

ORIGINAL ARTICLE

# Asbestos Exposure, Pleural Mesothelioma, and Serum Osteopontin Levels

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ABSTRACT

**BACKGROUND**

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We investigated the presence of osteopontin in pleural mesothelioma and determined serum osteopontin levels in three populations: subjects without cancer who were exposed to asbestos, subjects without cancer who were not exposed to asbestos, and patients with pleural mesothelioma who were exposed to asbestos.

**METHODS**

A group of 69 subjects with asbestos-related nonmalignant pulmonary disease were compared with 45 subjects without exposure to asbestos and 76 patients with surgically staged pleural mesothelioma. Tumor tissue was examined for osteopontin by immunohistochemical analysis, and serum osteopontin levels were measured by an enzyme-linked immunosorbent assay.

**RESULTS**

There were no significant differences in mean ( $\pm$ SE) serum osteopontin levels between age-matched subjects with exposure to asbestos and subjects without exposure to asbestos ( $30\pm 3$  ng per milliliter and  $20\pm 4$  ng per milliliter, respectively;  $P=0.06$ ). In the group with exposure to asbestos, elevated serum osteopontin levels were associated with pulmonary plaques and fibrosis ( $56\pm 13$  ng per milliliter) but not with normal radiographic findings ( $21\pm 5$  ng per milliliter), plaques alone ( $23\pm 3$  ng per milliliter), or fibrosis alone ( $32\pm 7$  ng per milliliter) ( $P=0.004$ ). Serum osteopontin levels were significantly higher in the group with pleural mesothelioma than in the group with exposure to asbestos ( $133\pm 10$  ng per milliliter vs.  $30\pm 3$  ng per milliliter,  $P<0.001$ ). Immunohistochemical analysis revealed osteopontin staining of the tumor cells in 36 of 38 samples of pleural mesothelioma. An analysis of serum osteopontin levels comparing the receiver-operating-characteristic curve in the group exposed to asbestos with that of the group with mesothelioma had a sensitivity of 77.6 percent and a specificity of 85.5 percent at a cutoff value of 48.3 ng of osteopontin per milliliter. Subgroup analysis comparing patients with stage I mesothelioma with subjects with exposure to asbestos revealed a sensitivity of 84.6 percent and a specificity of 88.4 percent at a cutoff value of 62.4 ng of osteopontin per milliliter.

**CONCLUSIONS**

Serum osteopontin levels can be used to distinguish persons with exposure to asbestos who do not have cancer from those with exposure to asbestos who have pleural mesothelioma.

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**P**LEURAL MESOTHELIOMA IS AN ASBESTOS-related cancer with a median survival of 8 to 18 months.<sup>1</sup> Persons with asbestos-related nonmalignant disease are an ideal cohort in which to study biomarkers for the early detection of pleural mesothelioma, because they are at high risk for the tumor; have had a measurable, identifiable exposure to the carcinogen; reside in defined and well-studied regions; and have a high rate of compliance in long-term follow-up studies.<sup>2</sup>

Retrospective studies of small numbers of patients with pleural mesothelioma have attempted to identify biomarkers that predate symptoms in a high-risk population. These markers include tissue polypeptide antigen, carcinoembryonic antigen, hyaluronic acid and ferritin,<sup>3</sup> hyaluronic acid alone,<sup>4-11</sup> cytokeratins such as soluble cytokeratin 19 fragment,<sup>12-16</sup> CA-125,<sup>17</sup> and soluble mesothelin-related protein.<sup>18</sup>

We have used gene-expression arrays to predict survival and recurrence patterns in patients with pleural mesothelioma<sup>19</sup> and to seek markers that would be useful for screening and diagnosis of pleural mesothelioma. The most promising biomarker was osteopontin, a glycoprotein that is overexpressed in lung,<sup>20</sup> breast,<sup>21</sup> colorectal,<sup>22</sup> gastric,<sup>23</sup> and ovarian<sup>24-26</sup> cancer and in melanoma.<sup>27</sup> Osteopontin mediates cell-matrix interactions and cell signaling through binding with integrin and CD44 receptors<sup>28</sup> and is regulated by proteins in cell-signaling pathways that are associated with asbestos-induced carcinogenesis. Moreover, high levels of osteopontin correlate with tumor invasion, progression, and metastases. Sandhu et al. reported that osteopontin is up-regulated in asbestos-induced tumors in a rat model of asbestos carcinogenesis and in cells exposed to asbestos *in vitro*.<sup>29</sup>

We undertook the present study to test our hypothesis that osteopontin is a useful biomarker in pleural mesothelioma and, more specifically, to compare serum levels of osteopontin in a cohort of subjects with asbestos-related nonmalignant disease with preoperative levels in patients with surgically treated pleural mesothelioma.

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## METHODS

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### STUDY POPULATION

We studied three groups of subjects: 69 subjects with a history of exposure to asbestos, evidence of asbestosis, or both; 45 current or former smokers with no exposure to asbestos who were undergo-

ing screening bronchoscopy; and 76 patients with pleural mesothelioma. All subjects provided serum samples and written informed consent. The study was approved by the ethics committee of each participating institution.

### SUBJECTS WITH EXPOSURE TO ASBESTOS

From July to September 2004, we enrolled 69 subjects with a history of exposure to asbestos, radiographic changes consistent with the presence of asbestosis, or both who were seen at the Center for Occupational and Environmental Medicine, Royal Oak, Michigan. Entry criteria for this cohort were similar to those described by Cullen et al.<sup>2</sup> Both exposure to asbestos and the duration of exposure were documented with the use of the American Thoracic Society Division of Lung Diseases 78 Adult questionnaire.<sup>30</sup>

Subjects either were employed in a trade associated with established habitual exposure to asbestos for which there is a documented increase in the risk of asbestos-related diseases (including insulation manufacturing or installation, sheet-metal work, plumbing, plasterboard application, shipfitting, electrical work on ships, boilermaking, and ship scaling)<sup>31</sup> or had occupational exposure to asbestos (as determined by the American Thoracic Society Division of Lung Diseases 78 Adult questionnaire) and radiographic evidence of changes consistent with a diagnosis of nonmalignant asbestos-related disease. These radiographic findings included benign pleural disease, defined as thickening or fibrotic plaques on the pleural surfaces of both lungs, with or without diffuse lung fibrosis manifested by small, irregular shadows in both lungs.

A plain chest radiograph was obtained from each subject and interpreted by a single trained radiologist (a B reader certified by the National Institute for Occupational Safety and Health) who was unaware of the patient's exposure status and who was proficient in the classification of chest radiographs for pneumoconioses according to the International Labor Office Classification System. The B reader specifically commented on the presence or absence of pleural changes, including plaques and lung fibrosis. Lung fibrosis was interpreted according to the International Classification of Radiographs of Pneumoconioses (available at [www.ilo.org/publns](http://www.ilo.org/publns)). This 12-point system classifies fibrosis according to the size and number of abnormal areas (from 0/0 to 3/3 in each lung); only results of 1/0 or greater were classified as indicative of asbestosis.

Of the 69 subjects in the group exposed to asbestos, 57 (83 percent) had been exposed as the result of working in an asbestos-related trade for at least five years, 7 (10 percent) had had such exposure for less than five years, and 5 (7 percent) had radiographic evidence of abnormalities consistent with the occurrence of exposure to asbestos but had no such exposure documented during an interview. The professions of the 64 participants with exposure to asbestos were as follows: 11 were foundry workers, 7 were pipe fitters, 7 were in building and construction, 6 had passive exposure in the construction business or from contact with a family member, 5 were involved in brake assembly or repair, 4 were involved in boiler repair, 4 had exposure to vermiculite insulation, 3 were machinist grinders, 3 were plumbers, 2 were in the tool and die industry, 2 were shipbuilders, 2 were millwrights, 2 were firefighters, 2 were brick makers, 2 were electricians, and 2 were involved in asbestos removal. Radiographic evidence of fibrosis was found in 23 of the 69 (33 percent), and pleural plaques were found in 50 of the 69 (72 percent); 6 participants with 5 to 37 years of exposure had no radiographic abnormalities, 53 had either plaques or fibrosis, and 10 had both plaques and fibrosis.

#### SUBJECTS WITH NO EXPOSURE TO ASBESTOS

To document serum osteopontin levels in an unexposed, but similar population, we obtained serum samples from 25 current smokers and 20 former

smokers (age, 33 to 74 years) who were undergoing screening bronchoscopy as an entry criterion for a chemoprevention trial. Occupational histories were obtained from all these subjects to document the absence of known exposure to asbestos; all these participants had normal chest radiographs.

#### PATIENTS WITH PLEURAL MESOTHELIOMA

Serum was obtained from 76 patients who were scheduled to undergo cytoreductive surgery for pleural mesothelioma, according to a protocol approved by the Wayne State University Human Investigation Committee (D1420). The oldest serum sample was obtained 77 months before analysis, and the most recent sample 3 months before. Exposure to asbestos was documented on the basis of an occupational history in 59 of the 76 patients (78 percent). All patients underwent complete surgical staging according to the staging system of the International Mesothelioma Interest Group (IMIG)<sup>32</sup>: 13 had stage I, 20 had stage II, and 43 had stage III disease. All were followed with the use of computed tomography of the chest every three to four months until death or to a follow-up date in April 2005. Tumors were classified as epithelial in 50 patients, sarcomatoid in 4, and mixed in 22 by two of the authors. Table 1 lists the characteristics of the group exposed to asbestos and the group with pleural mesothelioma.

#### IMMUNOHISTOCHEMISTRY

Immunohistochemical analysis was performed on a multitissue pleural-mesothelioma array, consisting of 2-mm representative areas of resected tumor and normal-tissue controls. Thirty-eight of the 76 samples of pleural tumors studied were on the array. The other 38 were not available when the array was constructed, since they were conscripted for asbestos litigation cases. Immunohistochemical analysis was performed with the use of the standard avidin-biotin complex technique. The primary antibody, a monoclonal antibody against osteopontin (clone OP3 N, Vector Laboratories), was applied to the array at a dilution of 1:150 for 90 minutes at room temperature. The secondary antibody, anti-mouse IgG (Vector Laboratories), was applied at a dilution of 1:200 for 30 minutes at room temperature. A positive control and a negative control (obtained by omitting the secondary antibody) were included in each run. Samples were scored separately for staining intensity on a three-point scale (with a score of 1 indicating low intensity, and a score of

**Table 1. Demographic Characteristics of Patients with Pleural Mesothelioma and Subjects with Asbestos-Related Nonmalignant Disease.**

Characteristic	Pleural Mesothelioma (N=76)	Asbestos-Related Nonmalignant Disease (N=69)
Age (yr)*	65±1	65±1
Sex (no.)		
Male	60	61
Female	16	8
Race (no.)†		
White	72	66
Black	4	3
Smoking history (no.)		
Yes	60	56
No	16	13

\* Plus-minus values are means ±SE.

† Race was recorded by the investigators.

3 high intensity) and for the percentage of positive tumor cells (with a score of 1 indicating 10 percent or less, a score of 2 indicating 11 to 49 percent, and a score of 3 indicating 50 percent or more).

#### OSTEOPONTIN ENZYME-LINKED IMMUNOSORBENT ASSAY

The Human Osteopontin Assay Kit (ImmunoBiological Laboratories) was used to determine the level of serum osteopontin; all samples were coded. Each specimen was tested in duplicate, and the results were quantitated in nanograms per milliliter with the use of a standard curve.

#### STATISTICAL ANALYSIS

Kaplan–Meier survival plots and log-rank tests were used to assess differences in survival according to the stage of disease among the patients with pleural mesothelioma. The ability of serum osteopontin levels to distinguish the patients with pleural mesothelioma from the subjects with asbestos-related nonmalignant disease was evaluated by means of descriptive statistics and receiver-operating-characteristic (ROC) curves.<sup>33,34</sup> The area under the ROC curve (AUC) was calculated, and 95 percent confidence intervals were used to test the hypothesis that the theoretical AUC is 0.5. An AUC with a confidence interval that did not include the 0.5 value was considered evidence that the laboratory test had some ability to distinguish between the two

groups.<sup>34,35</sup> We calculated differences between groups by using analysis of variance and multiple regression analysis in a stepwise fashion, entering only variables with a P value of less than 0.05 in the model. All statistical analyses were performed with the use of MedCalc software.

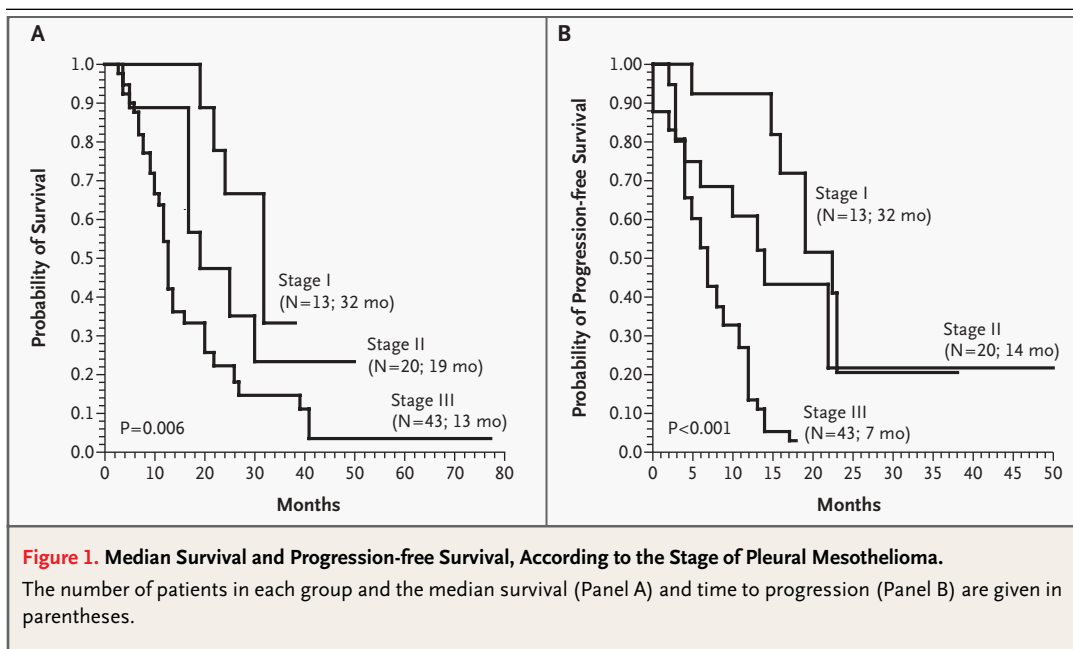
## RESULTS

#### SURVIVAL ACCORDING TO THE STAGE OF PLEURAL MESOTHELIOMA

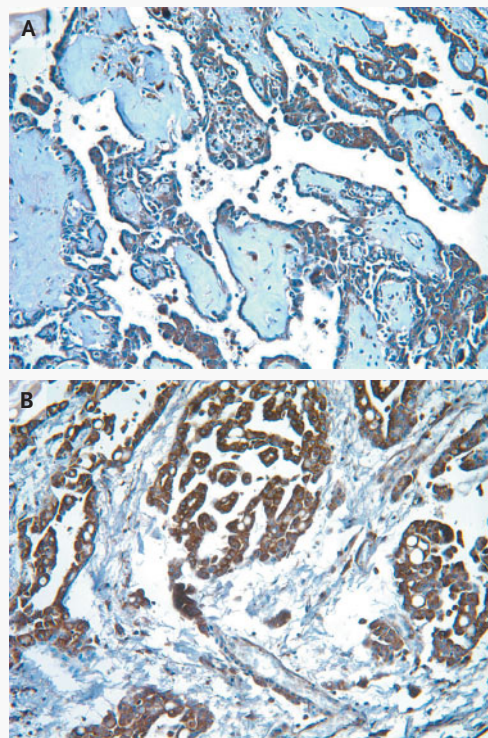
To determine whether the patients with mesothelioma in this study had outcomes similar to those in other series, survival and time-to-progression curves based on IMIG staging status were generated. Figure 1 shows significant differences in survival and progression according to stage; these results are consistent with those of other studies that used the IMIG staging system.<sup>36–38</sup>

#### IMMUNOHISTOCHEMICAL FINDINGS

Tumor tissue was available for osteopontin staining from 38 of the 76 patients with mesothelioma; 36 of the 38 samples were positive for osteopontin (Fig. 2). They showed cytoplasmic staining in at least 50 percent of tumor cells, and the staining intensity ranged from 1 in the case of 13 samples to 3 in the case of 15 samples; 8 samples had a staining intensity of 2. Osteopontin was seen in all pleural-mesothelioma variants: 19 of 20 epithelial tumors,







**Figure 2. Immunohistochemical Staining for Osteopontin in Two Samples of Pleural Mesothelioma.**

Osteopontin is highly expressed in tumor cells and there is minimal stromal reactivity (Panel A,  $\times 100$ ; Panel B,  $\times 400$ ).

15 of 16 mixed tumors, and 2 of 2 sarcomatoid tumors. Lung parenchyma and adjacent normal pleura were negative for osteopontin, fibroblasts in tumor-associated stroma were infrequently weakly positive, and the media and intima of vessels showed weak positivity.

#### OSTEOPONTIN LEVELS

##### *Subjects Exposed to Asbestos and Subjects without Exposure*

The mean ( $\pm$ SE) serum level of osteopontin in the entire group of subjects who were exposed to asbestos was  $30\pm 3$  ng per milliliter (range, 2 to 221; 95 percent confidence interval, 23 to 36) and did not differ significantly from that in subjects without exposure to asbestos ( $20\pm 4$  ng per milliliter,  $P=0.06$ ). The levels in age-matched controls with no exposure to asbestos and normal radiographs did not differ significantly according to age from those in the group exposed to asbestos (younger than 50 years of age,  $12\pm 5$  ng per milliliter and

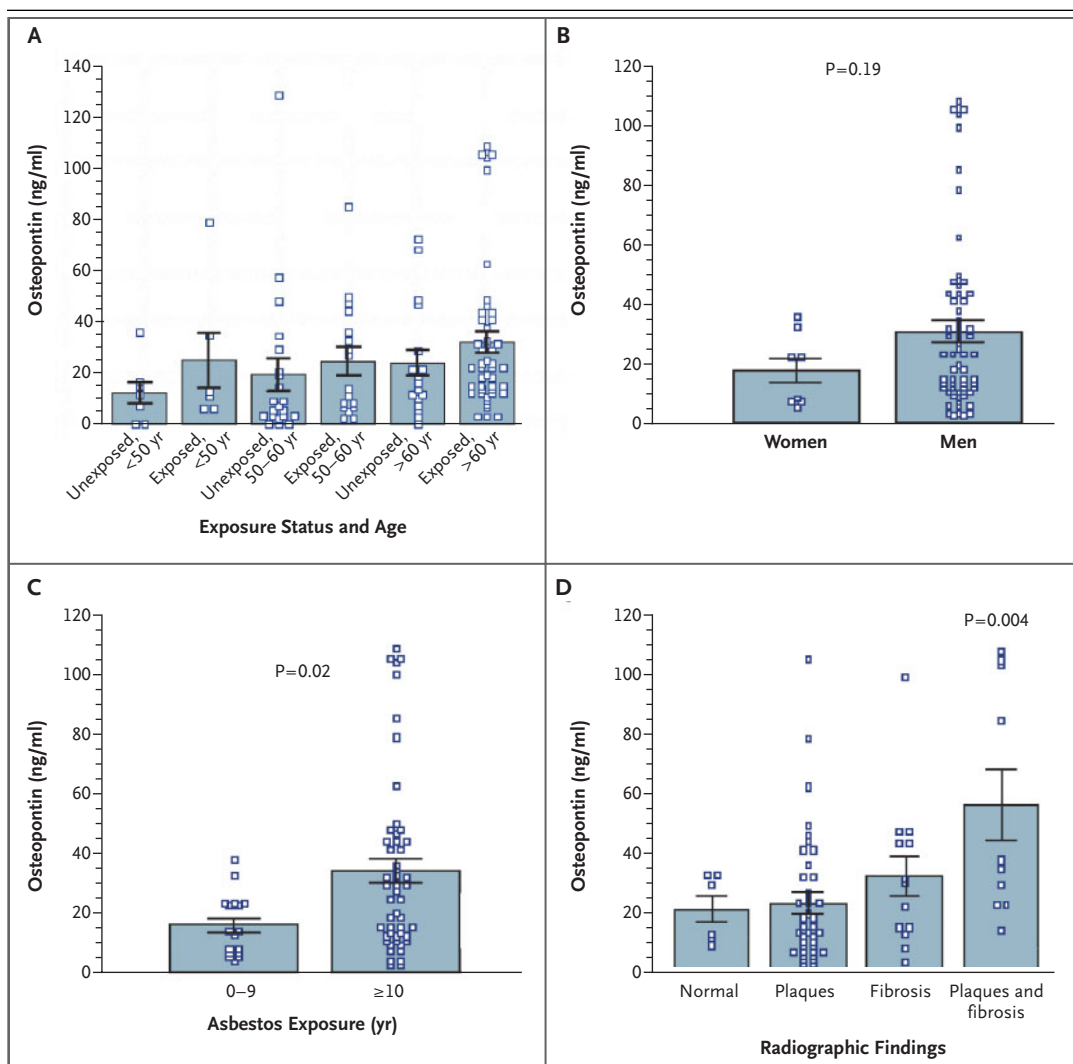
$25\pm 11$  ng per milliliter, respectively; 95 percent confidence interval for the difference,  $-16$  to  $41$ ;  $P=0.34$ ; 50 to 60 years of age,  $19\pm 6$  ng per milliliter and  $25\pm 5$  ng per milliliter; 95 percent confidence interval for the difference,  $-12$  to  $22$ ;  $P=0.56$ ; and older than 60 years of age,  $24\pm 5$  ng per milliliter and  $32\pm 4$  ng per milliliter; 95 percent confidence interval for the difference,  $-23$  to  $7$ ;  $P=0.29$ ) (Fig. 3A).

In the group with exposure to asbestos, there were no significant differences in osteopontin levels according to sex ( $P=0.19$ ) (Fig. 3B) or the presence or absence of pleural plaques ( $P=0.88$ ) (Fig. 3D). The subgroup with lung fibrosis had a significantly higher mean level of osteopontin than the subgroup without fibrosis ( $43$  ng per milliliter vs.  $23$  ng per milliliter; 95 percent confidence interval for the difference,  $7$  to  $33$ ;  $P=0.004$ ) (Fig. 3D), and the mean levels were significantly higher with 10 or more years of exposure than with fewer than 10 years of exposure ( $34$  ng per milliliter vs.  $16$  ng per milliliter; 95 percent confidence interval for the difference,  $4$  to  $33$ ;  $P=0.02$ ) (Fig. 3C). The highest levels of serum osteopontin were found in subjects who had both plaques and fibrosis ( $56\pm 13$  ng per milliliter). Serum osteopontin levels were significantly lower in age-matched unexposed controls than in subjects with asbestos exposure and plaques and fibrosis (mean age in both groups,  $64\pm 3$  years):  $14\pm 6$  ng per milliliter vs.  $56\pm 13$  ng per milliliter ( $P=0.03$ ). Among the subjects with exposure to asbestos, osteopontin levels were significantly lower in subjects with a normal chest radiograph ( $21\pm 5$  ng per milliliter), subjects with plaques ( $23\pm 3$  ng per milliliter), and subjects with fibrosis ( $32\pm 7$  ng per milliliter) than in those who had plaques and fibrosis ( $56\pm 13$  ng per milliliter,  $P=0.004$ ).

A multiple regression analysis that included age, the duration of exposure to asbestos, the presence or absence of fibrosis, the presence or absence of plaques, and the International Labor Organization radiography score was performed. Only the duration of exposure to asbestos and the radiographic findings were independently associated with osteopontin levels ( $P=0.001$  and  $P<0.001$ , respectively), with zero-order correlation coefficients of  $0.357$  and  $0.399$ , respectively.

##### *Patients with Pleural Mesothelioma*

The mean serum osteopontin level in the group with pleural mesothelioma differed significantly from that in the group exposed to asbestos ( $133\pm 10$  ng



**Figure 3.** Mean ( $\pm$ SE) Serum Osteopontin Levels According to Exposure Status and Age, Sex, Years of Exposure to Asbestos, and Radiographic Findings.

There were no significant differences in osteopontin levels between subjects exposed to asbestos and age-matched controls with no exposure to asbestos for subjects younger than 50 years ( $25 \pm 11$  ng per milliliter [range, 6 to 78; 95 percent confidence interval, -5 to 55] and  $12 \pm 5$  ng per milliliter [range, 0 to 36; 95 percent confidence interval, 1 to 24], respectively;  $P=0.34$ ), subjects 50 to 60 years of age ( $25 \pm 5$  ng per milliliter [range, 2 to 85; 95 percent confidence interval, 13 to 36] and  $19 \pm 6$  ng per milliliter [range, 0 to 128; 95 percent confidence interval, 6 to 33], respectively;  $P=0.56$ ), and subjects older than 60 years of age ( $32 \pm 4$  ng per milliliter [range, 3 to 108; 95 percent confidence interval, 24 to 41] and  $24 \pm 5$  ng per milliliter [range, 0 to 72; 95 percent confidence interval, 13 to 35], respectively;  $P=0.29$ ) (Panel A). No significant differences in osteopontin levels were noted with respect to sex (Panel B). Osteopontin levels rose as the duration of exposure to asbestos increased (Panel C) and the degree of radiographic abnormality increased (Panel D). The P value in Panel D is for the comparison of plaques and fibrosis with the other findings.

per milliliter [range, 6 to 385; 95 percent confidence interval, 113 to 154] vs.  $30 \pm 3$  ng per milliliter [range, 2 to 221; 95 percent confidence interval, 23 to 36],  $P < 0.001$ ). There were no significant differences in mean serum osteopontin levels among patients with stage I mesothelioma ( $147 \pm 26$  ng per millili-

ter; range, 10 to 341; 95 percent confidence interval, 92 to 204), stage II mesothelioma ( $158 \pm 22$  ng per milliliter; range, 14 to 385; 95 percent confidence interval, 99 to 216), or stage III mesothelioma ( $118 \pm 12$  ng per milliliter; range, 4 to 302; 95 percent confidence interval, 83 to 145;  $P > 0.15$  for all compari-

sons); however, the means in all these stages differed significantly from the mean in the group exposed to asbestos ( $30 \pm 3$  ng per milliliter; range, 2 to 221; 95 percent confidence interval, 23 to 36;  $P < 0.001$ ). Moreover, serum osteopontin levels in the subjects with exposure to asbestos and plaques and fibrosis differed significantly from those in the patients with pleural mesothelioma ( $56 \pm 13$  ng per milliliter vs.  $133 \pm 10$  ng per milliliter; 95 percent confidence interval for the difference, 49 to 114;  $P < 0.001$ ).

Mean osteopontin levels were similar in men and women with mesothelioma ( $136 \pm 12$  ng per milliliter and  $125 \pm 21$  ng per milliliter, respectively; 95 percent confidence interval for the difference,  $-61$  to 39;  $P = 0.66$ ) and did not vary according to the histologic characteristics of the tumor ( $128 \pm 13$  ng per milliliter among those with epithelial tumors, as compared with  $133 \pm 18$  ng per milliliter among those with nonepithelial tumors; 95 percent confidence interval for the difference,  $-28$  to 59;  $P = 0.49$ ) or the history of asbestos exposure ( $151 \pm 24$  ng per milliliter among those with exposure, as compared with  $128 \pm 12$  ng per milliliter among those without exposure; 95 percent confidence interval for the difference,  $-73$  to 28;  $P = 0.37$ ).

#### ROC CURVES

ROC analyses comparing the subjects with exposure to asbestos with patients with pleural mesothelioma showed an AUC of 0.888 (95 percent confidence interval, 0.826 to 0.934) (Fig. 4A). Subgroup analyses showed that the AUC values for patients with stage I, those with stage II, those with stage I or II, and those with stage III pleural mesothelioma were 0.906, 0.925, 0.917, and 0.865, respectively, as compared with subjects with exposure to asbestos. A cutoff value of 48.3 ng of osteopontin per milliliter (sensitivity of 77.6 percent and specificity of 85.5 percent) had the highest accuracy (minimal false negative and false positive results) for confirming mesothelioma (Fig. 4C). For the purpose of screening (i.e., early detection for mesothelioma), a cutoff value with the highest sensitivity (95 to 99 percent) might be most appropriate independent of specificity, and at a cutoff value of 10.9 ng of osteopontin per milliliter, the sensitivity was 96.1 percent, with a specificity of 23.2 percent (Fig. 4B). If screening were for the detection of stage I disease alone, a cutoff value of 9.5 ng of osteopontin per milliliter would provide 100 percent sensitivity, with a specificity of 21.7 percent. The most accurate cut-

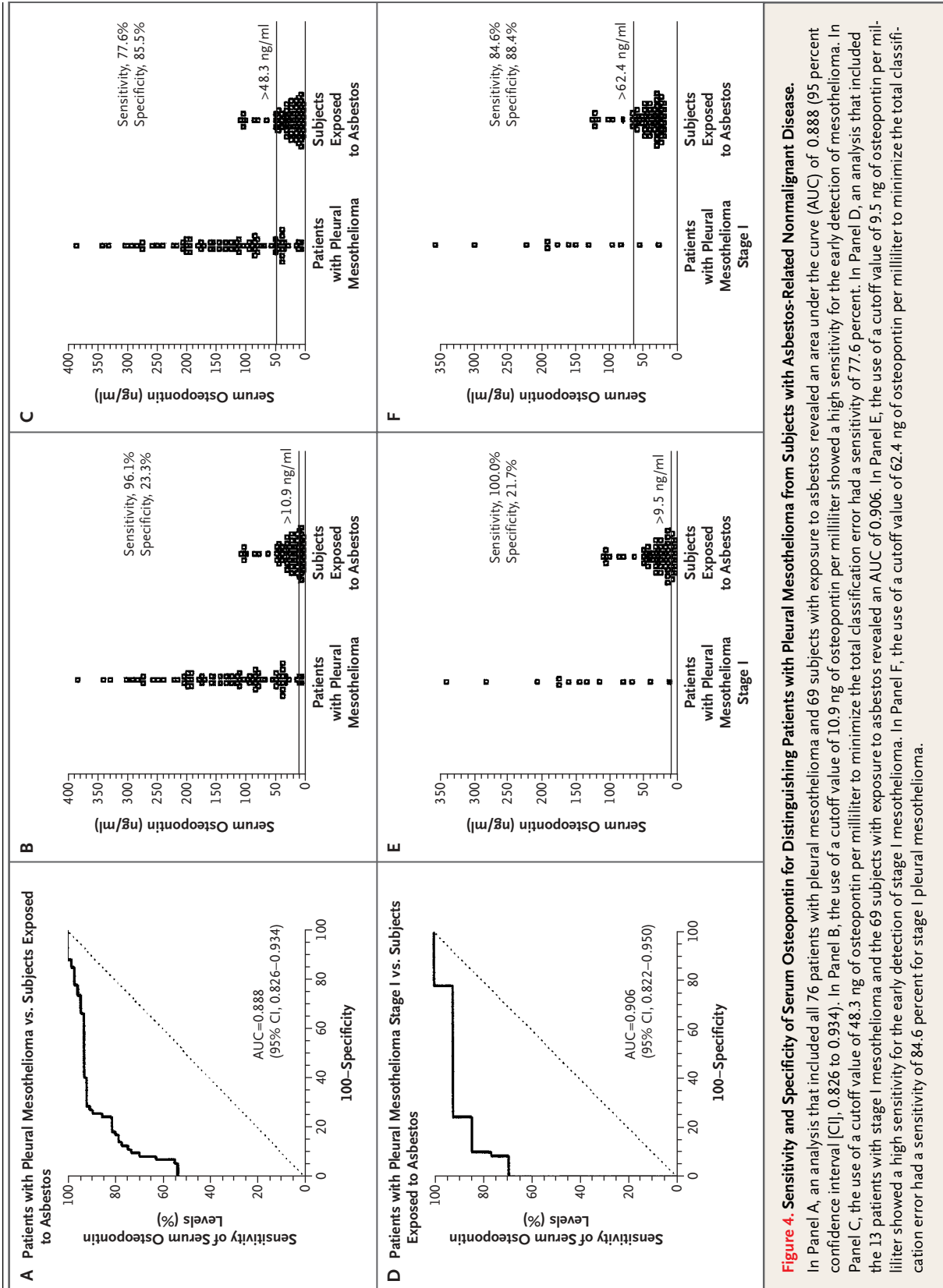
off value, however, for the detection of a stage I mesothelioma (with a sensitivity of 84.6 percent and a specificity of 88.4 percent) was 62.4 ng of osteopontin per milliliter.

#### DISCUSSION

The lifetime risk of pleural mesothelioma in a population with exposure to asbestos ranges from 4.5 to 10.0 percent. Workers at risk for high levels of exposure are miners, factory workers, carpenters, electricians, shipfitters, ships' electricians, boilermakers, insulation manufacturers, railroad workers, gas-mask manufacturers, and pipe insulators.<sup>39</sup> It has been estimated that as many as 7.5 million construction workers in the United States have used construction materials containing asbestos for fireproofing of buildings, acoustic control, ductwork, and pipe and boiler installation.<sup>40</sup> Moreover, asbestos is still a hazard for an estimated 1.3 million workers in the construction industry in the United States and for workers involved in the maintenance of buildings and equipment.<sup>41</sup> At present, there are no economically feasible, validated methods to screen all these persons at risk, since the estimated number of new cases of mesothelioma in the United States per year is only 2500 to 3000 (an age-adjusted incidence of 2 cases per 100,000 persons).<sup>42</sup>

The median survival after the diagnosis of pleural mesothelioma is 9 to 12 months; in advanced cases, resection of the tumor can prolong survival by about 3 months. Patients with stage IA disease, however, can survive for five or more years if the tumor is promptly resected. Unfortunately, the difficulty in detecting early disease means that less than 5 percent of patients with pleural mesothelioma present with stage IA disease. Hence, a marker or series of biomarkers that can predict the development of mesothelioma or detect pleural mesothelioma in its early stages in populations with exposure to asbestos would be of considerable value.

We found that osteopontin levels in the 69 subjects with exposure to asbestos did not differ significantly from those in age-matched controls and that osteopontin levels reflected the duration of occupational exposure and the extent of radiographic abnormalities. As compared with the subjects with exposure to asbestos, patients with pleural mesothelioma had significantly higher serum osteopontin levels. We carefully documented the type of exposure history, using a standardized occupational–environmental questionnaire, and interpre-



**Figure 4. Sensitivity and Specificity of Serum Osteopontin for Distinguishing Patients with Pleural Mesothelioma from Subjects with Asbestos-Related Nonmalignant Disease.**

In Panel A, an analysis that included all 76 patients with pleural mesothelioma and 69 subjects with exposure to asbestos revealed an area under the curve (AUC) of 0.888 (95 percent confidence interval [CI], 0.826 to 0.934). In Panel B, the use of a cutoff value of 10.9 ng of osteopontin per milliliter showed a high sensitivity for the early detection of mesothelioma. In Panel C, the use of a cutoff value of 48.3 ng of osteopontin per milliliter to minimize the total classification error had a sensitivity of 77.6 percent. In Panel D, an analysis that included the 13 patients with stage I mesothelioma and the 69 subjects with exposure to asbestos revealed an AUC of 0.906. In Panel E, the use of a cutoff value of 9.5 ng of osteopontin per milliliter showed a high sensitivity for the early detection of stage I mesothelioma. In Panel F, the use of a cutoff value of 62.4 ng of osteopontin per milliliter to minimize the total classification error had a sensitivity of 84.6 percent for stage I pleural mesothelioma.



tation of the radiographs by a B reader allowed classification of the population with exposure to asbestos according to important risk factors for pleural mesothelioma, including the duration of exposure.

A multiple regression analysis revealed that the duration of exposure and the radiographic findings were the most important influences on serum osteopontin levels and that longer exposure and more extensive radiographic abnormalities were significantly associated with elevated osteopontin levels. Fibrotic changes, but not pleural plaques, were also associated with elevated levels of osteopontin. The combination of radiographic findings and serum levels of osteopontin could be used to stratify the risk of pleural mesothelioma in populations with exposure to asbestos; close surveillance might be indicated in workers with a long history of exposure, pleural plaques and fibrosis, and elevated serum osteopontin levels.

The most important result of our study is the apparent ability of an enzyme-linked immunosorbent assay (ELISA) for osteopontin to identify early pleural mesothelioma (stage I). This finding, if confirmed, would have immediate clinical applications, because the use of therapy could potentially influence survival among patients with stage I pleural mesothelioma.

Immunohistochemical analysis showed that osteopontin was present in the tumor cells of pleural mesothelioma and not in the stroma. This finding provides support for the specificity of osteopontin as a marker for transformed mesothelial cells.

Osteopontin is being investigated as a biomarker in other types of cancer. Using immunohistochemical analysis, Coppola et al.<sup>43</sup> found high levels of osteopontin in gastric, colorectal, pancreatic, lung, and ovarian carcinomas, among others, and a strong correlation between osteopontin levels and the pathological stage. Schneider et al.<sup>44</sup> found that high tissue levels of osteopontin, measured by means of a real-time polymerase-chain-reaction

assay, correlated with decreased survival among patients with resected non-small-cell lung cancer. In both patients with pancreatic cancer<sup>45</sup> and patients with breast cancer,<sup>46</sup> serum osteopontin levels, as measured by ELISA, were elevated in patients with new or progressive neoplasms. The levels of osteopontin in these studies differed from those seen with our commercially available ELISA owing to technical differences, including our use of a monoclonal antibody system as opposed to a polyclonal ELISA and the measurement of serum osteopontin rather than plasma osteopontin, which led to levels that were lower than the manufacturer's standard levels for plasma determinations.

Our data suggest that serum osteopontin levels could be used to discriminate between persons with exposure to asbestos who do not have early pleural mesothelioma and those with exposure to asbestos who do have early pleural mesothelioma, regardless of the histologic type of the mesothelioma. Moreover, the fact that the AUC approached 0.9 suggests that the osteopontin level has a positive predictive power equivalent to that of CA-125 for ovarian cancer.<sup>47</sup> Osteopontin levels, however, are also elevated in other types of cancers, including gastrointestinal, laryngeal, and urinary neoplasms, and these cancers have been weakly associated with exposure to asbestos. The hypothesis that the osteopontin level may be increased in asbestos workers in whom cancers other than mesothelioma develop is being investigated. Nevertheless, we suggest that asbestos workers with high osteopontin levels who do not appear to have mesothelioma should be evaluated to rule out the presence of other cancers. Our data do not justify the global use of osteopontin measurements in persons other than those who have been exposed to asbestos as defined here or who have radiographic evidence of pleural disease.

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