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REVIEW ARTICLE

Serum Mesothelin for Diagnosing Malignant Pleural Mesothelioma: An Individual Patient Data Meta-Analysis

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ABSTRACT

Purpose
Mesothelin is currently considered the best available serum biomarker of malignant pleural mesothelioma. To examine the diagnostic accuracy and use of serum mesothelin in early diagnosis, we performed an individual patient data (IPD) meta-analysis.

Methods
The literature search identified 16 diagnostic studies of serum mesothelin, measured with the Mesomark enzyme-linked immunosorbent assay. IPD of 4,491 individuals were collected, including several control groups and 1,026 patients with malignant pleural mesothelioma. Mesothelin levels were standardized for between-study differences and age, after which the diagnostic accuracy and the factors affecting it were examined with receiver operating characteristic (ROC) regression analysis.

Results
At a common diagnostic threshold of 2.00 nmol/L, the sensitivities and specificities of mesothelin in the different studies ranged widely from 19% to 68% and 88% to 100%, respectively. This heterogeneity can be explained by differences in study population, because type of control group, mesothelioma stage, and histologic subtype significantly affected the diagnostic accuracy. The use of mesothelin in early diagnosis was evaluated by differentiating 217 patients with stage I or II epithelioid and biphasic mesothelioma from 1,612 symptomatic or high-risk controls. The resulting area under the ROC curve was 0.77 (95% CI, 0.73 to 0.81). At 95% specificity, mesothelin displayed a sensitivity of 32% (95% CI, 26% to 40%).

Conclusion
In patients suspected of having mesothelioma, a positive blood test for mesothelin at a high-specificity threshold is a strong incentive to urge further diagnostic steps. However, the poor sensitivity of mesothelin clearly limits its added value to early diagnosis and emphasizes the need for further biomarker research.

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INTRODUCTION

Malignant mesothelioma is an asbestos-related malignancy, predominantly arising from the surface serosal cells of the pleura and, to a lesser extent, the peritoneum, pericardium, and tunica vaginalis. The three main histologic subtypes of mesothelioma are epithelioid (60%), sarcomatoid (10%), and biphasic (30%), which combines epithelioid and sarcomatoid features.[1] Reported incidences of mesothelioma vary worldwide and are approximately nine per million inhabitants in the United States, 40 per million inhabitants in Australia, and 20 per million inhabitants in Europe, with large between-country differences.[2] In most developing countries, epidemiologic data are either unavailable or under-reported.[3] In developed countries, peak incidences are expected to occur within the next decade or have been reached already, for example in the United States.[2] Mesothelioma, nevertheless, will remain a global health issue for future generations because of the continued use of asbestos in some developing countries, the environmental asbestos exposure, and the long latency period (typically > 30 years) of this malignancy.

Mesothelioma primarily occurs in the older population, and patients currently face a poor prognosis. Current therapeutic options are limited, and mesothelioma is still considered fatal.[4] The natural history results in a median survival of 7 to 9

months.[2] When treated with standard of care chemotherapy, cisplatin and an antifolate (pemetrexed or raltitrexed), median survival is approximately 1 year.[5,6] Highly selected patients with early-stage epithelioid disease, treated with extrapleural pneumonectomy, alone or in combination with chemotherapy and/or radiation therapy, have a median survival of up to 2 years.[7]

Patients with malignant pleural mesothelioma typically present with symptoms of an underlying pleural effusion, including dyspnea and chest pain.[1] The initial diagnostic procedures involve a chest x-ray or computed tomography scan and pleural fluid cytology. The latter may be reliable in experienced hands,[8] but a definitive diagnosis typically requires the histologic analysis of pleural biopsies, obtained during thoracoscopy.[1,2] Because of the nonspecific presenting symptoms and insidious development of the tumor, mesothelioma is often diagnosed at an advanced stage.[1] Because early diagnosis and subsequent intervention are thought to improve disease outcome, there is a critical need for reliable and noninvasive tools that shorten this diagnostic delay.

Because of their noninvasive feature and relative inexpensiveness, serum tumor biomarkers are an attractive adjunct to this purpose. Serum mesothelin, previously referred to as soluble mesothelin-related protein, is currently the most studied and is considered the best available blood protein biomarker of mesothelioma. The mesothelin gene (*MSLN*) encodes a 69-kDa precursor protein, which is cleaved into a soluble 31-kDa fraction, megakaryocyte potentiating factor, and a membrane-bound 40-kDa glycoprotein, mesothelin.[9] The latter is a differentiation antigen that is normally present on the mesothelial cells lining the pleura, peritoneum, and pericardium but is highly expressed in mesothelioma (limited to the epithelioid tumor cells) and some other malignancies, including ovarian and pancreatic cancer.[9] Mesothelin has three presumed isoforms that can enter the circulation, either by shedding of the membrane-bound portion (variants 1 and 2) or by a frameshift mutation (variant 3; Appendix Fig A1, online only).[10,11] Serum mesothelin refers to all isoforms that are present in the circulation, although variant 1 is predominantly expressed and released from the membrane.[11] In 2003, Robinson et al[12] were the first to report serum mesothelin as a biomarker of mesothelioma, using an enzyme-linked immunosorbent assay (ELISA) that detects both variants 1 and 3. This assay became later commercialized as Mesomark (Fujirebio Diagnostics, Malvern, PA) and was approved in 2007 by the US Food and Drug Administration to aid in the monitoring of patients with epithelioid and biphasic mesothelioma.[13]

Serum mesothelin could be an added value to the current diagnostic process if it proves to shorten the diagnostic delay of mesothelioma and lead to an earlier diagnosis. However, despite numerous published studies, the diagnostic use of mesothelin remains under debate. To examine the diagnostic accuracy of mesothelin in the available studies and elucidate whether this biomarker can be an adjunct to an earlier diagnosis of mesothelioma, we performed an individual patient data (IPD) meta-analysis.

METHODS

Search Strategy and Study Selection

MEDLINE (PubMed database) and EMBASE were searched for studies between 2003, when the first study was published,[12] and July 2010 with the following keywords: "mesothelioma" and "mesothelin." In addition, the references of all publications were manually searched. Meeting abstracts were excluded because of their limited data. Only studies published in peer-reviewed journals and written in English were evaluated for eligibility. To avoid heterogeneity caused by different assay platforms, only studies that measured mesothelin in serum with the commercial Mesomark ELISA kit (Fujirebio Diagnostics) were included. To be eligible, studies also had to include patients with malignant pleural mesothelioma (patient cases) and one or more control groups. Investigators of all eligible studies were invited to join the Mesothelin Collaboration and share the IPD of their patient cases and participants in one of the following five control groups: healthy individuals without asbestos exposure; individuals with reported asbestos exposure and no obvious asbestos-related lesions; individuals with a benign asbestos-related disease; individuals with a benign nonasbestos-related respiratory disease; and individuals with lung cancer. IPD included the serum mesothelin levels (measured in nanomoles per liter), age, and sex of each study participant. In controls, type of control group and, in cases, tumor stage (I or II *v* III or IV) and histologic subtype (epithelioid, sarcomatoid, or biphasic) were also collected.

Study Quality Assessment

All eligible studies were assessed for methodologic quality using an adapted version of the Quality Assessment of Diagnostic Accuracy Studies tool.[14] Each report was evaluated using the following four questions, answered with yes or no: (1) Were the controls representative and well defined? (2) Were the patient cases representative and well defined? (3) Were mesothelin levels measured, blinded to the sample data? (4) Were mesothelin levels measured in the same laboratory? Question 1 was answered positive if the absence of asbestos exposure in healthy individuals, the absence of asbestos-related disease in healthy asbestos-exposed individuals, and the presence of benign asbestos-related diseases were adequately evaluated. Question 2 was scored as positive if patients with mesothelioma were diagnosed according to the reference standards (histopathology or cytology) and were enrolled before any anticancer treatment and if tumor stage and histologic subtype were reported. Questions 1 and 2 also evaluated whether the inclusion of participants was random or consecutive and thus free of selection bias. Questions 3 and 4 were answered positive if measurement bias and between-laboratory variance were avoided.[15] If a question could not be answered with the data available in the study, the corresponding author was contacted.

Statistical Analyses

To evaluate whether the distribution of mesothelin levels differed between studies, both in controls and patient cases, the Kruskal-Wallis test was applied. The correlation between age and mesothelin levels of controls was evaluated with the Spearman rank test. To document the between-study heterogeneity in diagnostic accuracy of mesothelin, sensitivity and specificity were determined in each study at a threshold of 2.00 nmol/L, which was arbitrarily chosen from the previously reported diagnostic thresholds.[16] The resulting pairs of sensitivity and specificity were meta-analyzed using the bivariate model with a random effects approach, to obtain summary estimates and 95% prediction intervals.[17] These predictions intervals show how the sensitivity and specificity of mesothelin are expected to vary in a new study that is comparable in design to the studies included in the meta-analysis. Such a prediction interval is centered at the summary estimate of the sensitivity or specificity, and its width accounts for the uncertainty of the summary estimate, the estimate of between-study variance in true sensitivities or specificities, and the uncertainty in the between-study standard variance estimate itself. The width of these 95% prediction intervals consequently aids to interpret the amount of between-study heterogeneity in the sensitivity and specificity of mesothelin.[18] All further analyses were based on IPD and used mesothelin as a continuous variable. Receiver operating characteristic (ROC) regression analysis was performed to examine the diagnostic accuracy of mesothelin and the factors affecting it.[19] Before ROC regression, mesothelin levels were standardized for differences in age between patient cases and controls within studies and for differences in the mesothelin levels in controls between studies.[19] The resulting regression coefficients were used to fit the ROC curves, of which the area under the curves (AUC), sensitivity, specificity, and likelihood ratios (LRs) could be derived.[20,21] Bootstrap resampling was performed (1,000 resamples) to obtain

the 95% CIs. The hierarchical nature of the data was preserved by first comparing mesothelin levels between controls and patient cases in each study. Controls of each study were consequently only included in the model if the appropriate patient cases were present, and vice versa. Each group of patient cases or controls had to contain at least 10 individuals. All hypothesis tests were performed two-sided at the 5% significance level. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Study Selection

The electronic search identified 189 studies in EMBASE and 119 studies in MEDLINE, resulting in a total of 187 unique studies (Appendix Fig A2, online only). After evaluating the inclusion criteria in title and abstract, 167 studies were excluded, and 20 studies were obtained for full-text evaluation. One study was excluded because of duplicate data,[15] and three studies were excluded for using an ELISA other than Mesomark to measure serum mesothelin levels.[12,22,23] One study reported mesothelin levels in plasma,[24] but the corresponding author provided the matching serum levels. As such, a total of 16 studies were included in the meta-analysis[16,24-38]; for all of these studies, the corresponding authors agreed to share IPD. One research group published four studies,[25,27,29,33] and another research group published three studies.[26,28,36] After consulting the investigators, their IPD were pooled into one data set per research group, thus excluding duplicate data. This resulted in a total of 11 IPD sets (Table 1).

Study Methodology

Question 1 on representative and well-defined controls was negatively scored in two studies,[32,34] because the absence of previous asbestos exposure in healthy controls was not ascertained (Appendix Table A1, online only). In the other studies, this was done with a questionnaire. In all asbestos-exposed controls, the presence of a benign asbestos-related condition was radiologically evaluated. Question 2 on representative and well-defined patient cases was negatively scored in six reports, because histologic subtype and, more frequently, tumor stage were not available (Appendix Table A1).[25,27,33,24,37,38] One report included a number of patients with mesothelioma with recurrent disease.[35] These patients were excluded from the IPD. All 16 studies reported the use of histopathology to diagnose mesothelioma,[16,24-38] although six of them also used cytology.[25,27,29,31,33,37] In two of these studies, cytology was only applied in a small number of patients (five [5%] of 111 patients[31] and one [3%] of 36 patients[37]). In the four other studies,[25,27,29,33] published by the same research group, 89 (36%) of 249 patients had a cytology-based diagnosis. Staging was done in accordance with the TNM scoring system of the International Mesothelioma Interest Group[39] or the earlier classifications of the Union for International Cancer Control.[40] All participants were included in a random or consecutive manner. Questions 3 and 4 on the blinded mesothelin analysis of the samples and the avoidance of interlaboratory variance, respectively, were positively scored in all reports (Appendix Table A1).

Study Population

The IPD of a total of 4,491 participants were obtained; 1,026 patients had malignant pleural mesothelioma and 3,465 participants were controls, including 909 healthy individuals, assumed without asbestos exposure, 775 healthy individuals with reported asbestos exposure, 736 patients with a benign asbestos-related disease (pleural plaques, diffuse pleural thickening, or asbestosis), 267 patients with a benign nonasbestos-related respiratory disease (asthma, chronic obstructive pulmonary disease, or pleural effusion), and 778 patients with lung cancer (non–small-cell and small-cell histology). The size and distribution of these control groups substantially differed across the studies. Healthy individuals, with or without asbestos exposure, were significantly younger than the other groups ($P < .001$; Table 1).

Table 1. Studies and Participants Included in the Meta-Analysis

Study and Age	Control Groups (No. of participants)					Malignant Pleural Mesothelioma (No. of participants)	Total Participants	
	Healthy	Healthy Asbestos Exposed	Benign Asbestos-Related Disease	Benign Respiratory Disease	Lung Cancer		No.	%
Creaney et al[25,27,29,33*]	38	79	121	184	47	249	718	16
Scherpereel et al[26]; Grigoriu et al[28,36*]	—	113	32	—	—	96	241	5
Di Serio et al[30]	109	26	66	10	30	24	265	6
Cristaudo et al[31]	65	203	122	27	215	111	743	17
van den Heuvel et al[32]	50	—	—	—	110	74	234	5
Amati et al[24]	54	85	33	—	—	22	194	4
Pass et al[34]	409	4	62	—	174	90	739	17
Schneider et al[35]	—	—	75	—	139	100	314	7
Rodriguez Portal et al[37]	48	176	102	—	—	36	362	8
Hollevoet et al[16]	101	89	123	46	63	85	507	11
Rai et al[38]	35	—	—	—	—	139	174	4
Total								
No.	909	775	736	267	778	1,026	4,491	
%	20	17	17	6	17	23		
Age, years								
Median	56	54	63	65	65	66	62	
Interquartile range	49-66	50-60	57-70	53-75	58-72	59-72	54-50	

*Studies were grouped into a single data set per research group.

Table 2. Characteristics of the Patients With Malignant Pleural Mesothelioma (n = 1,026)

Histologic Subtype and Tumor Stage	No. of Patients	%
Epithelioid		54
I-II	186	
III-IV	237	
NOS	133	
Sarcomatoid		8
I-II	17	
III-IV	28	
NOS	37	
Biphasic		12
I-II	56	
III-IV	34	
NOS	31	
NOS		26
I-II	7	
III-IV	11	
NOS	249	

Abbreviation: NOS, not otherwise specified.

Seventy percent of all participants were men, 15% were women, and data were missing for 15% of participants. Mesothelin levels were available from each participant, whereas data on age, mesothelioma tumor stage, and histologic subtype were missing in 18%, 44%, and 26% of participants, respectively. The majority of the patient cases had epithelioid and advanced stage III or IV mesothelioma (Table 2). Of the patients who lacked data on tumor stage, histologic subtype, or both, the diagnosis was cytology based in none of 201, four (22%) of 18, and 91 (37%) of 249 patients, respectively.

Between-Study Heterogeneity

Mesothelin levels differed significantly both within the controls ($P < .001$) and patient cases ($P < .001$) of the different studies. In controls, median mesothelin levels varied between 0.34 nmol/L (interquartile range [IQR], 0.23 to 0.53 nmol/L)[38] and 0.95 nmol/L (IQR, 0.73 to 1.26 nmol/L),[16] whereas in patient cases, median levels ranged between 0.80 nmol/L (IQR, 0.47 to 1.66 nmol/L)[37] and 3.41 nmol/L (IQR, 1.62 to 11.73 nmol/L).[24] The reported diagnostic thresholds of mesothelin to differentiate controls from patient cases varied widely between 0.93 nmol/L[26] and 2.50 nmol/L.[27] When applying a common threshold of 2.00 nmol/L, the resulting sensitivities in the different studies ranged from 19%[37] to 68%[24] (summary estimate, 47%), whereas the specificities varied from 88%[25,27,29,33] to 100%[38] (summary estimate, 96%; Fig 1). Similarly, the 95% prediction intervals of the sensitivity and specificity of mesothelin ranged widely from 26% to 70% and from 85% to 99%, respectively. Altogether, these findings reflected substantial between-study heterogeneity in the diagnostic accuracy of mesothelin.

ROC Regression Analysis

Besides differing among studies, mesothelin levels of controls were also significantly correlated with age ($r = 0.24$; $P < .001$). Therefore, before the ROC regression analysis, mesothelin levels were standardized for these factors. Because of missing data on age, two complete study populations (n = 536),[37,38] a group of healthy controls (n = 409),[34] and a group of healthy asbestos-exposed individuals (n = 113)[26] were omitted from the ROC regression analysis. One group of healthy asbestos-exposed individuals was excluded because of its limited size (n = 4; Table 1).[34] In total, 2,578 (74%) of 3465 controls and 851 (83%) of 1,026 patients with mesothelioma were available for the ROC regression analysis. Results indicated that the type of control group had a significant effect on the diagnostic accuracy of mesothelin levels (Fig 2). The highest AUCs were observed for differentiating patient cases from the two groups of healthy controls, either with or without asbestos exposure (AUC, 0.84; 95% CI, 0.81 to 0.87). Overall, the differences between the AUCs in the four control groups with no malignant disease were relatively modest. The lowest AUC was obtained when differentiating patient cases from patients with lung cancer (AUC, 0.76; 95% CI, 0.73 to 0.79; Fig 2). In addition, tumor stage (I or II *v* III or IV) and histologic subtype (epithelioid, sarcomatoid, or biphasic) significantly affected the diagnostic accuracy of mesothelin (Table 3). The highest AUC was observed for differentiating patients with epithelioid stage III or IV mesothelioma from controls (AUC, 0.84; 95% CI, 0.82 to 0.86). The lowest AUC was obtained for sarcomatoid stage I or II mesothelioma (AUC, 0.56; 95% CI, 0.51 to 0.60).

Mesothelin in Early Diagnosis

The use of mesothelin in early diagnosis was examined by constructing a clinically relevant ROC regression model, again with respect to the hierarchical structure of the data. This model included 217 patients with stage I or II histologically proven epithelioid (n = 185) or biphasic (n = 32) mesothelioma and 1,612 symptomatic or high-risk controls, including 318 healthy asbestos-exposed individuals, 480 patients with a benign asbestos-related disease, 83 patients with a benign nonasbestos-related respiratory disease, and 731 patients with lung cancer. These 1,829 individuals were retrieved from nine studies (Appendix Table A2, online only).[16,26,28,30-32,34-36] Healthy individuals without asbestos exposure were not included, because the use of mesothelin in these individuals is not clinically relevant. When differentiating patient cases from controls, mesothelin levels displayed an AUC of 0.77 (95% CI, 0.73 to 0.81), representing the overall diagnostic performance. In addition, mesothelin was evaluated in the following two specific settings: as an adjunct to rule in (through a positive blood test) or to rule out (through a negative blood test) mesothelioma diagnosis. For a positive test to do so, a high-specificity threshold is typically required. Because mesothelin levels were standardized for between-study differences and age, no thresholds in nanomoles per liter could be derived. Therefore, we opted for a specificity of 95% (ie, a false-positive rate of one out of 20), which resulted in a sensitivity of 32% (95% CI, 26% to 40%) and a positive LR of 6.40. For a negative test result to aid in excluding diagnosis, a high-sensitivity threshold is generally required. At a selected sensitivity of 95%, specificity was 22% (95% CI, 15% to 29%), yielding a negative LR of 0.23. Using the associated LRs, Table 4 illustrates how a mesothelin blood test result shifts the post-test probability of mesothelioma in two hypothetical patients with a pretest probability of 25% and 50%, respectively.

DISCUSSION

Mesothelin is currently the most studied serum biomarker of malignant pleural mesothelioma. To examine the reported diagnostic accuracies and evaluate whether this biomarker can be an adjunct to an

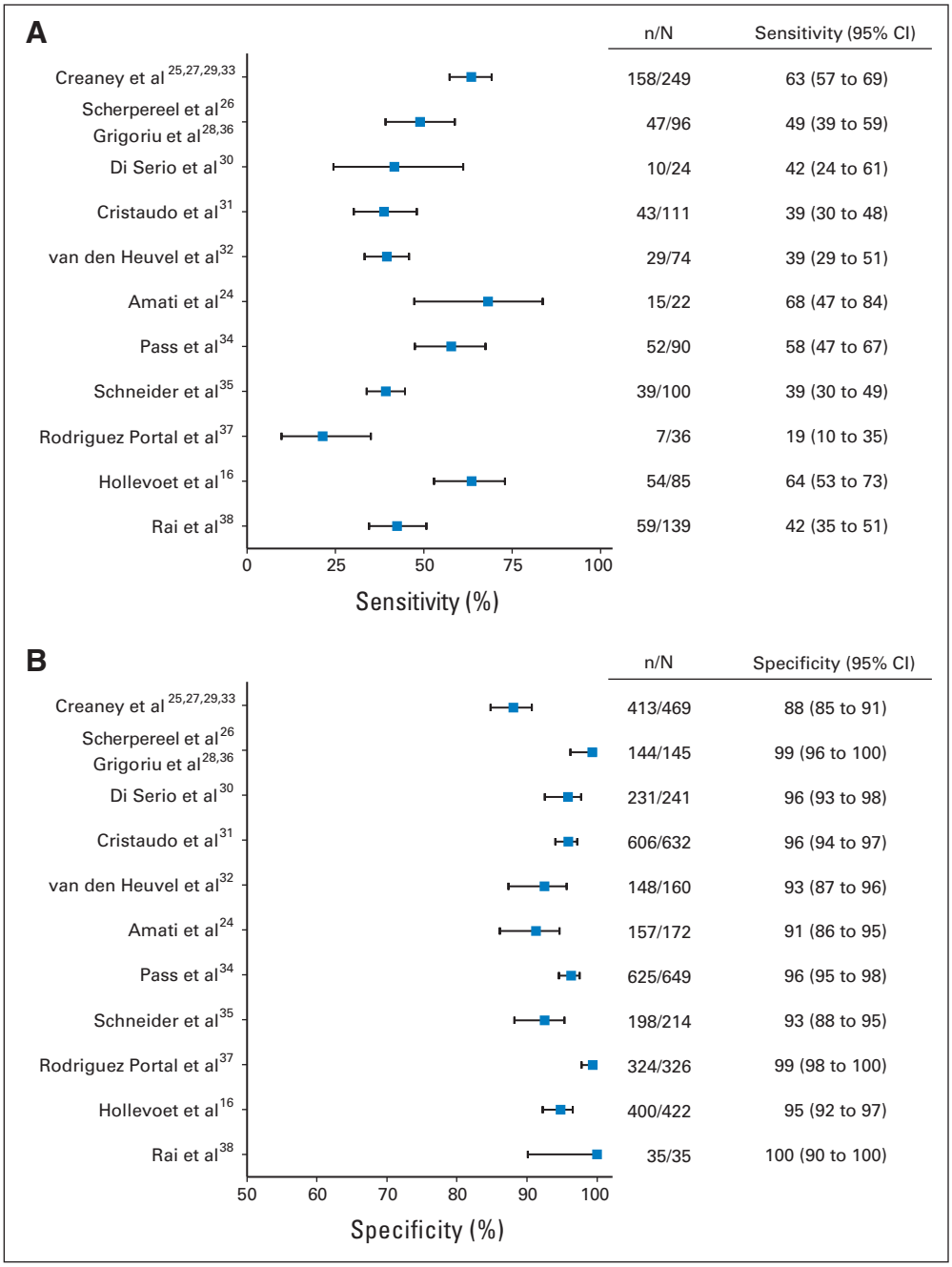
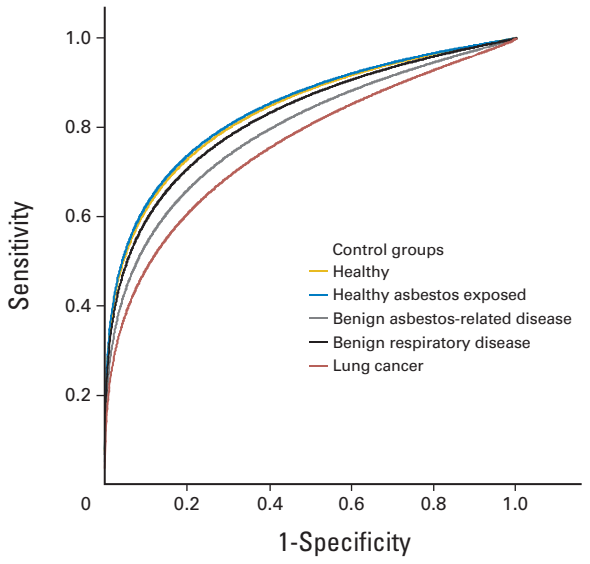


Fig 1. Forest plots of the (A) sensitivity and (B) specificity of serum mesothelin at a threshold of 2.00 nmol/L. (A) n, patients with mesothelioma with elevated mesothelin levels; N, all patients with mesothelioma. (B) n, controls with normal mesothelin levels; N, all controls. One research group published four studies,[25,27,29,33] and another research group published three studies[26,28,36]; thus, the studies from the same research group were grouped into a single data set per research group.

earlier diagnosis of mesothelioma, we performed an IPD meta-analysis on 16 published diagnostic studies, representing a total of 4,491 individuals.

On review, all studies had a good methodologic quality but did show large differences in number of participants, clinical characteristics (age, type of control group, mesothelioma stage, and histologic subtype), and reported diagnostic thresholds of mesothelin. In addition, clinically relevant information concerning mesothelioma stage and histology was often not available. Such heterogeneity cannot be adequately addressed in systematic reviews or meta-analyses based on aggregate mesothelin data.[41,42] Conducting an IPD meta-analysis allowed us to adequately quantify and address the observed between-study heterogeneity.

First, we evaluated how the diagnostic accuracy of mesothelin compared across studies. Interestingly, even when the large differences in the applied thresholds were eliminated, the sensitivity and specificity of mesothelin still displayed a substantial between-study heterogeneity. To gain more insight in the sources of this heterogeneity, we performed an ROC regression analysis. Before this analysis, mesothelin levels were standardized to account for the previously reported correlation with age[43] and between-laboratory differences.[15] Subsequent results showed that the between-study heterogeneity in diagnostic accuracy can be explained by differences in type of control group, mesothelioma stage, and histologic subtype. Mesothelin levels better differentiated patients with mesothelioma from controls without a malignancy than from those with lung cancer, whereas controls



Control groups	AUC (95% CI)	AUC compared with healthy
Healthy	0.84 (0.81 to 0.87)	—
Healthy asbestos exposed	0.84 (0.81 to 0.87)	$P = .91$
Benign asbestos-related disease	0.80 (0.77 to 0.82)	$P < .05$
Benign respiratory disease	0.82 (0.79 to 0.86)	$P = .53$
Lung cancer	0.76 (0.73 to 0.79)	$P < .01$

Fig 2. Receiver operating characteristic curves of standardized serum mesothelin levels for differentiating each control group from patients with malignant pleural mesothelioma. AUC, area under the curve.

Table 3. AUC of Standardized Serum Mesothelin Levels for Differentiating All Control Groups From Patients With Malignant Pleural Mesothelioma, Stratified According to Histology and Tumor Stage

Histologic Subtype and Tumor Stage	AUC	95% CI
Epithelioid		
I-II	0.79	0.77 to 0.81
III-IV	0.84*	0.82 to 0.86
Sarcomatoid		
I-II	0.56†	0.51 to 0.60
III-IV	0.64*†	0.60 to 0.68
Biphasic		
I-II	0.70†‡	0.66 to 0.75
III-IV	0.77*†‡	0.73 to 0.80

Abbreviation: AUC, area under the curve.
*$P < .001$ when differentiating stage III and IV patients from stage I and II patients in each histologic subtype.
†$P < .001$ when differentiating patients from the epithelioid subtype in the associated tumor stage.
‡$P < .001$ when differentiating patients from the sarcomatoid subtype in the associated tumor stage.

were better discriminated from patients with advanced epithelioid or biphasic mesothelioma than from those with early-stage or sarcomatoid disease. These results confirmed the findings of individual studies and are the consequence of mesothelin overexpression in some lung cancers, a lack of mesothelin expression in nonepithelioid mesothelioma, and the association of mesothelin levels with tumor burden.[9,12] Altogether, studies that include predominantly young healthy controls and older patients with an epithelioid histology and a more advanced disease are likely to report a high diagnostic accuracy of mesothelin, and vice versa.

To confine such over- or underestimation to a minimum, the use of mesothelin in early diagnosis was evaluated in a clinically relevant model, in which symptomatic or high-risk controls were differentiated from patients with stage I or II epithelioid and biphasic mesothelioma. Although the resulting AUC of mesothelin was acceptable, this value merely provides an indication of its overall diagnostic performance and is of little relevance towards its actual use in clinical practice.[20] A mesothelin blood test would especially be useful if it allows clinicians to efficiently steer further diagnostic steps and shorten the current diagnostic delay of mesothelioma. Therefore, we evaluated mesothelin in the following two specific clinical settings: as an adjunct to rule in (through a positive blood test) or to rule out (through a negative blood test) the diagnosis of early-stage mesothelioma. For a negative mesothelin test to aid in excluding mesothelioma, the use of a high-sensitivity threshold (typically in the range of 1.00 to 1.50 nmol/L) is a first requirement. However, our results indicated that at a sensitivity of 95% for mesothelioma, more than 75% of the controls would have false-positive test results, leading to an inordinate number of individuals undergoing unnecessary diagnostic work-ups or biopsies. This is especially relevant for mesothelioma, because this malignancy has a low prevalence, even in populations at risk. As a result, a negative mesothelin test cannot serve as an adjunct in excluding mesothelioma diagnosis, even at a high-sensitivity threshold. For a positive mesothelin test to serve as an adjunct to include diagnosis, the use of a high-specificity threshold (likely in the range of 2.00 to 2.50 nmol/L) is typically required. At a specificity of 95%, however, we found that approximately 70% of patients with early-stage epithelioid or biphasic mesothelioma would remain undetected—an unacceptably high proportion. Although a positive mesothelin test at a high-specificity threshold presents a strong incentive to urge ensuing diagnostic steps (eg, thoracoscopy), its poor sensitivity clearly limits the added value to early diagnosis.

Different approaches can be pursued and combined to anticipate this limited accuracy of serum mesothelin. First, clinicians could use sequential mesothelin measurements to monitor symptomatic patients for marked changes in their blood levels, rather than solely rely on a single mesothelin measurement and a fixed diagnostic threshold.[44] When comparing the latter approach with a longitudinal algorithm, a recent retrospective study indeed saw an increase in sensitivity for mesothelioma from 16% to 40%.[45] Second, accounting for clinical characteristics that affect serum mesothelin levels, like age, glomerular filtration rate, and body mass index, might also improve the performance of this biomarker.[43] Third, the current gold standard for the measurement of serum mesothelin, the Mesomark ELISA, should be critically looked at. In addition to challenging this assay with previously developed mesothelin ELISAs,[22,23] the development of more sensitive antibodies is also of interest. Fourth, the quest for more accurate biomarker panels should be pursued. Given the heterogeneity of mesothelioma, it is indeed plausible that a single biomarker

Table 4. Effect of a Serum Mesothelin Test Result on the Post-Test Probability of Malignant Pleural Mesothelioma in Two Hypothetical Patients With Different Pretest Probabilities

Model and Mesothelin Test Result	Likelihood Ratio	Post-Test Probability (%)	
		At 25% Pretest Probability	At 50% Pretest Probability
217 patients with stage I or II epithelioid or biphasic mesothelioma *v* 1,612 controls*			
Positive†	6.40	68	86
Negative‡	0.23	7	19

*Controls include healthy asbestos-exposed individuals and patients with a benign asbestos-related disease, a benign nonasbestos-related respiratory disease, or lung cancer.
†Using a 95% specificity threshold.
‡Using a 95% sensitivity threshold.

cannot provide the necessary sensitivity and specificity for clinical practice. However, none of the studied combinations with mesothelin, including megakaryocyte potentiating factor, osteopontin, carcinoembryonic antigen, CA-125, cytokeratins, and hyaluronic acid, so far managed to substantially outperform mesothelin.[16,27,32,33,36] Further biomarker research could therefore specifically focus on patients who lack elevated mesothelin levels. For any candidate biomarker (or combination) of mesothelioma, it will be essential to evaluate its accuracy in direct comparison with mesothelin in a sufficiently large study population including relevant controls and patients with early-stage mesothelioma. Serum mesothelin is currently also under investigation in other fields of mesothelioma management, including monitoring therapy response and estimating patient prognosis.[43,46-49] It is obvious that further biomarker research is equally relevant for these fields.

Our meta-analysis has some limitations that require consideration. First, IPD was collected from 4,491 individuals, but the ROC regression analyses were performed on smaller groups of participants because of missing data on age, tumor stage, and histologic subtype. Second, cytology was used in a number of studies to diagnose mesothelioma. This approach is typically considered to have a high risk of diagnostic error,[2] though experienced centers report reliable results.[8] Although the controversy remains, the consequences for our meta-analysis were limited, because only a small number of the patients with mesothelioma (9%) were actually diagnosed with cytology. In addition, these patients lacked data on tumor stage and histology and, therefore, were not included in most of our analyses. Third, we cannot exclude the possibility of a positive publication bias (ie, negative studies on mesothelin that never got published). Fourth, other factors that affect serum mesothelin levels, such as glomerular filtration rate and body mass index,[43] were not accounted for, because these were not reported in the original studies. Future studies on mesothelin are strongly encouraged to report all of these clinical characteristics to more efficiently match study participants and interpret the obtained results.

In conclusion, our IPD meta-analysis indentified the presence and the origin of a substantial between-study heterogeneity in the diagnostic accuracy of mesothelin and allowed to evaluate the use of mesothelin in a clinically relevant model. We found that, in symptomatic or high-risk individuals, a negative blood test for mesothelin is not a useful adjunct to exclude mesothelioma, even at a high-sensitivity threshold. Conversely, a positive blood test for mesothelin at a high-specificity threshold was found to be a strong incentive to urge further diagnostic steps and could possibly lead to an earlier diagnosis. However, the associated poor sensitivity of mesothelin for early mesothelioma clearly limits its added value to early diagnosis and emphasizes the need for further biomarker research.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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