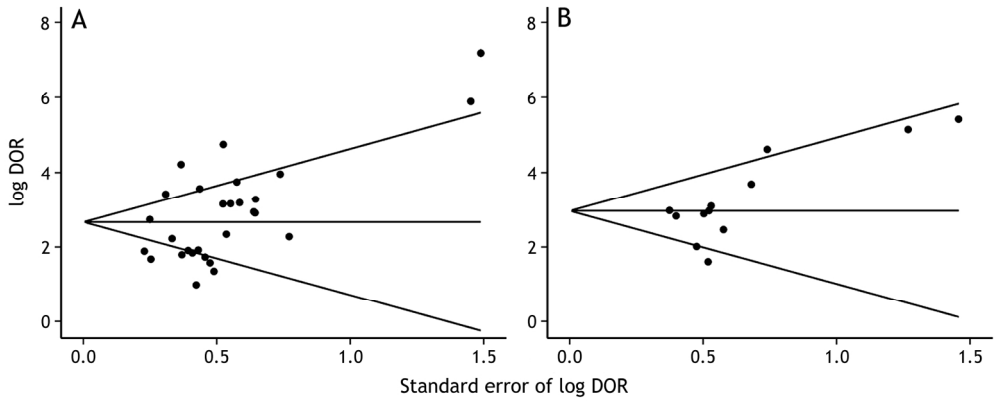


Figure 1. Funnel graphs for the assessment of potential publication bias in soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each solid circle represents each study in the meta-analysis. The line in the center indicates the summary DOR.
160x66mm (300 x 300 DPI)

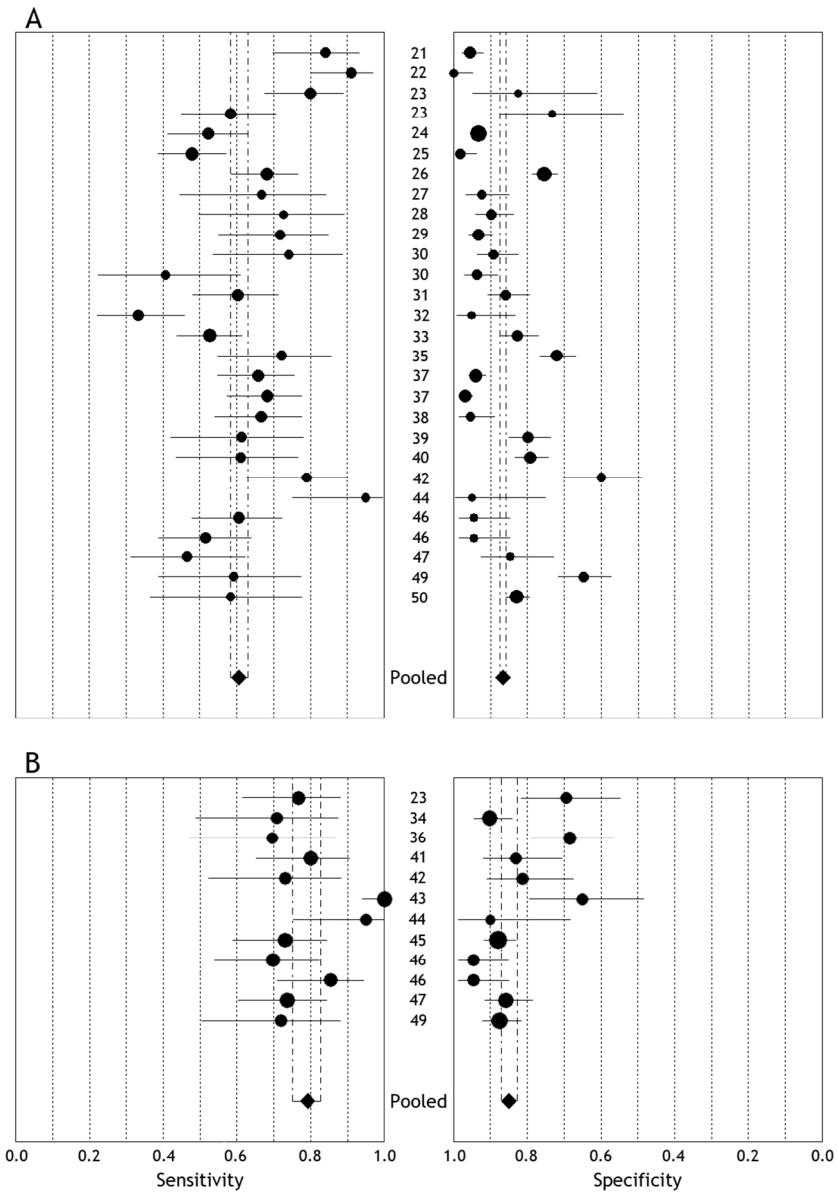


Figure 2. Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% confidence intervals. Numbers indicate the reference numbers of studies cited in the reference list.
94x134mm (300 x 300 DPI)

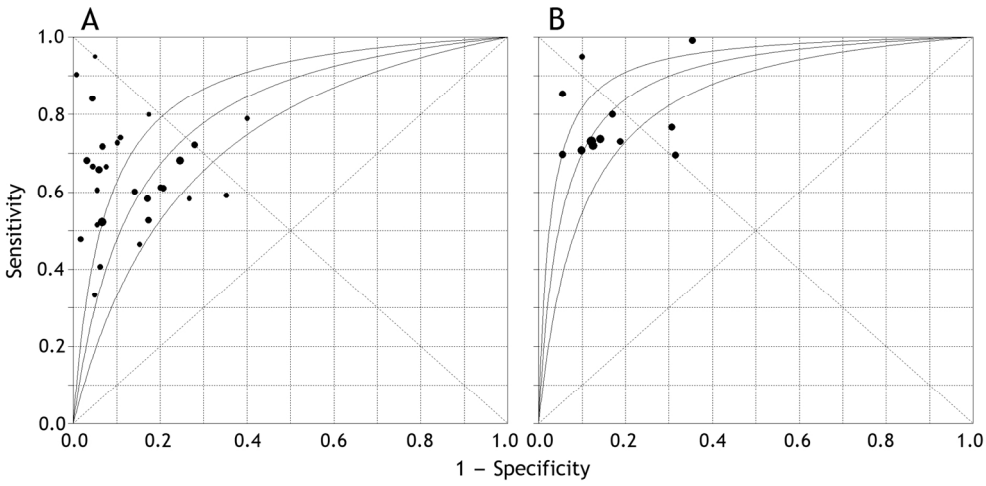


Figure 3. Summary receiver operating characteristic curves with 95% confidence intervals for soluble mesothelin family proteins in serum (A) and pleural fluid (B) for diagnosing malignant pleural mesothelioma. Each solid circle represents each study in the meta-analyses. The size of each study is indicated by the size of the solid circle. The regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy.
160x78mm (300 x 300 DPI)

**Online supplementary appendix 1**

**Excluded References**

One were excluded because it recruited less than 10 patients in one of study groups [1], nine were excluded because the same authors published several reports on the same patients, and only the best-quality study was considered [2-10], twenty-two were excluded because they did not allow the calculation of sensitivity or specificity [11-32].

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**Online supplementary appendix 2**

**The characteristics of subjects studied**

| Study | MPM Patients | Non-MPM Subjects |
|---|---|---|
| Robinson et al[21] | Epithelioid type (n = 25)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 15) | Healthy controls without asbestos exposure (n = 28)<br>Healthy controls with asbestos exposure (n = 40)<br>Patients with inflammatory non-pleural lung disease (n = 92)<br>Patients with non-MPM pleural diseases (n = 38)<br>Patients with non-pleural malignant lung disease (n = 30) |
| Onda et al[22] | Epithelioid type (n = 56) | Healthy controls (n = 70) |
| Scherpereel et al[23] | Epithelioid type (n = 55)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 13) | Patients with benign asbestos-related pleural diseases (n = 28)<br>Patients with pleural metastasis of carcinomas (n = 35) |
| Beyer et al[24] | Epithelioid type (n = 59)<br>Sarcomatoid type (n = 8)<br>Other or not specified (n = 21) | Healthy controls (n = 409)<br>Patients with non-MPM malignancy (n = 412)<br>Patients with nonmalignant conditions (n = 116) |

| | | |
|---|---|---|
| | | Subjects with asbestos exposure (n = 61) |
| Creaney et al[25] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 15)<br>Other or not specified (n = 67) | Healthy controls with asbestos exposure (n = 33)<br>Patients with benign asbestos-related diseases (n = 53)<br>Patients with benign pleural effusions (n = 30) |
| Cristaudo et al[26] | Epithelioid type (n = 72)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 25) | Healthy controls (n = 262)<br>Patients with benign respiratory diseases (n = 130)<br>Patients with lung cancer (n = 215) |
| Di Serio et al[27] | Epithelioid type (n = 20)<br>Sarcomatoid type (n = 2)<br>Other or not specified (n = 2) | Healthy controls with asbestos exposure (n = 26)<br>Patients with asbestos-related diseases (n = 66) |
| Amati et al[28] | Epithelioid type (n =11)<br>Sarcomatoid type (n = 6)<br>Other or not specified (n = 5) | Healthy controls without asbestos exposure (n = 54)<br>Subjects with asbestos exposure (n = 94) |
| Shiomi et al[29] | Epithelioid type (n = 21)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 9) | Patients with benign asbestos-related diseases and healthy controls with asbestos exposure (n = 201)<br>Patients with lung cancer (n = 45)<br>Others (n = 8) |
| Iwahori et al[30] | Epithelioid type (n = 13) | Healthy controls without asbestos exposure (n = 38) |

| | | |
|---|---|---|
| | Sarcomatoid type (n = 3)<br>Other or not specified (n = 11) | Healthy controls with asbestos exposure (n = 9)<br>Patients with lung cancer (n = 47)<br>Patients with other cancers (n = 35) |
| van den Heuvel[31] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 10)<br>Other or not specified (n = 20) | Healthy controls (n = 50)<br>Patients with lung cancer (n = 106) |
| Creaney et al[32] | Epithelioid type (n = 57)<br>Sarcomatoid type (n = 9) | Healthy controls without asbestos exposure (n = 10)<br>Healthy controls with asbestos exposure (n = 10)<br>Patients with benign asbestos-related diseases (n = 21)<br>Patients with lung cancer (n = 10) |
| Schneider et al[33] | Epithelioid type (n = 81)<br>Sarcomatoid type (n = 14)<br>Other or not specified (n = 34) | Patients with lung cancer (n = 139)<br>Patients with benign asbestos-related diseases (n = 75) |
| Davies et al[34] | Epithelioid type (n = 11)<br>Sarcomatoid type (n = 5)<br>Other or not specified (n = 8) | Patients with nonmesothelioma malignancy (n = 67)<br>Patients with benign pleural effusion (n = 75) |
| Rodriguex Portal et al[35] | Not specified (n = 36) | Healthy controls (n = 48)<br>Patients with asbestos exposure and no pleural disease (n = 177) |

| | | |
|---|---|---|
| | | Patients with benign asbestos-related diseases (n = 101) |
| Fujimoto et al[36] | Epithelioid type (n = 15)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 4) | Patients with lung cancer (n = 38)<br>Patients with benign asbestos pleurisy (n = 26)<br>Patients with tuberculosis pleurisy (n = 5)<br>Patients with no pleural diseases (n = 4) |
| Hollevoet et al[36] | Epithelioid type (n = 73)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Healthy controls (n = 101)<br>Healthy controls with asbestos exposure (n = 89)<br>Patients with benign asbestos-related diseases (n = 123)<br>Patients with benign respiratory diseases (n = 46)<br>Patients with lung cancer (n = 63) |
| Creaney et al[38] | Epithelioid type (n = 59)<br>Other or not specified (n = 7) | Patients with benign asbestos-related diseases (n = 47)<br>Patients with benign respiratory diseases (n = 42) |
| Cristaudo et al[39] | Epithelioid type (n = 31) | Healthy controls (n = 93)<br>Patients with benign respiratory diseases (n = 111) |
| Dipalma et al[40] | Epithelioid type (n = 29)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 3) | Healthy controls without asbestos exposure (n = 109)<br>Healthy controls with asbestos exposure (n = 26)<br>Patients with benign asbestos-related diseases (n = 48)<br>Patients with benign respiratory diseases (n = 110) |

| | | |
|---|---|---|
| | | Patients with lung cancer (n = 25) |
| Yamada et al[41] | Epithelioid type (n = 37)<br>Sarcomatoid type (n = 5)<br>Other or not specified (n = 3) | Patients with non-malignant pleural effusions (n = 24)<br>Patients with lung cancer (n = 29) |
| Ashour et al[42] | Not specified (n = 38) | Healthy controls with asbestos exposure (n = 32)<br>Patients with benign pleural diseases (n = 29)<br>Patients with pleural carcinomas (n = 24) |
| Blanquart et al[43] | Epithelioid type (n = 49)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 8) | Patients with adenocarcinoma effusions (n = 25)<br>Patients with benign pleural effusions (n = 15) |
| Amany et al[44] | Epithelioid type (n = 14)<br>Sarcomatoid type (n = 4)<br>Other or not specified (n = 2) | Patients with benign asbestos pleural effusions (n = 10)<br>Patients with tuberculosis pleural effusions (n = 10) |
| Canessa et al[45] | Epithelioid type (n = 35)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignant effusions (n = 94)<br>Patients with benign pleural effusions (n = 129) |
| Creaney et al[46] | Epithelioid type (n = 32)<br>Sarcomatoid type (n = 9) | Patients with non-MPM malignant effusions (n = 39)<br>Patients with benign disease (n = 37) |

| | | |
|---|---|---|
| | Other or not specified (n = 25) | Patients with chronic kidney disease (n = 53)<br>Healthy controls without asbestos exposure (n = 18) |
| Ferro et al[47] | Epithelioid type (n = 26)<br>Sarcomatoid type (n = 9)<br>Other or not specified (n = 8) | Patients with non-MPM malignancy (n = 23)<br>Patients with benign diseases (n = 36) |
| Filiberti et al[48] | Epithelioid type (n = 43)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 2) | Patients with malignant effusions (n = 64)<br>Patients with benign effusions (n = 56) |
| Hooper et al[49] | Epithelioid type (n = 23)<br>Sarcomatoid type (n = 3)<br>Other or not specified (n = 2) | Patients with non-MPM malignant effusions (n = 74)<br>Patients with benign asbestos-related effusions (n = 13)<br>Patients with benign pleural diseases (n = 100) |
| Bayram et al[50] | Not specified (n = 24) | Patients with pleural plaques (n = 279)<br>Healthy controls with asbestos exposure (n = 123)<br>Healthy controls without asbestos exposure (n = 120) |

MPM = malignant pleural mesothelioma.

## STARD checklist for reporting of studies of diagnostic accuracy

*(version January 2003)*

| Section and Topic | Item # | | On page # |
|---|---|---|---|
| TITLE/ABSTRACT/ KEYWORDS | 1 | Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity'). | 0-2 |
| INTRODUCTION | 2 | State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups. | 7,8 |
| METHODS | | | |
| *Participants* | 3 | The study population: The inclusion and exclusion criteria, setting and locations where data were collected. | 11 |
| | 4 | Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard? | 12 |
| | 5 | Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected. | 12 |
| | 6 | Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)? | 12 |
| *Test methods* | 7 | The reference standard and its rationale. | 12 |
| | 8 | Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard. | 12 |
| | 9 | Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard. | 11,12 |
| | 10 | The number, training and expertise of the persons executing and reading the index tests and the reference standard. | N/A |
| | 11 | Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers. | 12 |
| *Statistical methods* | 12 | Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals). | N/A |
| | 13 | Methods for calculating test reproducibility, if done. | N/A |
| RESULTS | | | |
| *Participants* | 14 | When study was performed, including beginning and end dates of recruitment. | N/A |
| | 15 | Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms). | 11 |
| | 16 | The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended). | N/A |
| *Test results* | 17 | Time-interval between the index tests and the reference standard, and any treatment administered in between. | N/A |
| | 18 | Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition. | 12 |
| | 19 | A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard. | 12-15 |
| | 20 | Any adverse events from performing the index tests or the reference standard. | N/A |
| *Estimates* | 21 | Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals). | 14,15 |
| | 22 | How indeterminate results, missing data and outliers of the index tests were handled. | N/A |
| | 23 | Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done. | 14,15 |
| | 24 | Estimates of test reproducibility, if done. | N/A |
| DISCUSSION | 25 | Discuss the clinical applicability of the study findings. | 18,19 |