

# Soluble Mesothelin-Related Peptide Level Elevation in Mesothelioma Serum and Pleural Effusions

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**Background.** Soluble mesothelin-related peptide (SMRP) is a potential marker for malignant pleural mesothelioma (MPM), which may be useful for screening high-risk asbestos-exposed individuals.

**Methods.** We evaluated SMRP in serum from MPM patients (n = 90), lung cancer patients (n = 170), age and tobacco-matched asbestos-exposed individuals (n = 66), and in MPM pleural effusions (n = 45), benign effusions (n = 30), and non-MPM effusions (n = 20) using the MesoMark enzyme-linked immunosorbent assay kit (Fujirebio Diagnostics, Malvern, PA). Receiver operating characteristic (ROC) curves were used to define true and false positive rates at various cutoffs.

**Results.** Mean serum SMRP levels were higher in MPM compared with lung cancer ( $5.67 \pm 0.82$  nM [mean  $\pm$  standard error of the mean vs  $1.99 \pm 0.43$  nM,  $p < 0.001$ ), and stage I MPM SMRP levels (n = 12;  $2.09 \pm 0.41$  nM) were significantly higher than those in asbestos-exposed

individuals ( $0.99 \pm 0.09$  nM,  $p = 0.02$ , respectively). Stage 2 to 4 SMRP serum levels were significantly higher than those for stage 1 MPM. The area under the ROC curve for serum SMRP was 0.81 for differentiating MPM and asbestos-exposed individuals; cutoff = 1.9 nM (sensitivity = 60%, specificity = 89%). The MPM pleural effusion SMRP was significantly higher than benign or other non-MPM pleural effusions ( $65.57 \pm 11.33$  nM vs  $27.46 \pm 11.25$  nM [ $p = 0.003$ ] and  $18.99 \pm 7.48$  nM [ $p = 0.044$ ], respectively).

**Conclusions.** These data support SMRP as a promising marker for MPM in both serum and pleural effusion fluid, and justify prospective screening studies of SMRP in combination with other markers for screening of asbestos-exposed cohorts.

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Malignant pleural mesothelioma (MPM) is an aggressive, asbestos-related tumor which is increasing in incidence and causes an estimated 15,000 to 20,000 deaths per annum worldwide. Malignant pleural mesothelioma has a median survival of seven to ten months and a clinical pattern that usually involves substantial pain and dyspnea. It presents at a clinically advanced stage in most patients so there is a need for new methods of early detection. The fact that asbestos is the main etiologic agent for MPM means that at-risk populations can be readily identified and studied, and these populations represent ideal cohorts in which to undertake early cancer detection studies.

There have been a number of studies attempting to define biomarkers that could predate symptoms in a “high risk for MPM” population and also distinguish MPM from other malignancies. Unfortunately, the majority of these studies have had very few patients of

various stages of MPM and the markers have not been prospectively evaluated. Some of these biomarkers include tissue polypeptide antigen, carcinoembryonic antigen, hyaluronic acid, and ferritin [1] as well as hyaluronic acid levels [2]. Other markers such as cytokeratins [3] and cancer antigen 125 (CA-125) [4] have been evaluated in MPM but have been inconclusive. One must conclude, therefore, that until recent reports of soluble mesothelin-related protein (SMRP) [5] and osteopontin [6] in MPM, there have been no reliable, validated serum or pleural effusion markers that can distinguish a high-risk, asbestos-exposed population without MPM from patients with established MPM, or to distinguish other malignancies from MPM.

The SMRP is related to the mesothelin family of molecules. Mesothelin is a 40-kD cell surface glycosylated phosphatidylinositol-anchored glycoprotein, which functions in cell-to-cell adhesion [7]. Data from our laboratory using serial analysis of gene expression revealed that the mesothelin levels were 49-fold increased over normal perito-

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neum [8]. Mesothelin is expressed by normal mesothelial cells [9]; however, it is highly overexpressed in cancers such as MPM [10, 11], pancreatic [12], or ovarian carcinoma [10, 13]. In 1999, Scholler and colleagues [14] described a monoclonal antibody 569, which binds to a 40-kDa molecule with an NH<sub>2</sub>-terminal sequence similar to mesothelin and is overexpressed in ovarian cancers and certain other tumors. Scholler and colleagues speculated that a mesothelin-like molecule must be released from tumor cells and demonstrated that this molecule was released into culture supernatants, sera, and malignant effusions from patients with certain tumors. The release of these soluble mesothelin-related proteins could be due to an abnormal splicing event resulting in a frameshift mutation of the protein, making it unable to stay attached at the cell surface. Another hypothesis for the origin of SMRP is that it represents a proteolytically cleaved fragment of membrane-bound mesothelin.

Evidence has been published indicating that an enzyme-linked immunosorbent assay using two antibodies to SMRP, OV569 and 4H3, holds promise for detection of MPM and that it favorably complements CA-125 for detection of ovarian carcinoma. Robinson and colleagues [5] were the first to investigate SMRP using a nonquantitative assay in the serum of MPM patients and a limited numbers of controls. He published that 37 (84%) of 44 patients with MPM had raised concentrations of SMRP compared with a limited number of non-MPM cancers (n = 30), asbestos-exposed individuals (n = 40), and nonasbestos-exposed individuals (n = 28). The SMRP was elevated in 1 of 30 cancers, 0 of 28 nonasbestos-exposed individuals, and 7 of 40 asbestos patients. In a recent report from Scherpereel and colleagues [7], serum SMRP level was significantly higher in patients with MPM than in subjects exposed to asbestos with pleural benign disease, or in those with metastatic disease to the pleura.

The Karmanos and New York University Thoracic Oncology Laboratories have had an interest in mesothelioma and has developed a large archive of serum, plasma, and pleural effusions from patients having surgery for the disease. As such, we felt it was important to corroborate the data regarding MESOMARK (Fujirebio Diagnostics, Malvern, PA) by defining the sensitivity and specificity of the marker in distinguishing patients with mesothelioma from patients exposed to asbestos but not having the cancer. By having a surgical archive with complete staging, we wanted to establish whether SMRP serum levels varied between early and late stage mesothelioma. Moreover, we wanted to define whether SMRP is elevated in other thoracic malignancies such as lung cancer. Finally, we felt that an investigation of SMRP levels as a biomarker for differentiating pleural malignancies was warranted.

## Patients and Methods

### Serum

Serum (n = 90) and pleural effusions (n = 45) were collected from mesothelioma patients having attempted cytoreduction under protocols between 1990 and 2005 at the National Cancer Institute, Bethesda, Maryland or at

the Karmanos Cancer Institute, Detroit, Michigan (Collection of Serum and Tissue Samples from Patients with Biopsy Proven or Suspected Malignant Disease, Human Investigation Committee 101399M1E). All Institutional Review Boards of the cooperating institutions approved the study, including the Center for Occupational and Environmental Medicine (MH, co-investigator). All patients or asbestos-exposed individuals, after giving consent, donated tumor, serum, and effusion either in the clinic or at the time of surgery, and volunteered information for an occupational/health history. For archival samples from the National Cancer Institute, Karmanos Cancer Institute waived the need for obtaining consent from those patients for this study who had already donated stored samples. A materials transfer agreement between the Karmanos Cancer Institute and Fujirebio Diagnostics allowed the use of the MESOMARK assay, 50 lung cancer sera, and age-matched normal sera.

Patients with mesothelioma were staged according to the International Mesothelioma Interest Group Staging System (IMIG). Sera from lung cancer patients having surgery at the Karmanos Cancer Institute (n = 120) and from the archives at Fujirebio Diagnostics (Malvern, PA [n = 50]), as well as serum from consenting normal individuals (n = 409) were analyzed for SMRP.

Clinical demographics including age, sex, histology, stage, time to recurrence, and survival time were available on patients, except for the lung cancer sera provided by Fujirebio for whom only sex and age were available. All specimens were obtained using the same model serum tubes and all sera were stored at -80°C.

Serum also was obtained from 66 consenting individuals with a history of asbestos exposure and (or) radiographic changes consistent with asbestosis at the Center for Occupational and Environmental Medicine, Royal Oak, Michigan from July 2004 to September 2004. Entry criteria for this cohort were similar to that described by Cullen and colleagues [15]. Briefly, exposure was documented by (1) employment in a trade with established regular asbestos exposure and published risk of asbestos-related diseases, or (2) occupational asbestos exposure

Table 1. Demographics of Patient Cohorts

Characteristics	MPM (n = 90)	Lung Cancer (n = 170)	Asbestos Exposed (n = 66)
Sex (M/F)	71/19	94/76	61/5
Age (years)	63 ± 1 (39-84)	66 ± 1 (33-87)	64 ± 1 (36-90)
Asbestos exposure	73/90 (81%)	NA	66/66 (100%)
Histology <sup>a</sup>			
Epithelial	58 (64%)	Adenocarcinoma (77)	—
Biphasic	29 (32%)	Squamous cell (40)	—
Sarcomatoid	3 (4%)	Small cell (3)	—

<sup>a</sup> Histology data available only on 120 of the 170 lung cancers.

MPM = malignant pleural mesothelioma.

Table 2. Soluble Mesothelin-Related Peptide (SRMP) Levels in Serum and Pleural Effusions (Mean ± Standard Error of the Mean)

Serum SMRP			
	MPM (n = 90)	Lung Cancer (n = 170)	Asbestos Exposed (n = 66)
Mean SMRP, nM	5.67 ± 0.82	1.99 ± 0.43	0.99 ± 0.10
Range	(0-32 nM)	(0-32 nM)	(0-32 nM)
Pleural Effusion SMRP			
	MPM (n = 45)	Other Cancers (n = 20)	Benign (n = 30)
Mean SMRP, nM	65.57 ± 11.33	27.46 ± 11.25	18.99 ± 7.48
Range	(0-255 nM)	(0-140 nM)	(0-151 nM)

MPM = malignant pleural mesothelioma.

in any job or occupation and evidence of radiographic changes consistent with a diagnosis of nonmalignant asbestos-related disease. These radiographic findings included (1) benign pleural disease, defined as thickening or fibrotic plaques on pleural surfaces of the lung bilaterally, and (or) (2) asbestosis, defined as diffuse lung scarring based on small irregular shadows bilaterally. Each consenting asbestos-exposed individual had a plain chest radiograph that was interpreted by a single trained B-reader (National Institute for Occupational Safety and Health [NIOSH] B Reader Program). The B-reader specifically commented on the presence of pleural changes including plaques as well as the presence in lung fibrosis. Lung fibrosis was interpreted according to the International Labor Organization scoring process [16].

### Pleural Effusions

Nonmesothelioma pleural effusions were obtained from 30 individuals with a benign etiology (postoperative day 3 effluent after benign thoracotomy or bypass [n = 20], pneumothorax treatment [n = 2], Wegeners granulomatosis, granulomatous disease, asbestosis, congestive heart failure, postinterleukin-2 effusion, sympathetic effusion, postchemoradiotherapy effusion, renal failure, [n = 1 each]), and from 20 individuals with nonmesothelioma malignant effusions (adenocarcinoma [n = 16]; lymphoma, breast, renal cell carcinoma, thymoma [1 each]). Effusions were processed by centrifugation with storage of the liquid component at -80°C.

### MESOMARK Assay

The MESOMARK assay was performed according to the manufacturer's instructions. Briefly, patient serum samples were diluted 1:101 using the assay diluent provided and 100 µL of the diluted samples were added in duplicates to a 96-well plate precoated with the 4H3 antibody. The samples were incubated on a plate-shaker for 60 minutes followed by a 5× rinse with wash buffer. The OV569-HRP conjugate was next added to the sample wells and the microwell plate incubated for a further 60 minutes on a plate-shaker. After a wash step, 100 µL of substrate was added to the reaction wells for 15 minutes before adding 100 µL of stop solution. The absorbance at 450 nm was used to quantify the SMRP levels by comparison to a six-point calibration curve. The MESOMARK values are expressed as nM (nanomolar).

### Statistical Analysis

Kaplan-Meier survival plots and log-rank tests were used to assess differences in survival for the MPM patients. All survival data were calculated from the date of the operation. The performance of SMRP in detecting MPM from

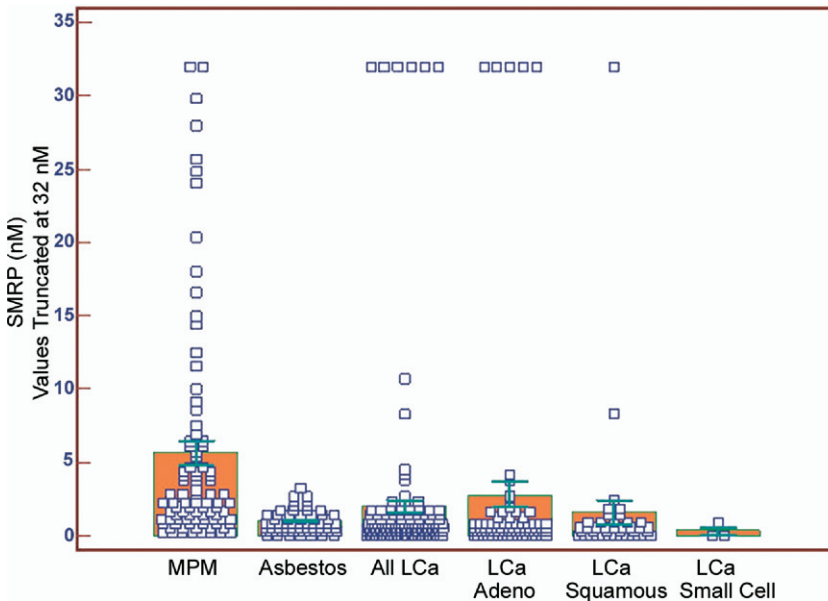


Fig 1. Soluble mesothelin-related peptide (SMRP) levels for groups studied. Bars represent mean ± standard error of the mean. (Adeno = adenocarcinoma; LCa = lung carcinoma; MPM = malignant pleural mesothelioma.)

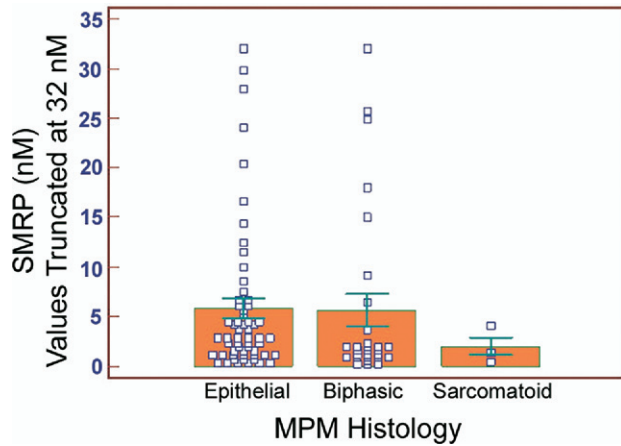


Fig 2. Soluble mesothelin-related peptide (SMRP) level for differing malignant pleural mesothelioma (MPM) histologies. Bars represent mean  $\pm$  standard error of the mean.

other groups was evaluated by descriptive statistics and by receiver operating characteristic (ROC) curves. The ROC curves defined the sensitivity and specificity for distinguishing between subgroups of patients with MPM and from the asbestos-exposed individuals and those with lung cancer [17, 18] by illustrating the true positive rate as a function of false positive rate at different cutoff points. The area under the ROC curve (AUC) was calculated, and 95% confidence intervals for the AUC were used to test the hypothesis that the theoretical area is 0.5. Areas under the curve whose confidence interval did not include the 0.5 value were considered evidence that the laboratory test had some ability to distinguish between the groups studied [18, 19]. As with any analyses using ROC curves, the AUC must for relevance had to be not only different than 0.5 but ideally significantly greater than 0.5. Differences between groups were calculated using the Kruskal-Wallis test and analysis of variance

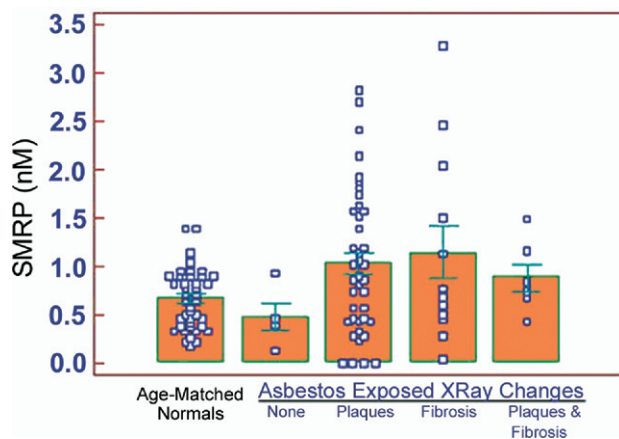
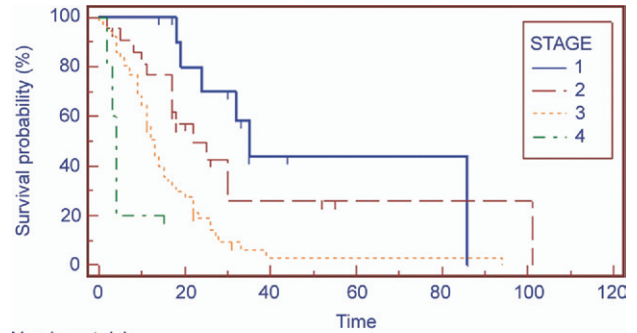


Fig 3. Soluble mesothelin-related peptide (SMRP) levels based on the radiographic characteristics of age- and sex-matched normal controls, not exposed to asbestos compared with asbestos exposed controls. Bars represent mean  $\pm$  standard error of the mean. See text for details.



Number at risk	0	20	40	60	80	100	120
Group: 1	12	8	2	1	1	0	0
Group: 2	22	8	3	1	1	1	1
Group: 3	50	13	1	1	1	0	0
Group: 4	5	0	0	0	0	0	0

Fig 4. Survival curves for all 90 malignant pleural mesothelioma patients in the study based on the International Mesothelioma Interest Group staging system reveals differences in survival based on stage of the patients.

(ANOVA). All statistical analyses were performed using MedCalc Software (Mariakerke, Belgium).

## Results

### SMRP in Mesothelioma and Controls and Influence of MPM Histology

The characteristics of the patients from whom serum was obtained are seen in Table 1. Serum SMRP levels were different among patients with MPM, lung cancer, and individuals exposed to asbestos ( $p < 0.0001$ , Kruskal-Wallis test; Table 2). The mean serum SMRP values for the 90 patients with MPM ( $5.67 \pm 0.82$  nM) was significantly higher than those for patients with lung cancer ( $1.99 \pm 0.43$  nM,  $p < 0.001$  by ANOVA) or from asbestos-exposed individuals ( $0.99 \pm 0.10$  nM,  $p < 0.001$  by

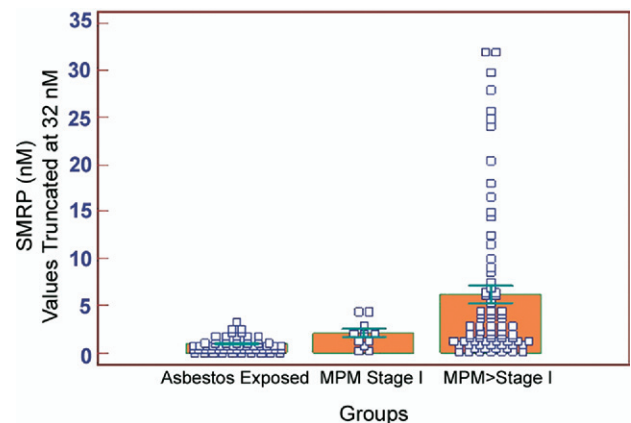


Fig 5. Soluble mesothelin-related peptide (SMRP) levels for asbestos-exposed individuals and various malignant pleural mesothelioma (MPM) stages.

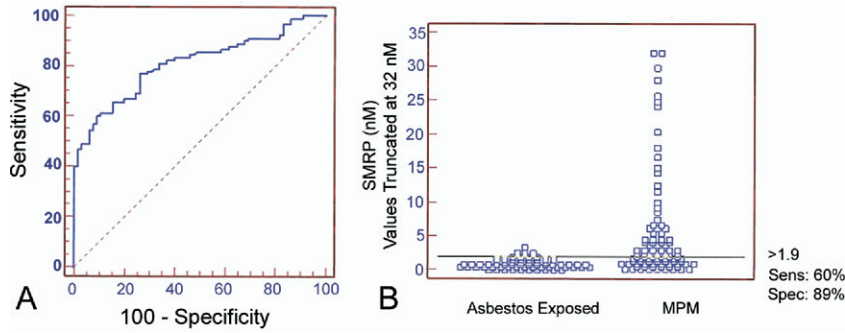


Fig 6. (A) Receiver operating characteristic (ROC) for serum soluble mesothelin-related peptide (SMRP) of malignant pleural mesothelioma versus asbestos-exposed individuals. Area under the curve was 0.81. (B) Interactive dot diagram of data represented by the ROC curve for serum SMRP in (A).

ANOVA). Lung cancer serum SMRP levels were not significantly different from the asbestos-exposed cohort ( $p = 0.173$ ).

#### SMRP and Cancer Histology

Figure 1 demonstrates the differences in serum SMRP levels between MPM, lung cancer and its various histologies, and the asbestos-exposed cohort. There were no differences between serum SMRP levels for adenocarcinoma (compared with squamous cell carcinoma or small cell carcinoma ( $p = 0.390$ )). For the MPM cohort, as seen in Figure 2, there were no differences seen in SMRP levels comparing epithelial ( $n = 58, 5.88 \pm 0.98$  nM) to biphasic ( $n = 29, 5.64 \pm 1.61$ ). There was a trend toward a lower SMRP level for sarcomatoid MPM but the small number of pure cases of sarcomatoid prevented any conclusions ( $n = 3, 2.01 \pm 1.08$ ).

#### Serum SMRP and Characteristics of the Asbestos-Exposed Cohorts

Of the 66 asbestos-exposed individuals, 62 had complete information regarding fiber exposure history and radiographic information. Forty-nine (79%) had an exposure in an asbestos-related trade for five years or more, and 13 (21%) had an exposure in such a trade for less than five years. Four (6%) asbestos-exposed individuals with exposure years of 5 to 37 years had no evidence of radiographic abnormalities, radiographic evidence of fibrosis was seen in 18 of 62 (29%), and pleural plaques were documented in 40 of 62 (65%). No differences in SMRP serum levels were noted for asbestos-exposed individuals based on age, sex, or exposure duration. The level of serum SMRP of 409 normal individuals (age 31 to 80 years, mean  $46 \pm 0.6$  years) was significantly lower than the 66 asbestos-exposed individuals ( $0.39 \pm 0.02$  vs  $0.99 \pm 0.09$ ,  $p < 0.001$  by ANOVA), but when age and sex were matched between the two groups the difference in serum SMRP was less pronounced but still significant ( $0.65 \pm 0.04$  vs  $0.89 \pm 0.09$ ,  $p < 0.01$ ). As seen in Figure 3, there was a trend toward elevation of serum SMRP levels comparing individuals with no plaques and fibrosis to those with evidence of radiographic asbestos changes.

#### Serum SMRP and Characteristics of the MPM Cohorts

Figure 4 depicts the Kaplan-Meier curves for the survival of all 90 MPM patients according to IMIG stage. Median

survivals were 35 months, 22 months, 13 months, and 4 months for stage I ( $n = 12$ ), stage II ( $n = 22$ ), stage III ( $n = 51$ ), and stage IV ( $n = 5$ ), respectively. These survival data of patients from whom the serum for SMRP were collected validate that the SMRP level reported should be representative of the IMIG stage of the patients because the survival curves are similar to those from other centers. No differences were seen in serum SMRP values for MPM patients based on age or sex. The SMRP levels did rise, however, comparing stage I MPM with the asbestos-exposed cohort ( $2.09 \pm 0.41$  vs  $0.99 \pm 0.09$ ,  $p = 0.02$ ). Moreover, as seen in Figure 5, MPM stages greater than stage I had significantly higher SMRP levels ( $10.61 \pm 3.89$ ,  $p = 0.03$ ) than stage I MPMs. Serum SMRP level, however, did not prove to be an independent predictor of survival by the Cox proportional hazards model.

#### ROC Analyses: Serum SMRP

The ROC analysis of serum SMRP revealed an AUC of 0.810 (95% confidence interval [CI], 0.739 to 0.868) for distinguishing between MPM and asbestos-exposed individuals with the best statistical cutoff at 1.9 nM (specificity, 89.2%; sensitivity, 60%; accuracy, 73%; Fig 6). The AUC for differentiating MPM from lung cancer was 0.820 (95% CI, 0.768 to 0.865) with the best statistical cutoff at 1.1

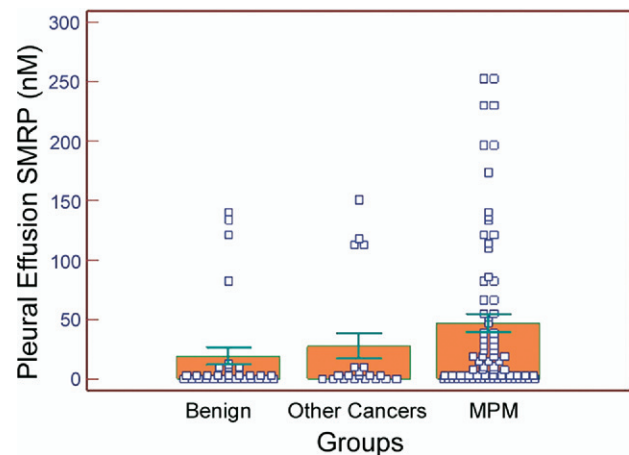
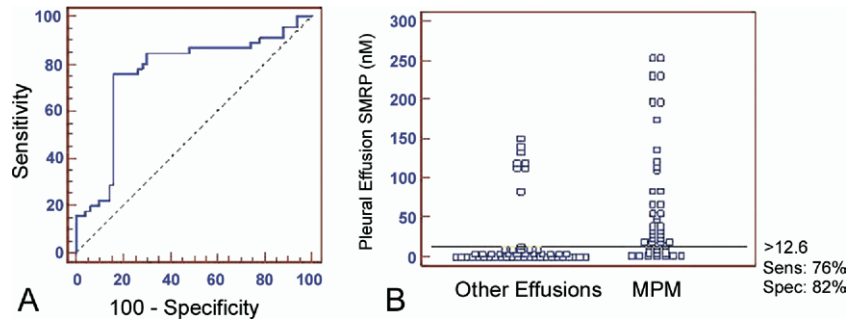


Fig 7. Pleural effusion serum soluble mesothelin-related peptide (SMRP) levels for malignant pleural mesothelioma (MPM), benign, and other malignancies.

Fig 8. (A) Receiver operator characteristic (ROC) for pleural effusion serum soluble mesothelin-related peptide (SMRP) of mesothelin-related peptide (MPM) versus other cohorts. Area under the curve was 0.76. (B) Interactive dot diagram of data represented by the ROC curve for pleural effusion SMRP in (A).



nM (specificity, 76.4%; sensitivity, 78.9%; accuracy, 77%). The AUC for distinguishing stage I MPMs from the asbestos-exposed individuals was 0.741 (95% CI, 0.630 to 0.834, with the best statistical cutoff at 2.0 nM (specificity, 91%; sensitivity, 58%; accuracy, 85%). The AUC for MPM versus all other groups, including the normal individuals, was 0.891 (95% CI, 0.866 to 0.913) with the best statistical cutoff at 1.075 nM (specificity, 87.1%; sensitivity, 81.1%; accuracy, 79%).

#### Pleural Effusion SMRP

The SMRP levels were significantly higher in the pleural effusions than in the serum of the patients. The SMRP levels of the MPM, benign group, and other cancers differed significantly by the Kruskal-Wallis test,  $p < 0.0003$ , as seen in Figure 7. Mean MPM pleural effusion SMRP was significantly elevated over benign effusion ( $65.57 \pm 11.33$  nM vs  $18.99 \pm 7.48$  nM,  $p = 0.003$ , respectively) and over other cancer pleural effusion SMRP ( $27.46 \pm 11.25$  nM,  $p = 0.044$ ). The AUC for distinguishing MPM from benign pleural effusions was 0.78 (95% CI, 0.664 to 0.863), and the ideal cutoff point was 12.6 nM, with a sensitivity of 75.6% and a specificity of 83.3% (accuracy, 79%). The AUC for distinguishing MPM from all other effusions was 0.76 (95% CI, 0.662 to 0.842) (Fig 8) at a best cutoff level of 12.6 nM (sensitivity, 75.6%; specificity, 82%; accuracy, 79%).

#### Comment

Soluble mesothelin-related peptide is currently a biomarker for mesothelioma, which has now been studied by at least three independent groups on three different continents. The most promising data were originally presented by Robinson and colleagues [5] in which a sensitivity of 84% was presented with close to a 100% specificity using a nonquantitative form of the MESO-MARK assay. Robinson and colleagues noted that SMRP was elevated in 7 of 40 (18%) individuals with asbestos exposure or asbestosis while the majority of individuals with "pleural disease," including those with plaques or fibrosis, did not have elevated serum SMRPs. In a prospective, multiinstitutional study performed by Scherpereel and colleagues [7], patients were recruited to donate serum with or without pleural effusion who either (1) had mesothelioma, (2) had a pleural biopsy for lesions that were associated with asbestos exposure, or (3) had a

diagnosis of pleural metastases. The degree of pleural changes in these patients was not quantitatively expressed according to either the degree of plaque or fibrosis, nor were the demographics of the asbestos exposure clearly documented. The data from Scherpereel and colleagues are encouraging, demonstrating AUCs between 0.693 to 0.872 for discriminating MPM patients from non-MPM cancer patients or from individuals with asbestos-related pleural "lesions." Cutoffs were defined in the study by Scherpereel and colleagues as being optimal at 0.93 nM/L for distinguishing asbestos-exposed from MPM with a sensitivity of 80% and specificity of 82.6%.

The SMRP investigation conducted by our group used archives that were accumulated by one of the authors (HIP) over a 16-year period, with supplementation of 50 lung cancers from a commercial source. A portion of these archives were previously used to investigate osteopontin as a marker for mesothelioma in the hope of combining multiple markers for the early detection and classification of mesothelioma. All of the serum cohorts used for these investigations had well-documented demographics. For the mesotheliomas all of the patients had attempted cytoreduction of the disease with careful intraoperative staging, and 120 of 170 lung cancer patients had resection of their tumor with mediastinal lymph node dissection by one of the authors (HIP). The asbestos-exposed population, although small, was specifically recruited for this study in 2004 not only to compare the values with cancer bearing populations, but also to see if any of the mesothelioma markers being investigated were influenced by parameters such as age, sex, or duration of asbestos exposure (by history). One could argue that this is not a representative group of study subjects because all of the groups had sera or pleural effusion collected in the past, and do not represent a new, prospective collection. Such a prospective collection is being planned by the Early Detection Research Network as a validation for markers such as SMRP and will serve as a reference set for determining how robust combinations of markers can be in detecting or classifying mesothelioma.

Our study reveals, like those of Robinson and colleagues [5] and Scherpereel and colleagues [7], that serum SMRP levels are significantly higher in patients with MPM than in an asbestos-exposed cohort which either does or does not have radiographic evidence of the exposure. In this surgically staged group of mesotheliomas, SMRP levels rise dramatically comparing early

stage I mesotheliomas with those serum levels seen with more advanced disease, which confirms data from the Robinson group in noncytoreduced patients [5]. Normal individuals ( $n = 409$ ) with a median age of 46 years have significantly lower levels for serum SMRP than asbestos-exposed or MPM bearing individuals. The marker is different from osteopontin, in that SMRP serum levels are not significantly different among asbestos-exposed individuals based on their duration of exposure, and although the marker is elevated in individuals with pleural plaques, there is no further elevation as the degree of radiographic changes increases (ie, with addition of interstitial fibrosis). These findings would imply that SMRP should be a more robust marker for mesothelioma because it seems not to be influenced by inflammatory cascades, as osteopontin may be.

The discriminatory power of SMRP between MPM and asbestos-exposed cohorts in our study compared with those of Scherpereel and colleagues [7] and Robinson and colleagues [5] is not as clearly defined, and our AUC, although very respectable at 0.81, is less than that seen in the study by Scherpereel and colleagues (0.89) and the most recent publication by Robinson and colleagues (0.89). One possible explanation for this is that the patients in our series, all of which had blood taken prior to cytoreductive surgery, had a different stage distribution than those seen in the other studies in the literature. The possibility exists then that our series of patients had less bulk of disease, and hence had lower levels of SMRP (some closer to levels seen in asbestos-exposed individuals). This is difficult to sort out considering that the published data of Robinson and colleagues is not described as nM levels and the study by Scherpereel and colleagues uses median values for serum SMRP as opposed to mean. Moreover, the number of asbestos-exposed individuals and the characterization of the asbestos-exposed population are more detailed in the present study than in the others. The ideal cutoff for differentiating the asbestos group from the mesothelioma group in our study was 1.9 nM, with a sensitivity of 60% and a specificity of 89%. Using Scherpereel and colleagues' cutoff of 0.93, we would have had a sensitivity of 83% and a specificity of 60% with our data. In order for this marker to be used for early detection, the sensitivity must obviously be close to 100% at an acceptable specificity. Our serum data suffer from the same limitations as Scherpereel and colleagues' in that adjusting our sensitivity to 95% at a cutoff of 0.36 nM would lead to a specificity of 17%. The only way in which such a low specificity marker could be adapted presently into the clinical arena would be to demonstrate conclusively that the marker rises with time as the degree of disease increases, or that the marker functions effectively as a monitor of treatment of the disease. For SMRP, there are only anecdotal, but extremely interesting data from Robinson and colleagues at this time that SMRP will rise months in advance of the development of clinical symptoms of mesothelioma (Robinson, personal communication). Validation studies are presently being designed using retrospective collections of sera from well-

described asbestos-associated archives, which were generated during large prospective screening or chemoprevention studies in the United States. By measuring the longitudinal changes in a given patient known to have developed mesothelioma who was entered on these trials, we will better be able to define whether SMRP or any other marker for mesothelioma alone or in combination can signal the development of the disease.

This study tries to distinguish mesothelioma from lung cancer specifically. Mesothelin has been described to be associated with a number of cancers including pancreatic and ovarian cancer, and mesothelin staining has been identified in non-small cell lung cancers in up to 27%. The data are encouraging that discrimination exists between lung cancer and mesothelioma serum SMRP in that the AUC was 0.820 with a respectable specificity of 76.4% and sensitivity of 78.9% at 1.1 nM. This is especially important for asbestos-exposed cohorts who use tobacco products and will have an elevated risk not only for mesothelioma but for lung cancer. A clinical scenario characterized by a developing pleural effusion in a smoker with or without obvious pleural metastases in which the SMRP is high would probably point to the development of mesothelioma, while a low SMRP could not rule out stage IIIB lung cancer. Future prospective studies which combine SMRP with novel markers for both mesothelioma and lung cancer could further enhance this discrimination.

Short of video-assisted thoracoscopy with tissue biopsy, the characterization of an isolated new effusion in a patient without a previous history of malignancy depends on cytologic description with or without specialized immunopanel. The pleural effusion data which attempt to characterize such pleural effusion presented in the manuscript should be considered very preliminary but concordant with the results described by Scherpereel and colleagues [7]. One of the problems with performing such an analysis is the characterization of the comparator groups, and the definition of a benign pleural effusion. The description of these effusions is only tangentially alluded to in the article by Scherpereel and colleagues, and the benign effusions used in our data represent a wide variety of pathologic conditions that may very well be influenced by pleural homeostasis. The obvious benign conditions that must be represented in future studies include effusions related to the following: (1) primary organ failure including congestive heart failure, renal failure, and cirrhosis; (2) inflammatory-infectious conditions including postpneumonic exudative effusions; and (3) autoimmune-related effusions. These conditions are not well-represented in this manuscript and a different approach was taken (ie, collection of postthoracotomy or postcardiopulmonary bypass effluents from uncomplicated futile or benign thoracotomies). Despite some variation, over 75% of the 30 benign pleural effusions had an SMRP level less than 20 nM. The majority of the cancer effusions were weighted toward adenocarcinoma of the lung, and further discrimination between mesothelioma effusions and effusions from mesothelin expressing tumors, including ovarian cancer, should be performed. Nevertheless, the 82% specificity and 76% sensitivity

discriminating mesothelioma from all the "other" effusions is encouraging enough to warrant a multiinstitutional United States prospective study assessing the role of SMRP in classifying new onset pleural effusions.

These preliminary North American data investigating SMRP demonstrate the need for prospective reference samples that can be used for the validation of markers associated with mesothelioma. The design of prospective trials in which the level of SMRP may decide intervention must, by definition, incorporate reference ranges for "nonpathologic" levels of SMRP in the serum or in the pleural effusion. Whether these nonpathologic ranges are influenced by such factors as geographic location of the subjects, type of fiber the individual is exposed to, and duration of exposure to the fiber, is not clearly defined such that SMRP levels can be used to guide the definitive management of the patient. In the interim such prospective reference samples are being formulated and will be used to insure that SMRP has the desired characteristics as a putative marker for mesothelioma either alone or in combination with other novel proteins.

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## DISCUSSION

**DR THOMAS J. WATSON** (Rochester, NY): I have one question. Did you check other values for the serum SMRP (soluble mesothelin-related peptide) cutoff? You said you used 1.9 for your cutoff and that gave you only 60% sensitivity and a slightly better specificity. Did you look at other cutoffs to try to increase the sensitivity?

**DR PASS:** Sure, but in the context of a screening trial, those are the numbers you want to use. You don't want to have a lot of sensitivity that's going to be too low because you're going to have a lot of patients that you're going to work up, so you want good specificity. So what I was shooting for was to have a high specificity with whatever the sensitivity is, and those are the ideal numbers. You can lower the two and then even it out, but then you're losing the value of the marker as an early detection marker.

**DR LAWRENCE R. GLASSMAN** (Manhasset, NY): I have a question regarding the availability of the assay. Is it generally available, and if it's not, when will it be available?

**DR PASS:** Well, a week ago, based on these data from our laboratory in which it showed that you could actually use this marker for monitoring of mesothelioma. Here you see mesothelioma levels before and then after surgery and then rising after recurrence in patients who have had operations. This marker is now going to be available through a reference laboratory. The physician will be able to see a patient with mesothelioma, patients who are under treatment. So the indication for this marker now is not for screening. It will be to follow patients who have had treatment for mesothelioma to see if the marker decreases or if the marker then starts to rise while the patient is on therapy.